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Comparing the role of garlic and folic acid on hematological parameters of male rabbits

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Abstract:

Thinks about have appeared that garlic has restorative and antimicrobial properties, speeds up assimilation and broadly utilized as additives, zest and condiment in numerous homes. It has powerful antioxidant properties fundamental for the stabilization of natural layers, securing cells from oxidative stretch and represses angiogenesis. The display work was conducted to explore the modifications in hematological components in male rabbits after orally organization a single measurements of garlic (GA) by gavage at a measurements of 40 mg/kg B.W/day, and folic corrosive by gavages' at a measurements 27.6 Kg), were±of 5 mg/kg B.W/day for 12-week. Twenty male rabbits weighting (1.891 isolated into four bunches with 5 creatures. The result demonstrated that the treatment with garlic, and folic corrosive essentially expanded critical increment in red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelet tally (PLT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH).While, cruel cell hemoglobin concentration (MCHC) expanded. The in general point of this inquire about was to discover the comparative impact of the garlic and folic corrosive on hematological parameters of male rabbits.

Keywords: Garlic, folic acid, hematological parameters, Rabbits

Introduction

Garlic, Allium sativum is portion of the Liliaceae family and contains a long history in people pharmaceutical in a assortment of societies over the world.

In spite of the fact that numerous of these societies created autonomously of each other, the wide accessibility and strength of garlic in a assortment of conditions have created comparative employments of garlic in a few diverse areas and times in history. Garlic is local to the Tien Shan and Pamir-Alai mountains of southern central Asia. Since of its compact and solid structure, garlic is thought to have been a nourishment source for the roaming hunter-gatherers more than 10,000 a long time prior (Cumo, 2015). Garlic contains more than 200 chemicals. It contains sulfur compounds (allicin, alliin and agoene), unsteady oils, proteins (allinase, peroxidase and miracynase), carbohydrates (sucrose and glucose), and minerals (selenium). It as well contains amino acids (cysteine, glutamine, isoleucine and methionine), which offer help to guarantee cells from the harms of free radicals, bioflavonoids (quercetin and cyanidin, allistatin I and allistatin II and vitamins C, E and A), which offer help to secure us from oxidation administrators and free radicals (Aldeeb *et al.,* 2021). Garli one of the foremost imperative local plants in Iran and has noteworthy restorative esteem (Hussein *et al.,* 2013). Garlic plant has a few antimicrobial, anti-carcinogenic, antifungal and anti-stress properties conjointly known as a calculate in progressing dietary indices, immune and development stimulants, cancer prevention agents, additionally adjusting blood weight (Fazlolahzadeh *et al.,* 2011). Among the foremost vital garlic compounds, allicin, phosphoric compounds, soluble chemicals, peroxidase, ajuvin, citral and granulated are said. A few ponders have appeared that garlic utilization increments the generation of cytokines, the movement of macrophages and lymphocytes, and eventually makes strides and invigorates the resistant framework (Khodadadi *et al.,* 2013). B vitamins bunch are a water-soluble counting folic corrosive (FOL), thiamine, biotin, riboflavin, pyridoxine, cobalamin, inositol, choline, and para-aminobenzoic corrosive (Ponce *et al.,* 2012). Folate is required for purine and pyrimidine nucleotides union (antecedents of DNA and RNA, individually), and for the digestion system of a few amino acids counting homocysteine (Lucock, 2000). The insufficiency of folate may lead to folate-dependent metabolic pathways disturbance, causing the improvement of clinical variations from the norm beginning from frailty to development impediment (Krishnan and Kiernan, 2009). A few of these vitamins are utilized alone or in combination with diclofenac or other Non-steroidal anti-inflammatory drugs (NSAIDs) for numerous difficult maladies like polyneuropathies and the spinal column degenerative illnesses, and the aggravation treatment coming about from the insufficiency of the vitamin (Mibielli *et al.,* 2009). This study was aimed to compare roles of garlic and folic acid on hematological parameters of male rabbits**.**

Materials and Methods

In this study folic acid was purchased from a pharmacy n El -Bayda-Libya and garlic were used. Garlic oil was purchased from public market for

medicinal herbs in Al-Bayda city.Mature male New Zealand White rabbits age of 6 months and initial weight of (1.891 ± 27.6 Kg) were used. The animals were individually housed in cages and weighed weekly throughout 3-months experimental period. Feed and water were provided ad libtum. Rabbits fed pellets which consisted of 30 % berseem (*Trifoliumalexandrinum*) thay, 25 % yellow corn, 26.2% wheat bran, 14 % soybean meal, 3 % molasses, 1 % $CaCl_2$, 0.4 % NaCl, 0.3 % mixture of minerals and vitamins, and 0.1 % methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.). The chemical analysis of according to Smith and Mayer. (1955**) s**howed that they contained 15.8 % crude protein, 11.3 % crude fiber, 3.7 % ether extract, 7.2 % ash, 92.9 % organic matter and 62.4 % nitrogen free extract % as DM basis. Fifteen mature male rabbits were randomly divided into three equal groups (each five rabbits) as follows: G**roup I:** Rabbits were used as control for 12 successive weeks**. Group II:** Rabbits were treated daily with folic acid 5 mg/kg BW (Mohammed *et al.*, 2022). **Group III :** Rabbits were treated daily with garlic 40 mg/kg BW (Khaled and Qataf, 2021). Blood samples were collected from the ear vein of all animals every other week throughout the 12-week experimental period. The samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in tube containing heparin to obtain plasma. The samples were used for complete blood count (CBC). All CBC tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc (Turgeon, 2012). CBC were performed on EDTA as anti-coagulated samples. Differential cell counts were performed manually using Dif-Quik-stained blood smears. The data obtained was recorded according to the following categories: white blood cell (WBC); red blood cell (RBC); hemoglobin (HB); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis:

Where applicable, statistical analysis was carried out in Minitab software (version17)/ statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

Results

Results indicated that treatment with garlic, and folic acid Significantly increased the red blood cells (RBC), white blood cells (WBC), packed cell

volume (PCV), platelet count (PLT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH). While, mean cell hemoglobin concentration (MCHC) increased.

Table1: Values of complete blood counts Red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelets count (PLT), hemoglobin (Hb), mean cell volume (MCV; fi), mean cell hemoglobin (MCH; pg) and mean cell hemoglobin concentration (MCHC; g/dl) of male rabbits treated with Folic acid and Garlic**.**

Parameter			
	C	***FOL***	***GAR***
RBC ×10⁶ (µl)	5.69± 0.149[a]	6.06±0.117[a]	5097± 0.169[a]
WBC ×10³(µl)	8.65 1± 0.26[b]	10.10± 0.49[a]	10.51± 0.32[a]
HCT×10³(µl)	41.33±0.573[b]	43.92± 0.638[a]	43.21± 0.527[ab]
PLAT ×10³(µl)	267.00±0.473[b]	290.08±9.559[ab]	304.49±7.641[a]
Hb (g/dl)	12.55± 0.21[c]	14.25± 0.19[a]	14.00± 0.17[ab]
MCV(fl)	77.77±1.94[a]	69.77±1.67[b]	68.39±1.61[b]
MCH(pg)	25.13± 0.91[a]	23.13± 0.97[a]	22.93±0.66[a]
MCHC(dl)	27.86±1.077[b]	33.71± 0.557[a]	33.48±0.520[a]

Values were expressed as means ± SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c) were significantly different, $p<0.05$.

DISCUSSION

The significance of garlic is due to utilize not as it were for culinary but too for helpful and restorative purposes in both conventional and advanced pharmaceutical. It is expended either as crude vegetable (new takes off or dried cloves), or after handling within the shape of garlic oil, garlic extricates and garlic powder with contrasts in chemical composition and bioactive compounds substance between the different shapes (Lanzotti *et al.,* 2014). The common increment in PCV, RBC, WBC, and Hb of rabbits encouraged garlic supplemented diets shows that garlic may contain blood shaping variables which will have fortified more blood generation This too proposes that this herbs may have made a difference in boosting the safe framework of the rabbits. Garlic treatment expanded the number of RBCs, WBCs checks and Hb concentration in male rabbits (Al-Jowari, 2014). Garlic altogether anticipated the lessening of RBCs caused by lead intoxification (Ouarda and Abd-Ennour, 2011) in rabbits. Be that as it may, Suleria *et al.* (2013) detailed that rabbits treated with garlic appeared inconsequential diminishment in RBCs check. Fazlolahzadeh *et al*. (2011) recommended that garlic contains a few constituents that will play a part within the work of organs related to blood cell arrangement such as thymus, spleen, and bone marrow to invigorate more blood generation. Onu and Aja. (2011) detailed that garlic might offer assistance in boosting the resistant framework of the rabbits. Iranloye. (2002) proposed the anti-infection properties of garlic that fortify safe capacities.

Moreover, garlic have a few critical phytochemicals such as flavonoids, steroidal glycosides, alkaloids, saponins, tannins, phenolics, pectin and amino acids, with their organic and physiological parts to invigorate the resistant framework and organs related to blood cell arrangement especially the bone marrow (Jeong and Lee., 1998). Treatment of rabbit with folic corrosive altogether ($P<0.05$) expanded hemoglobin (Hb) concentration, hematocrit value, count of RBCs and platelets, whereas altogether ($P<0.05$) diminished tally of WBCs as compared to control, being the most excellent for folic corrosive medicines (Table 1). In agreement with the display comes about, Sharma and Chowdary, (2015) shown that organization of folic corrosive (20 mg/kg LBW) for 15 days improved hematopoiesis counting RBCs, Hb concentration and stuffed cell volume in mice. Too, organization of folic corrosive (10, 20 and 40 mg/kg) to diabetic rats essentially ($P<0.05$) lifted Hb concentration and RBCs check (Daniel, 2015). Normal antioxidant medications moved forward hematological parameters (RBCs tally and Hb concentration) of rabbit does treated with green tea extricate (El-Ratel *et al.,* 2017) and rabbit treated with propolis (Hashem *et al.,* 2013).

In conclusion, treatment of rabbit with garlic and folic acid verbal organization for 12 weeks enhances the hematological parameters.

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The Toxic Effect of some Environment Polusion (Phathalate and Aluminum) on Oxidative Stress in Rabbits

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Abstract:

Phthalates (PAEs) are esters of phthalic corrosive; in specific, they act as plastic added substances and include versatility to mechanical polymers. The response between phthalic anhydride and liquor causes the arrangement of phthalates. Aluminum (Al) may be a abundant component within the earth's layer and is broadly dispersed all through the environment. As of now, aluminum salts are included in greasepaints, nourishment dealing with and pressing too utilized in different nonprescription drug. Different shapes of aluminum (Al) are natural xenobiotics that initiate free radical-mediated cytotoxicity. Therefore, this think about pointed at explaining the poisonous impacts of phathalate and aluminum chloride ($AlCl_3$) in male rabbits. The gather 1 gotten as control. Gather 2 gotten 34 mg $AlCl_3$/kg bw (1/25 LD50). Gather 3 was managed 500 mg phathalate/kg bw/day (1/50 LD50).Treatment was proceeded for three month. Treatment with phathalate and aluminum chloride ($AlCl_3$) caused noteworthy ($P<0.05$) diminish within the movement of GST, Turf and CAT in plasma and testicles homogenates compared to control. Whereas, caused a significant ($P<0.05$) increment in blood plasma and testicles homogenates TBARS as compared with control.

Keywords: Phathalat; Aluminum; Antioxidant enzyme; Rabbits.

Introduction:

Between 90 and 95% of all phthalates are utilized as plasticisers for the generation of adaptable PVC (Bi *et al.*,2021).Nowadays, plastic materials can be considered the foremost all inclusive utilized fabric for their flexibility, moo fetched and strong character. In any case, their nonstop utilize and destructive transfer are tireless toxins in each environmental specialty of the world (Bratovčić *et al.*, 2015). In 2017, plastic utilization come to 350 million tons (Gholamhosseini *et al.*, 2023). China can be considered the biggest

plastics creating nation (Bratovcic, 2019). Plastic materials are polymer and are utilized as a key fabric in buildings and developments, transportation, and logical gear. Some squanders are found in revealed and secured landfill destinations and will remain there for a few a long time. The essential land-based sources of plastics are bottles and packs, untreated sewage and expendable (Alimba and Faggio, 2019). Plastics buried in covered landfill locales will stay for a few decades, posturing potential issues within the long run. Furthermore, 'landfill mining', where flotsam and jetsam is burned as fuel to create vitality, has been proposed. While the transfer of plastics in landfills does sequester nearly 100 % of the carbon from the environment, this too suggests that both the fabric and the vitality stored in plastics are misplaced within the long-term prepare (Waring *et al.,* 2018). Aluminum (Al) could be a ample component within the earth's layer and is broadly conveyed all through the environment. As of now, aluminum salts are included in greasepaints, nourishment taking care of and pressing too utilized in different nonprescription drugs (Buddy *et al.*, 2012). A few creators assign that an over the top and delayed aluminum introduction specifically influences hematological and biochemical parameters, interrupts lipid peroxidation and diminishes the exercises of the antioxidant chemicals in plasma and tissues of creatures models (Lukyanenko et al. , 2013). Aluminum may be a neurotoxic component included within the etiology of a few neurodegenerative clutters like Alzheimer's maladies (Osman *et al.,* 2019).This study was designed to find out the toxic effect of some environment polusion (phathalate and aluminum) on oxidative stress in rabbits

Materials and chemicals:

In this study aluminum chloride and phathalate were used. Aluminum chloride was brought from chemistry department, faculty of science, Omar AL-Muktar University and phthalate (purity 99.0%) was purchased from Sigma–Aldrich (USA).

Experimental animals

Fifteen adult male rabbits weighting (2.917 ± 28.9 kg)were obtained from shahat city. Animals were housed (5 rabbits in each group) at the animal facility at chemistry Department, Faculty of Science, Omar Al-Mukhtar University, El -Beyda-Libya. In clean and cages and kept under standard situation. The rabbits were fed with rodent pellets and tap water ad libitum.

Experimental design:

The animals were equally divided into three groups (n = 5). The first group served as control (G1) and received distilled water, rabbits of the second group were received phathalate (G2) at the dose of 500 mg/kg /day (Mokhtar*et al.*, 2018) the therid group were received aluminium chloride (G3) at the dose of

34 mg/kg /day (Krasovskii *et al.*, 1979) by gavage for 12-week days. At the end of the experimental period animals were left night fasted and at the next day they were euthanized following protocols and ethical procedures.

Sample collection and biochemical assays

Blood samples were collected from the ear vein of all animals every other week throughout the 12-week experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in tubes, containing heparin to obtain plasma. Plasma was obtained by centrifugation of samples at 860×g C) until used for analysis. Storedfor 20 min, at - 80°C. Plasma samples were analyzed for glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig *et al.* (1974). Catalase (CAT; EC 1.11.1.6) activity was determined using the Luck method involving the decomposition of hydrogen peroxide (Luck, 1974). Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to Al-Ailla and Khaled, (2019). Plasma 40 thiobarbituric acid-reactive substances (TBARS) were measured by the method of Tappel and Zalkin, (1995).The testes was quickly removed and weighed. One testis from each rabbits was frozen at -20ºC, homogenized and assayed for Catalase (CAT), Glutathione *S*-transferase (GST), Superoxide dismutase (SOD). Also, Reduced glutathione (GSH), Malondialdhyde (MDA) on were assayed in testicular homogenate.

Statistical analysis

In the present study, all results were expressed as Mean ± S.E of the mean. One-way analysis of variance (ANOVA) was used to assess significant differences among treated groups and controls using Graph Pad Prism 7 (La Jolla, CA, USA). The Tukey Test was used to compare all groups with each other and to show the significant effect of treatment. Values were considered statistically significant when $p<0.05$.

Results:

The effects of of phathalate and aluminium chloride ($AlCl_3$) on plasma and testes homogenates glutathione S- transferase (GST), catalase (CAT) and superoxide dismutase (SOD) activities during the 12-week experimental period are shown in tables (1,2) represents the biweekly mean values of these parameter expressed as absolute values. Treatment with of phathalate and aluminium chloride ($AlCl_3$) caused significant ($P<0.05$) decrease in the activity of GST, SOD and CAT in plasma compared to control. While, increase in the level of TBARS in blood plasma and testes homogenates.

Table (1). Average of plasma glutathione S-transferase (GST; μmol/hr), glutathione catalase (CAT; U/min/ml), superoxide dismutase (SOD; U/ml) and thiobarbituric acid-reactive substances (TBARS) of male rabbits treated with phathalate and aluminium chloride ($AlCl_3$) (means ± SE).

Parameters	Animal Groups		
	G1	G2	G3
(GST; μmol/hr)	1.020 ± 0.018^{b}	0.572 ± 0.020^{c}	0.900 ± 0.018^{c}
(CAT; U/min/ml)	1.006 ± 0.019^{b}	1.120 ± 0.020^{b}	0.828 ± 0.023^{c}
(SOD; U/ml)	1.154 ± 0.022^{b}	1.041 ± 0.029^{c}	0.958 ± 0.033^{c}
(TBARS)	1.728 ± 0.025^{b}	1.978 ± 0.063^{a}	2.154 ± 0.070^{a}

Values are means ± SE of 5 rabbits in each group
Mean with different letters (a, b and c) are significantly difference ($p \leq 0.05$).
Mean with the same letters (a, b and c) are non significantly difference ($p \geq 0.05$).

Table (2). Average of testes homogenates glutathione S-transferase (GST; μmol/hr), catalase (CAT; U/min/ml), superoxide dismutase (SOD; U/ml) and thiobarbituric acid-reactive substances (TBARS) of male rabbits treated with phathalate and aluminium chloride ($AlCl_3$) (means ± SE).

Parameters	Animal Groups		
	G1	G2	G3
(GST; nmol/min/gT)	0.298 ± 0.18^{b}	0.060 ± 0.01^{c}	0.052 ± 0.8^{c}
(CAT; nmol/min/gT)	5.1 ± 0.24^{bc}	3.94 ± 0.8^{c}	4.2 ± 0.35^{c}
(SOD; U/mgT)	12.7 ± 0.99^{bc}	9.02 ± 0.8^{c}	9.9 ± 0.65^{c}
(TBAS;nmol/gT)	60.05 ± 0.99^{b}	72.57 ± 0.8^{a}	71.5 ± 3.85^{a}

Values are means ± SE of 5 rabbits in each group
Mean with different letters (a, b and c) are significantly difference ($p \leq 0.05$).
Mean with the same letters (a, b and c) are non significantly difference ($p \geq 0.05$).

Discussion:

Glutathione S-transferase plays a key part in cellular detoxification by catalyzing the response of glutathione with toxicants to make an S-substituted glutathione (Townsend and Tew, 2003) Superoxide dismutase has an antitoxic impact against the superoxide anion; Grass quickens the dismutation of superoxide to H2O2 which is evacuated by catalase (Usoh *et al.*, 2005). In this way Grass can be acting as essential defense and avoids advance era of free radicals. Whereas, catalas are catalyzes the expulsion of H2O2 that shaped amid the response by Grass (Ramanathan *et al.*, 2002).Treatment with AlCl3 and phathalate caused significant ($P \leq 0.05$) diminish within the exercises of plasma and testicles catalase (CAT) are glutathione S-transferase (GST), plasma and testicles superoxide dismutase (Turf) expanded the levels of thiobarbituric acid-reactive substances (TBARS) compared to control

animals.AlCl3intoxicated creatures appeared a number of markers of oxidative stretch, which incorporates increment within the level of TBARS and diminish in Grass, GST and catalase within the testes. The comes about of show consider demonstrate a design of AlCl3-induced oxidative push comparative to that found within the past ponders, which shows aluminum, initiated oxidative push including free radical era (Yousef, 2004). Be that as it may, Al is considered to be a non-redox dynamic metal, it advances natural oxidation both in vitro and in vivo since of its pro-oxidant movement (Yousef *et al.*, 2007; Turner and Lysiak, 2008). Expanded responsive oxygen species (ROS) were detailed in past considers amid aluminum introduction, which was ascribed to electron spillage, improved mitochondrial movement and expanded electron chain movement. ROS along these lines assault nearly all cell components counting layer lipids and creating lipid peroxidation (Greenery *et al.*, 2003). In this manner, it can be hypothesized that oxidative stretch may be one of the contributing components for aluminium-induced testicular brokenness (Turner and Lysiak, 2008). Lipid peroxidation (LPO) is one of the most signs of oxidative harm and it has been found to play an vital part within the harmfulness of numerous xenobiotic. The display think about proposes a significant increase in TBARS in testicles, which embroils free radicals-induced oxidative cell harm in interceding the harmfulness of aluminum (Yousef, 2004). The show think about appeared that phathalate caused diminished within the movement and concentration of antioxidant chemical GST, Turf and CAT in plasmaand testicles. The diminish in antioxidant protein certified the discoveries of Acharya *et al.* (2004) who found a diminish within the activities of testicular antioxidant chemicals in mice. Too, those creators detailed ROS-induced disability of Leydig cells, which play a significant part in steroidogenesis driving to diminished union of testosterone. Hence, the watched increment in free radicals may be ascribed in portion to the concomitant decrease of antioxidant protein and GST movement taking after phathalate treatment.

In conclusion,it is clear from the obtained results that phathalate and aluminium chloride induced pronounced hazardous effects in antioxidaint enzymes.

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Evaluation of Paraphenylenediamine in Kohl and black Lipstick samples Collected from Libyan Local Markets using High-Performance Liquid Chromatography

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Abstract:

Cosmetics are known to contain a long list of toxic chemicals. The aim of this study was to detect and determine the toxic substance para-phenylenediamine (PPD) in some cosmetics such as Kohl and black Lipstick using Reversed Phase-High Pressure Liquid Chromatography (RP-HPLC). In this study, ten cosmetics samples were collected from some local markets in the Libyan city of El-Beida. A simple, rapid and reliable method was developed and validated for the determination of PPD in kohl and lipstick samples using solution of methanol (50%) as solvent. The method has been validated over a wide linear range of 5-25 µg/ml with correlation coefficients being consistently greater than 0.997. The lowest PPD level was observed in Hashmi black Kohl sample (0.0058 % w/w), while the Flormar Eyeliner powder sample showed the highest PPD content (0.0105 % w/w). The RP-HPLC measurments indicated that the PPD content in four samples under study (Hashmi (Kajal) Kohl, Kohl Homemade from Massa, Libya, Kohl Homemade from El-Beida, Libya and Pupa Pencil Eyeliner) were free of PPD (Not Detected). The PPD content in all samples analyzed in this study is well below the permissible limits set by the US Food and Drugs Administration, and documented values.

Keywords: Kohl; Lipstick; PPD; Methanol; HPLC.

Introduction:

The toxic substance paraphenylenediamine (PPD) is widely used as a permanent hair dye (Puri and Puri, 2013). p-Phenylenediamine (PPD) is a chemical substance containing a monocyclic aryl amine compound; the chemical formula of PPD is $C_6H_8N_2$ and its molecular weight is 108.15 g/mol.

When exposed to air, the powder, which is initially white to light purple, oxidizes and turns red, then brown, and finally black (HSDB, 1993). At a maximum concentration of 4.0%, it is mainly utilized as an ingredient in oxidative hair coloring products. PPD is also present in rubber compounds as an antioxidant, in hannan, fur or textile dyes, and as a developing agent for photography. PPD can be manufactured or used in the course of an individual's job, and exposure can happen through ingestion, skin and/or eye contact, or inhalation (SCCP, 2006). Humans may experience acute effects of high PPD exposure, such as severe dermatitis, gastritis, asthma, renal failure, vertigo, tremors, convulsions, and coma. Humans may develop eczematous contact dermatitis as a chronic effect of prolonged exposure (Lepoittevin and LeCoz, 2007; Jacob *et al.*, 2008; Kind *et al.,* 2012). PPD is frequently added to cosmetics like henna and is present in over a thousand hair dye formulas sold worldwide (Stanley et al., 2005). According to epidemiologic studies, people who use hair dye, work in the rubber and textile industries, or are barbers are more likely to develop bladder cancer, multiple myeloma, non-Hodgkin's lymphoma, or hematopoietic cancers (Thun *et al.,* 2005). Typically, carcinogens damage DNA, exposing cells to the possibility of either apoptosis or genomic proliferation that could result in the development of cancerous cells (Steller, 1995).

Literature review indicates that some analytical methods have been developed to the determine PPD by HPLC (Yoshiaki and Masa-aki, 2000; Vincent *et al.,* 2002; Al-Suwaidi and Ahmed, 2010), GC/MS (Stambouli *et al.,* 2004; Di Gioia *et al.,* 2005), voltametric method (Gao *et al.,* 2011), emission spectroscopy (Ngamdee *et al.,* 2012) and several reports of spectrophotometric methods have been made. While spectrophotometric techniques offer advantages over other approaches, they require a lot of time due to the diazotization and coupling with N-(1-naphthyl) ethylenediamine processes (Jadhav *et al.,* 2010), and involve oxidation of the compound converted in to salt that is measured colorimetrically (Hilton, 1960), coupling of triclosan with reagent 2-aminonaphthalene-4, 8- disulfonic acid with low level detection (Kysliak and Smyk, 2010). The other method relied on the reaction of sodium nitrite with p-sulfanilic acid in an acidic medium to form diazonium ion, with which triclosan also forms an azo compound in an alkaline medium (Lu *et al.,* 2009). Determination of triclosan in antiperspirant gels by first-order derivative spectrophotometry has also been developed (Du *et al.,* 2011). This study aims to develop a simple, sensitive, rapid, reproducible, precise and accurate HPLC method to evaluate PPD in kohl and black lipstick samples collected from Libyan markets. The reliable method developed in our study was based on other methods recommended by other researchers with some modifications, in order to isolate and determine PPD in Kohl, hair dyes and

henna (Al-Suwaidi and Ahmed, 2010; Saranya *et al.*, 2014; Elmanfe *et al.*, 2019; Elmanfe *et al.*, 2022). The method used is faster and simpler compared to other methods (Al-Suwaidi and Ahmed, 2010; Saranya *et al.*, 2014). The analyses have been developed and validated for RP-HPLC measurements.

Materials and Methods:

Chemicals and reagents:

All chemicals, reagents, solvents and analytical standards used during this study were of analytical grade and highly pure. Para-Phenylenediamine (PPD) was purchased from (India-ResearchLab) with purity 97 % (for research and development). Also, other chemicals and solvents were used including Methanol (Riedel-Dehaen AG Seelze Hannover) with purity 99.9 % (for HPLC) as solvent ; Ammonia (BDH-Laboratory) to adjust the pH for the mobile phase; Acetic acid (Riedel-Dehaen AG Seelze Hannover) with purity 99.8 % is used as mobile phase.

Chemicals preparation:

Standard solution of PPD: 0.01 g in 100 ml (0.10 mg ml^{-1}) solution was prepared. Working standards were prepared by appropriate dilution of the stock. (5, 10, 15, 20 and 25 µg ml^{-1}). *Aqueous methanol solution 50 %*. *Acetic acid solution 0.05 M:* 2.88 ml of acetic acid is diluted with 1000 ml distilled water and the pH is adjusted to 5.9 with ammonia, this solution is used for HPLC mobile phase.

Instrumentation (HPLC):

The HPLC system (Thermo Series P2000 Pump) Autosampler, Series 200 UV/VIS Detector with a wavelength of 240 nm (the spectrum range from 190 to 1000 nm, The Series 200 Autosampler, Series 200 Analytical Pump, Series 200 Column Oven, and 20 µl loop injector. The stationary phase represents the analytical column which was a Brownlee Bio C18 column of 250 mm x 4.6 mm and 5 µm particle size.

HPLC operating conditions: Before selecting the final operating conditions, the HPLC was operated with several solvents (mobile phases), included buffer solutions, and choose the ratios A% and B% and change the wavelenght (λ_{max})…etc. The best separation conditions were the following:

Mobile Phase: A: 85% acetic acid buffer; pH ≈ 5.9 (adjusted with ammonia), B: 15% methanol; *Flow rate* was*:* 1.5 ml min^{-1}; *Injection:* 20 µl; *Tr*: 1.53 min for PPD; *Temperature* was*: 30 °C* and *Pressure* was*: 135 bar.*

Standard Solutions of PPD (HPLC Calibration curve):

The calibration curve for standard solutions in the range 5-25 µg/ml was prepared by serial dilution from the 100 µg/ml mixed standard as shown in figure (2). The figure (1) shows the chromatograms for different concentrations of PPD (5 - 25 µg/ml) at 240 nm and retention time Tr = 1.53.

The standard linear calibration curve obtained from the analysis of the standard solutions shown in the figure (2), showed a good linear relationship between the peak area and concentrations of the standard solutions of PPD.

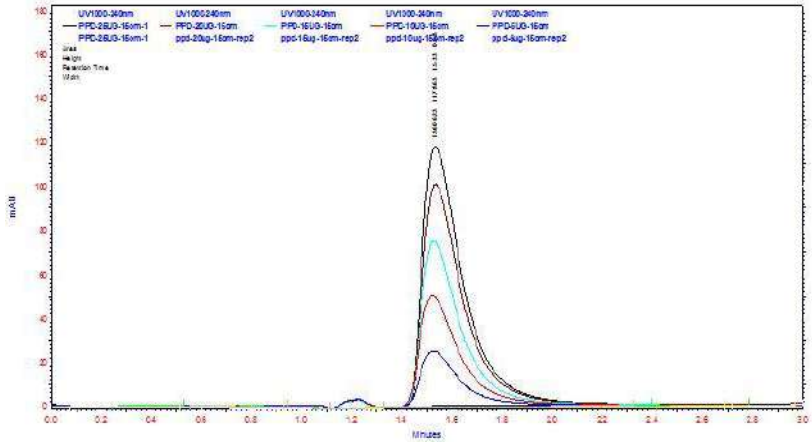

Figure 1: Chromatograms of different concentrations of PPD by RP-HPLC

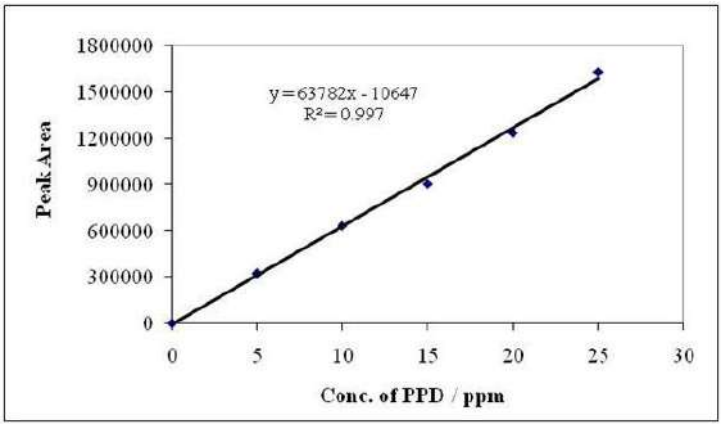

Figure 2*:* Calibration curve for PPD standard solutions, expressed on a linear scale**.**

Collecting samples under study:

Ten samples were collected from different markets in the Libyan city of El-Beida. Some of samples were Libyan products, and other samples were imported from another countries. Some of these samples were random samples of mixed Kohl (homemade). The table (1) shows the collected samples under study.

Table 1: Collected samples under study

N^0	Name of samples	Type of samples	Source
S1	Anagfasia (Beautiful Fashion)	Smooth Eyeliner (Kohl)	P.R.C
S2	Music Flower	Eyebrow and Eyeliner (Kohl)	P.R.C
S3	Flormar (water soluble)	Eye liner powder (Kohl)	France
S4	Karite Matte	Velvet Lipstick	France
S5	Hashmi Black Kohl	Kohl	Pakistan
S6	Hashmi (Kajal)	Kohl	Pakistan
S7	Kohl Homemade	Kohl	Massa- Libya
S8	Kohl Homemade	Kohl	Elbieda-Libya
S9	Huda Bauty	Pencil Eyeliner	London – UK
S10	Pupa	Pencil Eyeliner	Italia

PPD Extraction (preparation of samples for analysis):

The PPD extraction method was performed with some development and modification, based on the other studies in order to isolate and determine PPD in henna, hair dyes and other cosmetics (Al-Suwaidi and Ahmed, 2010; Saranya *et al.,* 2014; Mounika and Kinnera, 2015; Elmanfe *et al.,* 2019; Elmanfe *et al.,* 2022). One gram of each sample was weighed into a 50 ml volumetric flask and diluted with 50 ml of an aqueous solution of methanol (50%). This solution was then filtered after 15 minutes. Finally one ml of this solution was diluted to 5 ml with 50% aqueous methanol solution and the final solution was analyzed for PPD by RP-HPLC. To confirm the identity of the PPD in the samples under study, one ml of the standard was diluted to five ml with 50% aqueous methanol solution and the solution was analyzed to determine its retention time before analyzing any sample.

Results and Discussion:

The results obtained in our study indicate that the PPD contents in kohl and lipstick samples were in the range of 0.0058 % w/w - 0.0105 % w/w. These results are shown in table (2) and figure (3). The HPLC measurements indicate that the lowest PPD content was observed in the Hashmi black kohl sample (S5) from Pakistan (0.0058 % w/w), while the maximum PPD content was observed in the Flormar (Eye liner powder Kohl) sample (S3) from

France (0.0105 % w/w). There are some samples that were free from PPD (not detected) for example: (Hashmi (Kajal) Kohl from pakistan (S6), Kohl Homemade (S7) (Massa - Libya), Kohl Homemade (S8) (El-Beida - Libya) and Pupa Pencil Eyeliner (S10) from Italia). The concentration of PPD in the samples under study was well below the permissible limits set by the US Food and Drugs Administration and the Scientific Committee on Consumer Products (SCCP, 2006).

Table 2: PPD content (% w/w) in the studied samples using RP-HPLC.

n°	Name of samples	PPD content (%w/w)
S1	Anagfasia (Beautiful fashion)	0.0096
S2	Music Flower	0.00907
S3	Flormar (water soluble)	0.0105
S4	Karite Matte	0.00996
S5	Hashmi Black kohl	0.0058
S6	Hashmi (Kajal)	ND
S7	Kohl Homemade	ND
S8	Kohl Homemade	ND
S9	Huda bauty	0.00913
S10	Pupa	ND

ND = (Not Detected)

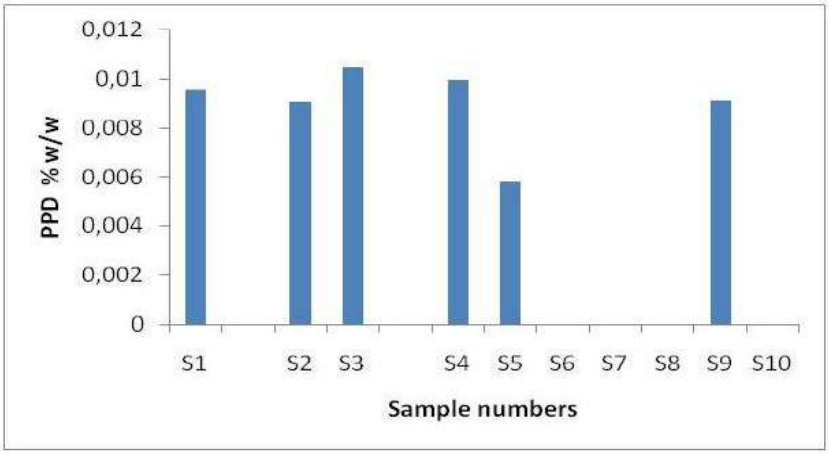

Figure 3: PPD content (% w/w) in the samples under study using RP-HPLC

Validation of the method used in this study:

Several factors were used to validate the analytical methods including: the linearity, limit of detection (LOD), limit of quantitation (LOQ), relative standard deviation (%RSD), accuracy and precision …etc.

Linearity: Standard solutions of PPD were prepared to determine the linearity of RP-HPLC response. Good linear correlations between peak areas and concentrations were obtained in the chosen range from 5 to 25 ppm (µg/ml). The linearity of the calibration standard curve was validated by the high value of correlation coefficients of the regression graph. The calibration curve was prepared and showed a linear response (R^2 =0.997) over the range of 5 – 25 µg/ml (ppm).

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The limits of detection (LOD) and the limit of quantification were determined to be 1, 21 and 3.67 µg/ml respectively, which was more sensitive than the previously mentioned method (Mounika and Kinnera, 2015).

Accuracy and precision : The precision of the proposed method was also determined by running the calibration series solutions at 5–25 µg/ml and then it was evaluated in term of repeatability and expressed as the relative standard deviation (RSD %). The results of the precision were ranged between 0.09 and 1.15 %, indicating good repeatability.

The validation parameters for the quantification of PPD standard can be summarized as follows:

The stationary phase (column) was: Brownlee BIO C18 column (250 mm x 4.6 mm and 5 µm particle size); *the mobile phase* was: acetic acid buffer (A) and methanol (B) with ratio (A: B = 85:15); *the detector* was: UV/VIS at wavelength of 240 nm; t_R = 1.53 min; *capacity factor* (k') = 0.12; LOD = 1.21 µg/ml; LOQ = 3.67 µg/ml; *coefficient of regression* (R^2) = 0.997 (y = 63782 x – 10647); *N (plates)* = 148.14 (592.56) *N expressed in plates per meter* and the *precision* (% RSD) for *N=3* were: (0.23; 0.29; 0.09; 0.33 and 1.15)% for the concentrations of (25 µg/ml, 20 µg/ml, 15 µg/ml, 10 µg/ml and 5 µg/ml) respectively.

Conclusion:

The validated method has been successfully applied to determine the PPD in different samples of kohl, eyeliners and lipsticks. PPD content in the samples under study were ranged from 0.0058 % w/w to 0.0105 % w/w. The contents of PPD in homemade samples (random samples) and some of other samples were free from PPD (not detected). The results obtained for PPD analysis in the samples under study using HPLC showed that there are differences in the levels of PPD in these samples. The maximum PPD content was observed in Flormar (Eye liner powder Kohl) sample, while the minimum PPD content was observed in Hashmi black kohl sample. The PPD levels in all the samples under study were well below the permissible limits set by the US Food and Drugs Administration, and documented values. Caution should be taken when

using cosmetics such as kohl, lipstick and other dyes, especially those of black color and unknown origin. We advise to avoid using the cosmetics that contain chemicals, some of which are toxic and harmful, and we recommend using natural materials for cosmetic symptoms or treatment.

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Role of Vitamin E in Enhancing the Chloropyrfios Actuated Changes in Body and Organs Weight in Male Rabbits

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ABSTRACT

Chlorpyrifos (CPF) is one of the foremost broadly utilized organophosphate (OP) insecticides in Libya. It is additionally the foremost broadly examined (OP) compound. The utilize CPF is still on the increment, with its orderly result on the wellbeing and well-being of human creatures and the environment. Since the use of CPF is on the increment, particularly in farming, the got to identify agents that would relieve the antagonistic wellbeing result postured by long term introduction to this chemical pesticide. Vitamin E may be a bunch of eight fat dissolvable compounds that incorporate four tocopherols and four tocotrienols. Vitamin E may be a fat-soluble antioxidant which may offer assistance secure cell films from responsive oxygen species. In this manner, the display test was embraced to decide the adequacy of vitamin E in easing the poisonous quality of chloropyrfios on body and organs weight of male rabbits. Creatures were alloted to 1 of 4 bunches: control; 33.3 mg CPF/kg b. w.; 100 mg vitamin E/kg b. w.; CPF (33.3 mg/kg b. w.) furthermore vitamin E (100 mg/kg b. w.), separately. Rabbits were orally managed the particular measurements each other day for 12 weeks. The gotten comes about appeared that vitamin E alone caused increment in body weight, weight of liver, lung, heart and kidney weight.

Key words: Chlorpyrifos, Vitamin E, Body weight, Rabbits.

Introduction

Chlorpyrifos (CPF), moreover known as Chlorpyrifos ethyl, is an organophosphate pesticide that has been utilized on crops, creatures, and buildings, and in other settings, to slaughter a few bugs, counting creepy

crawlies and worms. It acts on the apprehensive frameworks of creepy crawlies by repressing the acetylcholinesterase enzyme (Sparks *et al.,* 2020). Chlorpyrifos is considered tolerably dangerous to people (Course II) by the World Wellbeing Organization based on intense harmfulness data dating to 1999 (WHO, 2019). Introduction outperforming suggested levels has been connected to neurological impacts, tireless formative clutters, and immune system clutters. Presentation amid pregnancy may hurt the mental advancement of children (Israel, 2012). Chlorpyrifos presentation may lead to intense harmfulness at higher measurements. Determined wellbeing impacts take after intense harming or from long-term presentation to moo dosages, and formative impacts show up in fetuses and children indeed at exceptionally little measurements (Rauh *et al.,* 2012). In tests with rats, early, short-term low-dose presentation to chlorpyrifos brought about in enduring neurological changes, with bigger impacts on enthusiastic preparing and cognition than on engine abilities. Such rats shown behaviors steady with misery and diminished uneasiness (Timofeeva and Levin, 2010). In rats, low-level introduction amid advancement has its most noteworthy neurotoxic impacts amid the period in which sex contrasts within the brain create. Presentation leads to diminishments or inversions of typical sex contrasts (Connors *et al.,* 2008). Presentation to moo levels of chlorpyrifos early in rodent life or as grown-ups too influences digestion system and body weight (Slotkin, 2011). Vitamin E may have different parts as a vitamin. Numerous organic capacities have been hypothesized, counting a part as a fat-soluble antioxidant (Traber *et al.,* 2019). In this part, vitamin E acts as a radical forager, conveying a hydrogen (H) iota to free radicals. At 323 kJ/mol, the O-H bond in tocopherols is almost 10% weaker than in most other phenols (Lide, 2004). This frail bond permits the vitamin to give a hydrogen iota to the peroxyl radical and other free radicals, minimizing their harming impact. The thus-generated tocopheryl radical is reused to tocopherol by a redox response with a hydrogen giver, such as vitamin C (Traber *et al.,* 2019). Because it is fat-soluble, vitamin E is consolidated into cell layers, which are hence ensured from oxidative harm.

Materials and methods

Tested compounds

CPF was purchased from public market for pesticides in El-Beida city. Vitamin E was purchased from pharmacy in El-Beida, Libya. Twenty male

New Zealand white rabbits (6 months old) were individually housed in cages and weighed weekly throughout 12 weeks' experimental period.

Study design

Twenty mature male rabbits were randomly divided into four equal groups (n=5 for each group) as follows:

Group I: Rabbits were used as control and received an equivalent of 1 ml of the vehicle (corn oil) alone by oral gavage twice per week for 12 successive weeks.

Group II: In this group, rabbits were given oral administration of vitamin E with a dose of 100 mg/kg/day (Minardi *et al.,* 2020).

Group III: These rabbits received orally with a dose of CPF 3.3 mg/kg/day (Aldeeb *et al.,* 2022).

Group IV: These rabbits were administered orally with a combination of CPF and vitamin E.

At the end of the experimental period body weight of rabbits were recorded. Animals were sacrificed by decapitation and organ weight were immediately removed and weighed then the organs weight ratio was calculated. The relative weight of organs (%) was calculated as g/100 g body weight.

Statistical analysis

The data obtained were expressed as mean ±SEM. The significant differences were assessed by one-way ANOVA and Tukey test. After the detection of the normal distribution of the data and appropriate P-values, less than 0.05 is considered significant.

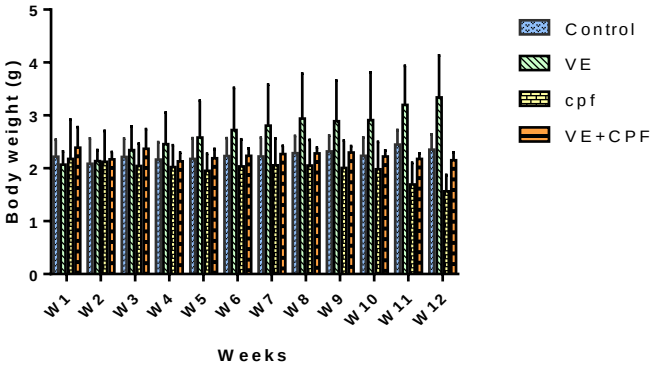
Results

The changes in body weight (BW) and the relative weights of liver, kidney, lung and heart of male rabbits were appeared in Table 1. Generally implies demonstrated that treatment with CPF aused noteworthy diminish in BW and relative weight of liver, kidney compared to control creatures. On the other hand the BW and relative weight of liver, kidney, lung and heart were altogether expanded in rabbits treated with vitamin E alone as compared to control creatures. The combination between vitamin E and CPF caused increment within the diminishment of BW and advancement in relative organ weights.

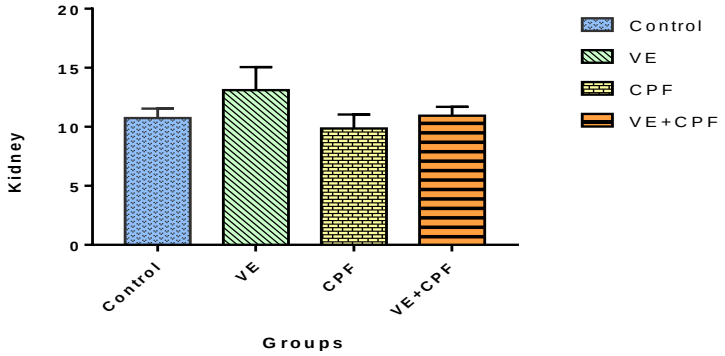
Table 1. Body weight (BW) and relative weight of kidney, liver, lung and heart of male rabbits treated with vitamin E, CPF and their combination

Parameter	Experimental groups			
	CON	VE	CPF	VE+CPF
BW (gm)	2.25±0.039^{b}	2.700±0.1^{a}	1.969±0.88^{c}	2.211±0.0190^{b}
Kidney (g/100gm)	10.740±0.357^{b}	13.100±0.877^{a}	9.860±0.527^{b}	10.940±0.337^{ab}
Liver (g/100gm)	56.600±0.170^{ab}	60.840±1.2844^{a}	50.500±2.872^{b}	55.200±2.538^{a}b
Lung(g/100 gm)	8.600±0.330^{a}	9.460±0.728^{a}	6.600±0.170^{b}	8.280±0.193^{a}
Heart(g/100 gm)	6.240±0.350^{ab}	6.480±0.460^{a}	4.740±0.357^{b}	6.262±0.358^{ab}

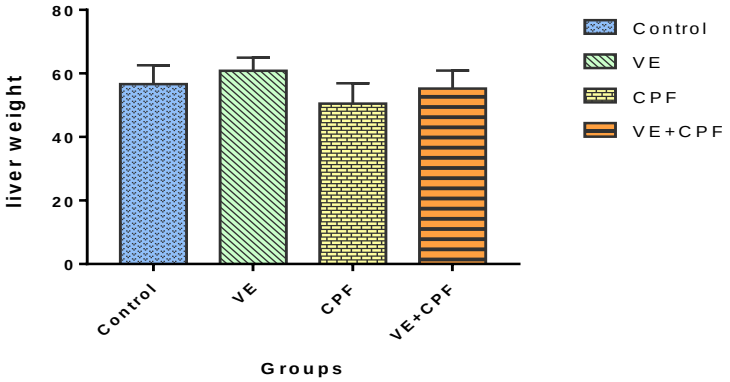
Values are expressed as means ± SE; n=5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p<0.05$.



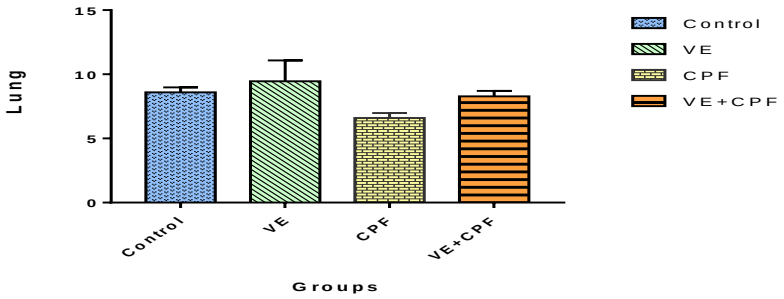
Figuer 1. Changes in body weight treatment of male rabbits with vitamin E, chlorpyrifos and/or combination**.**



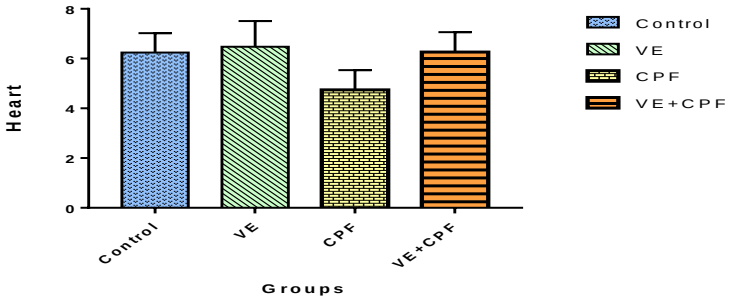
Figuer 2. Changes in weight of kidney treatment of male rabbits with vitamin E, chlorpyrifos and/or combination.



Figuer 3. Changes in weight of liver treatment of male rabbits with vitamin E, chlorpyrifos and/or combination**.**



Figuer 4. Changes in weight of lung treatment of male rabbits with vitamin E, chlorpyrifos and/or combination**.**



Figuer 5. Changes in weight of heart treatment of male rabbits with vitamin E, chlorpyrifos and/or combination**.**

Discussion

The display comes about demonstrate that treatment with (CPF) caused noteworthy decreases in body weight (BW) and relative organs weight (Push) of (Table 1 and Figure 1 to 5). The diminishment in BW and Push of the CPF

treated rabbits is in understanding with that gotten by Carr *et al.* (2001) and Raees (2007). The lessening in body weight in reaction to chlorpyrifos admissions may be a result of the combined activity of cholinergic and oxidative stretch and/or due to extend corruption of lipids and proteins as a coordinate impact of organophosphours compound introduction (Goel *et al.*, 2005; Mansour and Mossa, 2010; Mossa *et al.*, 2011 and Heikal *et al.*, 2012). Vitamin E scavenges for responsive oxygen species (ROS), avoids cellular harm and progresses development (Wolf, 2005). This perception is in line with the report of Corino *et al.* (2011) who watched that dietary vitamin E expansion in rabbit's eat less brought about in higher body weight and nourish transformation proficiency in rabbits.

In conclusion, the comes about of the show think about convincingly illustrated that CPF introduction brought about in shifting degree in organs weight. Vitamin E treatment advancement the body and organs weight.

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Mineral Analysis and Some Chemical Composition Estimation for the Breast Milk

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Abstract

This study aimed to measure the level of some elements and heavy metals and to measure the percentage of protein, fat, and lactose sugar in the breast milk of some women in the city of El-Beida. The sodium concentration in the samples ranged between (72.14-193 mg/ml), and the potassium concentration ranged between (180.80-2501.9 mg/ml), the concentrations of copper and iron ranged between (3.236-12.726 mg/ml) and (2.050-3.053 mg/ml), respectively. The study also show discrepancy in the percentage of protein, as the results showed the highest percentage in sample number S1 (3.95 mg/ml) and the lowest percentage in sample number S7 (2.91 mg/ml). The percentage of fat was measured, which ranged between (0.83-5.17 mg/ml), and the percentage of lactose, which ranged between (4.37-5.92 mg/ml). Through the previous results, a difference was observed between the concentrations of these elements in the samples. We can say that it is due to what the mother takes in vitamins during the breastfeeding period, and the diet followed by the mother during breastfeeding.

Keywords: Heavy Metals, Breast Milk, Sink Tester Ltd.

Introduction

The optimum food for a newborn's nourishment is human milk. Human milk does not have a perfect composition, nor is it simple to regulate how much and how complexly breastfed newborns receive in terms of nutrition. Charts of newborn growth (weight, size and head circumference) and neurodevelopment criteria are used by pediatricians and nutritionists, and they take into account the food that these babies are fed. These charts show the physiology of the infant first, and they also probably show the makeup of human milk when a baby is nursed. Correlating infant growth or neurodevelopment with the content of breast milk is more challenging in cases of preterm birth because of the physiological impact on the mother. While several indicators (lipids and oligosaccharides) have been found in breast

milk, their exact roles are still being investigated. Improving our understanding of the potential effects of human milk on newborn development over the medium and long term, and how this relates to nutritional programming, is a difficult but necessary step towards improving infant nutrition management, particularly for the most vulnerable preterm babies (Barker *et al.*, 1993).

Breastfeeding:

In this particular situation, the World Health Organization (WHO) advises exclusive breastfeeding, beginning within the first hour of life, for a maximum of six months. The exclusive breastfeeding rate is still quite low, particularly in low and middle-income countries (only 37 % of infants younger than 6 months are exclusively breastfed), despite WHO recommendations and pro-breastfeeding messaging provided in hospitals and maternity hospitals (Godfrey and Barker, 2000; Victora *et al.*, 2016). There is wide scientific agreement that breastfeeding has health benefits that protect an infant's development in the first few weeks of life. These impacts are either short- or medium-term: 2 % lower risk of baby death when compared to those who are not breastfed, indicating a significantly protective effect on infant mortality (Sankar *et al.*, 2015). A reduction in gastrointestinal and respiratory infections in the first several weeks of a newborn's life (Victora *et al.*, 2016), most likely because of the combination of breast milk and colostrum, which is an immature milk given to a baby for the first three days of life and provides immune protection. Lastly, there is also general agreement regarding the benefit of breastfeeding for improving neurodevelopment in children born on term (Bernard *et al.*, 2013), premature (Rozé *et al.*, 2012), or premature (Der *et al.*, 2006). Numerous studies pertaining to premature infants indicate a favorable correlation between the amount of breast milk consumed while hospitalized and the development of the nervous system (Vohr *et al.*, 2006). Even though they caught up to non-breastfed children at three years old, prematurely breastfed children showed slower growth (weight, height) during hospitalization, despite having superior psychomotor development at two or five years old compared to non-breastfed children (Rozé *et al.*, 2012). Dubbed this the "breastfeeding paradox" because earlier research had linked neurodevelopment to a newborn's growth rate during hospitalization (Ehrenkranz *et al.*, 2006). Because breastfeeding lasts for a long time, all of these effects are more noticeable, emphasizing a "dose" impact. Nonetheless, benefits for neurodevelopment have been linked to both the length of nursing

and the quantity received, indicating a dose-response relationship (Belfort *et al.*, 2016). Furthermore, even in the long run, we frequently overlook the advantages of nursing for the mother in addition to these benefits for the child: Minimized risk of ovarian and breast malignancies (Chowdhury *et al.*, 2015; Victora *et al.*, 2016); Minimized risk of type 2 diabetes, where lactation duration has a significant impact (Gunderson *et al.*, 2018).

Human Milk Composition:

Because it is made up of active and unique biological molecules, breast milk provides all the nutrients a baby needs for healthy growth and development. However, the exact makeup of breast milk is still unknown. The following re some of the most significant elements of breast milk that are known (Tackoen, 2012). Metals, water, proteins, fat, vitamins, carbohydrates, antibodies, and hormones.

Materials and Methods

Sampling:

Nine samples were collected from lactating mothers inside the city of El-Beida, Libya, to estimate components and compare the obtained results. The samples were illustrated in the table (1).

Table 1: The Types of the studied samples

NO.	Age	Infants gender
1	Four months	Female
2	Ten months	Male
3	One week	Male
4	Seven months	Male
5	Eleven months	Female
6	Four months	Male
7	Five months	Female
8	Month	Female
9	Eleven months	Male

Sample preparation:

5 ml of each sample was transferred into a conical flask, the samples were Digested by conc. HNO_3 (5 ml) until dryness, then the 10 ml of water was added. After heating for 20 min, the samples were filtered and diluted to 50 ml in the measuring flask.

The metal content measurements:

Minerals and heavy metals Na and K were measured by used flam photometer, while Fe and Cu were measured using UV-VIS spectrophotometer and fanadine was used as a reagent.

Measurement of the percentage of the protein, fat and lactose:

The percentage of protein, fat and lactose was measured using a device from Sino Tester Ltd located in the confidence center.

Instruments:

UV/VIS is spectrum photometer, flame photometer, Sino Tester Ltd.

Results and Discussion

The concentrations of the studied metals and heavy metals in breast milk samples are illustrated in table (2).

Table 2: The concentrations of the studied metals and heavy metals :

Conc. / Sample	Na mg/ml	K mg/ml	Cu mg/ml	Fe mg/ml
1	193.0	1516.9	5.900	2.257
2	177.2	1706.8	9.096	2.877
3	183.3	2501.9	9.955	2.371
4	151.0	1329.3	3.236	2.319
5	183.3	1107.7	9.722	2.298
6	184.4	1800.9	9.265	2.433
7	72.14	180.80	7.997	2.050
8	165.4	1525.3	6.765	2.763
9	129.5	1149.6	12.726	3.053

The minerals:

The results recorded in this study showed that the levels of sodium of the selected samples fluctuated in the range of (72.14-193.0 mg/ml), where the result recorded that all samples containing high levels of potassium. Also, the results showed that the contents of potassium fluctuated in the range of (180.80-2501.9 mg/ml). The results of minerals were given in table 3 and figures (1 and 2).

Table 3: The concentration (mg/ml) of the minerals in the studied sample.

Conc. / Sample	Na mg/ml	K mg/ml
1	193.0	1516.9
2	177.2	1706.8
3	183.3	2501.9
4	151.0	1329.3
5	183.3	1107.7
6	184.4	1800.9
7	72.14	180.80
8	165.4	1525.3
9	129.5	1149.6

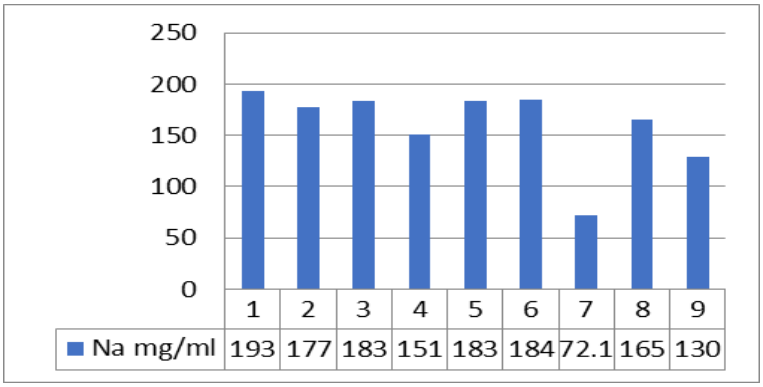

Figure 1: The distribution of sodium in the studied samples.

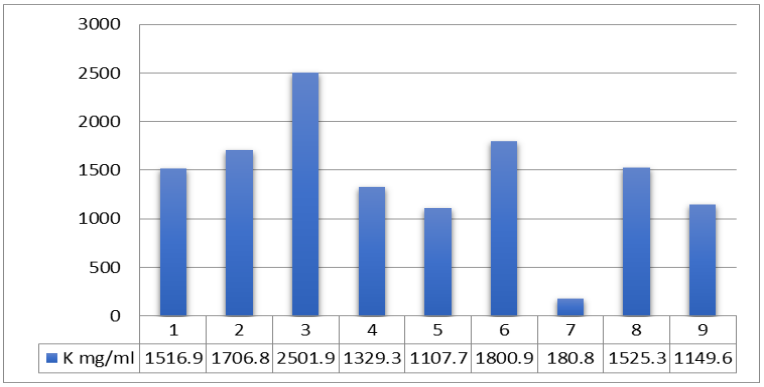

Figure 2: The distribution of potassium in the studied samples.

The heavy metals (iron and copper):

The results indicated that the breast milk samples containing different values of cupper and iron and ranged between (3.236-12.726 mg/ml) and (2.050-3.053 mg/ml) respectively (Table 4 and figure 3 and 4).

Table 4: The concentration (mg/ml) of the heavy metals in the studied sample.

Conc. / Sample	Cu mg/ml	Fe mg/ml
1	5.900	2.257
2	9.096	2.877
3	9.955	2.371
4	3.236	2.319
5	9.722	2.298
6	9.265	2.433
7	7.997	2.050
8	6.765	2.763
9	12.726	3.053

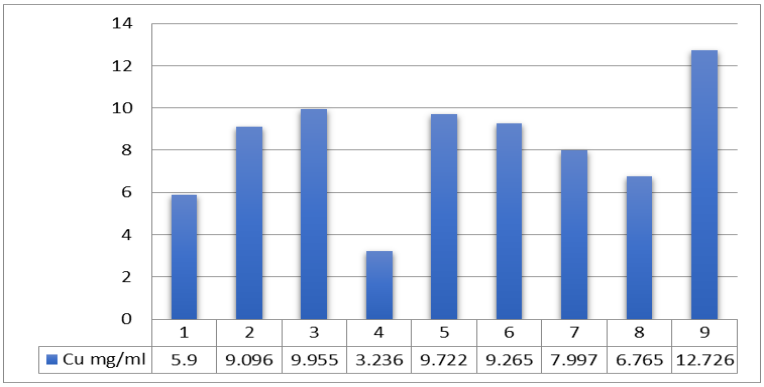

Figure 3: The distribution of copper in the studied samples.

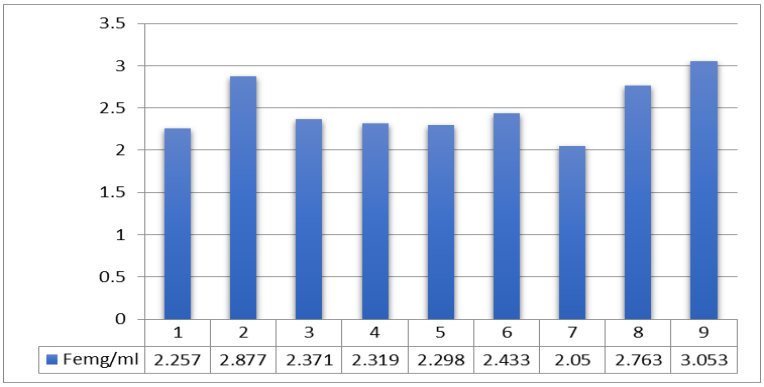

Figure 4: The distribution of iron in the studied samples.

Our findings about major nutrients are compared to other countries (USA) (Dubascoux *et al.*, 2018), and Sweden (Björklund *et al.*, 2012). For Na our result has highest value (193 mg/ml) and Sweden (192 mg/ml), the least value belongs to USA (145.5 mg/mL); and also for K our result has highest value (2501.9 mg/L), USA has second (433.5 mg/mL), the least value belongs to Sweden (307 mg/ml). Our copper result is (12.763 mg/ml) and Sweden (3.524 mg/ml), while the United States was lower. As for iron concentration in Libya, is the third highest value after Sweden and USA.

The percentage of the studied proteins, fat and lactose in breast milk samples is illustrated in table (5).

Table 5: The percentage of the protein, fat and lactose.

Percentage / Sample	Protein %	Fat %	Lactose %
1	3.95	2.82	5.92
2	3.19	2.08	4.78
3	3.45	0.83	5.17
4	3.20	1.16	4.80
5	3.35	5.17	5.01
6	3.44	1.46	5.16
7	2.91	3.60	4.37
8	3.28	3.23	4.92
9	3.66	2.60	5.48

Through the data record in table (5), the percentage of protein in mother milk ranges between (2.91-3.19 %), and the percentage of fat ranges or fluctuates between (0.83-5.17 %). While the percentage of lactose, which showed high values compared to the percentage of protein and fat, which ranges between (5.92 %) as a maximum and (4.37 %) as a minimum as shown in figure (5).

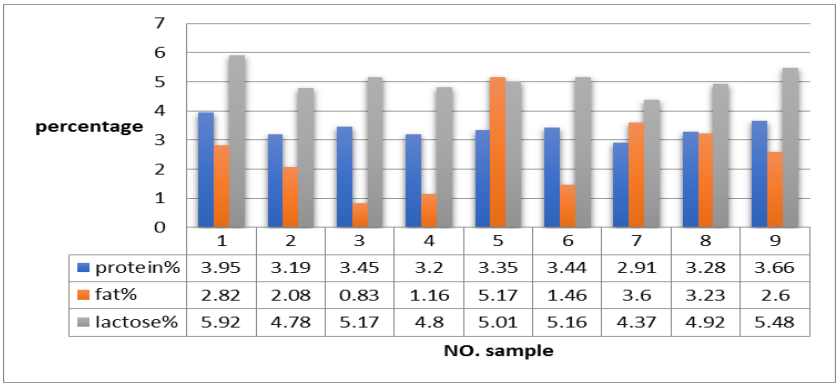

Figure 5: The percentage of the protein, fat and lactose.

Conclusion:

The samples had sodium concentrations ranging from 72.14 to 193 mg/ml and potassium concentrations between 180.80 and 2501.9 mg/ml. The ranges of concentrations for iron and copper were 2.050-3.053 mg/ml and 3.236-12.726 mg/ml, respectively. The study also revealed a disparity in the percentage of protein, with sample number S1 exhibiting the highest percentage (3.95 mg/ml) and sample number S7 exhibiting the lowest percentage (2.91 mg/ml). Were used to measure the percentages of fat and lactose, respectively, which ranged from (0.83-5.17 mg/ml) to (4.37-9.92 mg/ml). A variation in these elements' concentrations across the samples was noted by the earlier findings.

We can speculate that it results from the mother's vitamin intake during nursing as well as the mothers' diet during breastfeeding.

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Assessment of Mineral and Heavy Metal Content in Spices: Comparative Analysis and Health Implications

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Abstract

This study systematically investigated the mineral and heavy metal content in various spices, addressing both their nutritional benefits and potential health risks. Contamination often occurs during manufacturing and storage, posing public health concerns especially for heavy metals like lead, cadmium, and mercury. Key findings include Al-Anwar Company (Cn) Sample having the highest sodium level at 0.002 ppm and Attar agile (Cag) Sample containing lead at 24.579 ppm, far exceeding the WHO limit of 0.02 ppm. The study was aimed to guide consumer choices and inform regulatory actions to ensure public health safety.

Keywords: Spices, Heavy Metals, Public Health, Regulatory Guidelines.

Introduction:

Herbs, medicinal plants, spices, and perfumes have all seen large rises in popularity in recent years, which has had a huge impact on a wide variety of economies and cultures all over the world (Smith *et al.*, 2021). These components have worked their way into the social fabric, influencing both the culinary and medicinal traditions of the culture in which they have become embedded. Within the scope of this comprehensive category, the role that spices play is particularly essential. According to Opara and Chohan (2014), they do more than only improve the flavor profiles of a variety of foods; in addition, they contribute to the visual attractiveness, which engages numerous senses and elevates the whole dining experience (Opara and Chohan, 2014). In addition to the culinary benefits associated with using spices, there are also potential health benefits associated with using spices due to their distinct aromatic qualities (Raghavan, 2006). Due to the myriad ways in which they contribute to our lives, spices are an indispensable component of our routines, which is why this study delves so deeply into the complexities of their

environment. In this context, one of the primary areas of interest is the chemical composition of spices, specifically with regard to the kinds and amounts of minerals and heavy metals that they might contain. This is extremely important because, although spices improve the flavor of the food we eat and contribute to our health in a variety of different ways, they also have the potential to be sources of potentially harmful components like heavy metals (Tapsell *et al.*, 2006). Given the broad applications of spices that extend beyond the kitchen to medicinal and even cosmetic industries, the concerns about their safety are far-reaching (Leja and Czaczyk, 2016). They bring vivid flavors, colors, and fragrances to dishes in forms that range from whole to ground (García-Casal *et al.*, 2016). The rich history and diverse origins of spices, tracing back to various global regions like the Mediterranean and the tropics, add layers of complexity to their current universal usage (Behera *et al.*, 2020). The central question driving this research is the assessment of beneficial mineral concentrations and potentially hazardous heavy metal levels in commonly utilized spices within El-Beida, while also evaluating their alignment with global safety standards. To achieve this, the study aims to perform quantitative analyses on the levels of essential elements such as sodium, potassium, and lead in specific spice samples. Subsequently, these concentrations will be compared against safety guidelines established by the World Health Organization (WHO). The ultimate goal is to derive actionable regulatory recommendations based on these findings, with the overarching objective of ensuring the protection and enhancement of public health.

Materials and Method:

Chemical Solutions for the Experiment

Table (1) shows the chemical solutions used, which were all of analytical-grade to ensure the highest level of accuracy in the study.

Table 1: Chemical Solutions Used in the Experiment

Chemicals	Molecular formula
Nitric acid	HNO_3 65%
Hydrochloric acid	HCl, 37%
Distilled water	H_2O

Collection of Samples:

The process of collecting samples was carried out between the months of April and May in the year 2023. The samples were gathered from a variety of retail

markets and specialist spice shops located inside the city of El-Beida. There were a total of six distinct companies that contributed their data for this study, and each type of spice was represented by three distinct samples. These establishments provided a diverse selection, which included not one but four perfumer's shops and two distinct enterprises. The subsequent data that were received from the samples that were taken were meticulously organized, and they are shown in table (1) with as much specificity as possible. The experimental methodology incorporates a methodical approach to sample collecting, which ensures the production of a reliable and accurate dataset for the purposes of subsequent analyses and interpretations.

Sample Preparation:

Sample preparation was done carefully to ensure the integrity of the following analyses (Zimmerman and Weindorf, 2010). All sample preparation devices and glassware must be cleaned with distilled water and immersed in a strong nitric acid solution. Using distilled water, repeated washing cycles removed leftover contaminants. After then, sample digestion commenced (Van Loon, 1985).

Sample Digestion:

Wet digestion of the material involves the following steps. First, 0.5 grams of dry material was carefully placed in a 250 ml beaker. The mixture was heated under controlled conditions with 5 ml of strong nitric acid (HNO_3) to evaporate brown oxides (Ishak *et al.*, 2015). After halving the volume by evaporation, 5 ml of strong hydrochloric acid (HCl) was added. After evaporation and dilution, the mixture was filtered and placed in a 100 ml volumetric flask. To level the volume, distilled water was added.

Instrumentation:

Using UV-Visible spectrophotometric analysis (DU 800, BECKMAN COULTER), spice samples were tested for heavy metals. In addition, a Flame Photometer identified mineral metals. These advanced technologies provided precise elemental data on spice samples. The UV-Visible Spectrophotometer and Flame Photometer were calibrated to measure elemental concentrations precisely.

Table 2: shows the Scientific, common, source and code names of studied spices

Common name	Scientific name	Used part	Source name	Sample code
Paprika	Capsicum annual red	Seeds	Attar Al-Shalhoob	A_{sh}
			Attar Al-Zain	A_Z
			Attar Agile	A_{ag}
			Attar Cub	A_C
			Al-Anwar company	A_n
			Al-Weser company	A_W
Coriander	Corundum sativa	Seeds	Attar Al-Shalhoob	B_{Sh}
			Attar Al-Zain	B_Z
			Attar Agile	B_{ag}
			Attar Cub	B_C
			Al-Anwar company	B_n
Hararat (spices)	Baharat	Mix	Attar Al-Shalhoob	C_{Sh}
			Attar Al-Zain	C_Z
			Attar agile	C_{ag}
			Attar Cub	C_c
			Al-Anwar company	C_n
			Al-Weser company	C_W

Standard Preparation:

The typical preparation began with a 1000 ppm stock solution of lead, iron, copper, chromium, and other elements. Additionally, 5000 ppm copper was made (Zaleznik *et al.*, 2006). From this stock solution, serial working solutions at 4, 6, 8, and 10 ppm were made. To facilitate flame photometer analysis, sodium and potassium standard solutions were generated at 1000 ppm and serially diluted at 4, 6, 8 and 10 ppm.

Results:

This study provides a comprehensive examination of mineral and heavy metal concentrations in an array of spices from El-Beida local markets. The evaluation is aligned with the permissible limits set by international bodies like the World Health Organization and the Food and Agriculture Organization (WHO/FAO).

Table 3: Summary of Highest and Lowest Concentrations

Element	Highest Concentration (ppm)	Sample (Highest)	Lowest Concentration (ppm)	Sample (Lowest)	WHO/FAO Permissible Limits (ppm)
Na^+	0.002	Cn	0.000	Csh	5,000,000
K^+	0.034	Ash	0.011	Cc	3,500,000
Fe^{+3}	23.1	Cc	15.224	Ash	300
Cu^{+2}	8.824	Cn	0.000	Ash	30
Pb^{+2}	24.579	Cag	0.000	Ash	0.02
Cr^{+3}	2.724	Bn	0.000	Ash	45

Discussion:

In our analysis, all spice samples displayed sodium and potassium concentrations well within the WHO/FAO guidelines, indicating their safe consumption and potential positive impact on cardiovascular health. However, a significant concern emerged as the sample labeled "Cag" exhibited a lead concentration of 24.579 ppm, far surpassing the WHO/FAO permissible limit of 0.02 ppm. This alarming finding raises grave public health concerns, particularly due to the potential harm lead toxicity can pose, especially to young children. Immediate regulatory action is imperative to address this issue. When compared to similar studies in El-Beida, Al-Basra, and Misurata, our research unveiled variable heavy metal concentrations across regions, with some El-Beida samples showing higher lead levels than those in Misurata but lower than those in Al-Basra. These disparities underscore the urgent need for a more stringent regulatory framework, emphasizing continuous monitoring and robust quality assurance mechanisms to safeguard public health effectively.

Conclusion:

This study offers a thorough analysis of mineral and heavy metal content in spices, highlighting both their health benefits and risks. Most spices were found to be within safe limits for beneficial elements like sodium and potassium. However, the alarming levels of lead in some samples point to a critical need for immediate intervention by regulatory bodies. These findings underscore the importance of regular monitoring and stringent quality control to safeguard public health.

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The Protective Role of Melatonin against the Toxic Effect of Sodium Arsenite on Hematological Parameters in Rabbits

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Abstract:

Nowadays arsenic is considered as a genuine toxicant metallic poison of wide wellbeing concern and is unpredictably accessible in ground water by common way and in agrarian runoff and mining handle by anthropogenic way. Melatonin appeared solid antioxidant movement and had defensive properties against oxidative stretch. The show think about was attempted to assess the restorative viability of melatonin in terms of normalization of modified hematology parameters taking after sodium arsenite treatment in rabbits. Creatures were divided into four bunches. The primary bunch was utilized as control. Whereas, bunches 2, 3 and 4 were orally treated with melatonin (ME, 10 mg/kg BW), sodium arsenite (Sa, 5 mg/kg BW) and sodium arsenite additionally melatonin, separately. Comes about appeared that Sa essentially (PB/0.05) diminished hemoglobin (Hb), add up to erythrocyte number (TEC) and pressed cell volume (PCV), whereas add up to leukocyte count (TLC) expanded. Melatonin deliver a noteworthy diminish within the Stuffed Cell Volume (PCV) and Hemoglobin Concentration (Hb) treated rabbits. Be that as it may a critical increment was watched within the platelets tallies and add up to lymphocyte tally of the melatonin treated rabbits when compared to the controls. We conclude that whereas melatonin treatment may not be of any right hand to an frail persistent, it'll unquestionably be of gigantic advantage for those who require platelet and lymphocyte substitution. Comes about illustrated the advantageous impacts of melatonin in decreasing the negative impacts of Sa on blood hematology parameters of male rabbits.

Keywords: Rabbits; *melatonin*; sodium arsenite; hematological parameters.

Introduction:

Sodium arsenite more often than not alludes to the inorganic compound with the equation NaAsO2. Too called sodium meta-arsenite, it is the sodium salt of arsenous corrosive. Sodium ortho-arsenite is Na3AsO3 (Greenwood and Earnshaw, 2012). The compounds are colorless solids. Sodium arsenite can

be breathed in or retained through the skin. At the side its known carcinogenic and teratogenic impacts, contact with the substance can surrender side effects such as skin aggravation, burns, tingling, thickened skin, hasty, misfortune of shade, destitute craving, a metallic or garlic taste, stomach torment, sickness, spewing, loosebowels, shakings, diminished blood weight, and migraine. Serious intense harming may lead to anxious framework harm coming about in shortcoming, destitute coordination, or "pins and needles" sensations, possible loss of motion, and passing (Jing *et al.,* 2012). After assimilation into the body, the primary tissue experiences with arsenic is blood. The collection of arsenic in erythrocytes has been found to cause iron deficiency connected with leucopenia, neutrophil consumption and thrombocytopenia (Li *et al.,* 2015). Arsenic-induced consumption within the checks of erythrocyte, leukocyte, and platelet together with add up to Hb substance has been detailed by a number of investigators (Ghosh *et al.,* 2017). Encourage, the lessening in PCV, Hb, and RBC checks as a result of restraint of porphyrin or heme union has too been detailed in arsenic-induced harmfulness (Ola *et al.,* 2016). Decreased deformability and untimely devastation of RBCs in iron deficiency are exceptionally common in irritation due to arsenic introduction (Straat *et al.,* 2012). Melatonin (N-acetyl-5-methoxytryptamine) is primarily synthesized from the amino corrosive tryptophan by the pineal organ in warm blooded creatures and people (Tan *et al.,* 2015). Firstly, tryptophan is hydroxylated by tryptophan-5-hydroxylase to create 5-hydroxytryptophan. At that point, it is decarboxylated to 5-hydroxytryptamine (serotonin) by L-aromatic amino corrosive decarboxylase. After serotonin acetylation, N-acetylserotoninis delivered. At final, N-acetylserotoninis converted to N-acetyl-5-methoxytryptamine (melatonin) within the pineal organ (Ren *et al.,* 2017). But for endogenous melatonin, exogenous melatonin can be devoured from everyday eat less. There are parcels of melatonin-rich nourishments, such as acrid cherries, walnuts, and orange juice Melatonin seem direct the circadian beat, and reduce sleep deprivation and fly slack (Kennaway, 2017). In expansion, melatonin appeared a assortment of administrative impacts on sexual behavior, safe work, vitality digestion system, the cardiovascular framework, the regenerative framework, and the neuropsychiatric framework (Sun et al., 2015). Melatonin too displayed anticancer and anti-osteoarthritic exercises. In addition, melatonin appeared solid antioxidant action and had defensive properties against oxidative stretch (Bali *et al.,* 2016). Melatonin is the center of numerous investigate regions due to its capacity to rummage free oxygen radicals and subsequently ensure cells and tissues from radical harm (Reiter *et al.,* 2016).

Materials and Methods:

In this study sodium arsenite (Sa) and melatonin were used. Sodium arseniteIt was brought from chemistry department, faculty of science, and melatonin was purchased from pharmacy in El -Bayda-Libya. Mature male New Zealand White rabbits (age of 6 months and initial weight of (2089±97.66Kg) were used. Twenty mature male rabbits were randomly divided into four equal groups: Group I: Rabbits were used as control for 12 successive weeks Group II: Rabbits were treated with melatonin which was given daily by gavage at a dose of 10 mg/kg b.w. / day 2 h before lights of **(El-Sayed *et al.*, 2019)** for 12 successive weeks. Group III: These rabbits will be treated orally with Sa 5mg/kg/day by gavage **(El-Demerdash *et al.*, 2009)** for 12 successive weeks. Group IV: Rabbits were given with Sa daily at a dose of 5 mg/kg/day BW by gavage like group III and given melatonin concurrently daily at a dose of 10 mg/kg b.w. / day 2 h before lights off by gavage like group II for 12weeks. All groups were administrated the treatments orally daily morning, except melatonin was administrated between 09.00-10.00pm. Haematological Parameters Blood samples were collected from the ear vein of all animals every week throughout the 3-month experimental period. Blood samples were obtained in the morning before accesses to feed and water. Values derived from complete blood count (CBC). All CBC tests were performed by automatic blood cell analyzer (XP-300 Automated Hematology Analyzer, Sysmex American, Inc **(Turgeon, 2005).** CBCs were performed on EDTA as anti-coagulated samples. Differential cell counts were performed manually using DifQuik-stained blood smears. At the end of the experimental period, all rabbits were weighed then sacrificed under ether anesthesia to prepare bone marrow smear. Statistical Analysis Where applicable, statistical analysis was carried out in Minitab software (version17)/ Graph Pad prism 8; statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

Results:

Table1 and figure 1 to results showed that Sa significantly (PB/0.05) decreased hemoglobin (Hb), total erythrocytic count (TEC) and packed cell volume (PCV), while total leukocyte count (TLC) increased. Melatonin produce a significant decrease in the Packed Cell Volume (PCV), Hemoglobin Concentration (Hb), Mean Cell Hemoglobin (MCH) and Mean Cell Volume (PCV) of treated rabbits. However a significant increase was observed in the platelets counts and total lymphocyte count of the melatonin treated rabbits when compared to the controls. We conclude that while melatonin treatment may not be of any assistant to an anemic patient, it will definitely be of immense benefit for those who require platelet and lymphocyte replacement.

Results demonstrated the beneficial influences of melatonin in reducing the negative effects of Sa on blood hematology parameters of male rabbits.

Table1. Changes Complete blood counts red blood cells RBCs, white blood cells WBCs, haemoglobin Hb, packed cell volume PCV, **platelets**, of male rabbits treated with melatonin, sodium arsenite and their combination.

Parameter	***Experimental groups***			
	CON	***Me***	***Sa***	***Sa+Me***
RBC ×10^6 (µl)	5.54±0.126^a	5.63±0.205^a	4.13±0.133^b	5.38±0.136^a
WBC ×10^3(µl)	8.11±0.19^b	9.52±0.30^b	7.72±0.41^b	8.32±0.23^{ab}
HCT×10^3(µl)	39.93±0.354^a	35.32±1.272^b	33.48±1.066^b	40.29±0.757^a
PLAT ×10^3(µl)	268.49±8.502^a	283.77±15.440^a	188.13±10.712^b	268.55±11.6^a
Hb (g/dl)	13.72±0.17^a	12.98±0.13^a	11.43±0.25^b	12.90±0.25^a

Values are expressed as means ± SE; n = 5 for each treatment group.Mean values within a row not sharing a common superscript letter (a, b) were significantly different, p<0.05.

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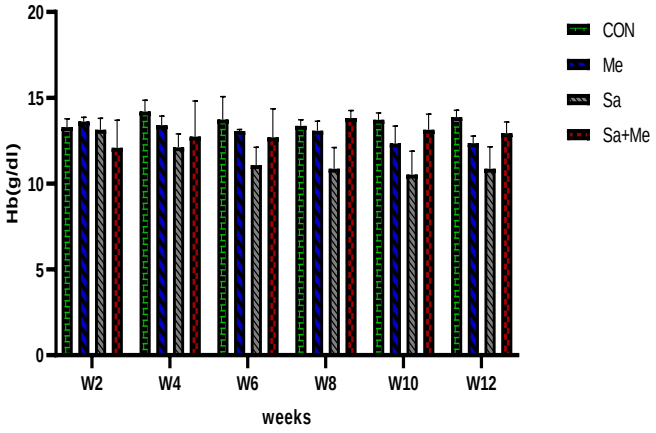

Figure 1: Change in haemoglobin Hbduring treatment of male rabbits with melatonin, (Sa) and/or their combination.

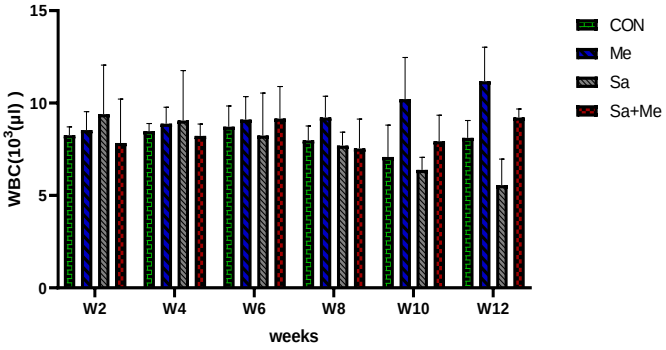

Figure 2: Change in white blood cells WBCs during treatment of male rabbits with melatonin, (Sa) and/or their combination.

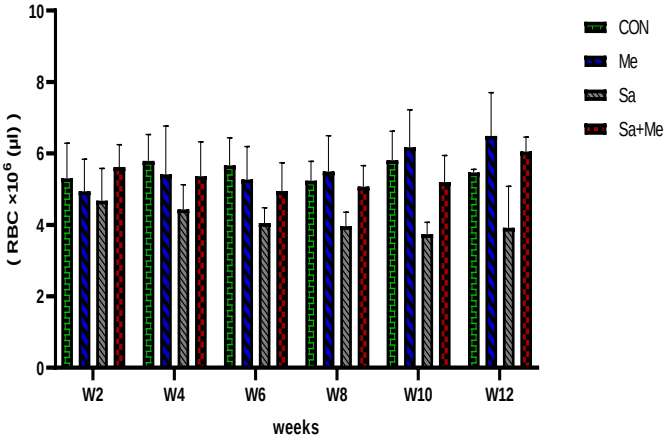

Figure 3: Change in white blood cells RBCs during treatment of male rabbits with melatonin, (Sa) and/or their combination.

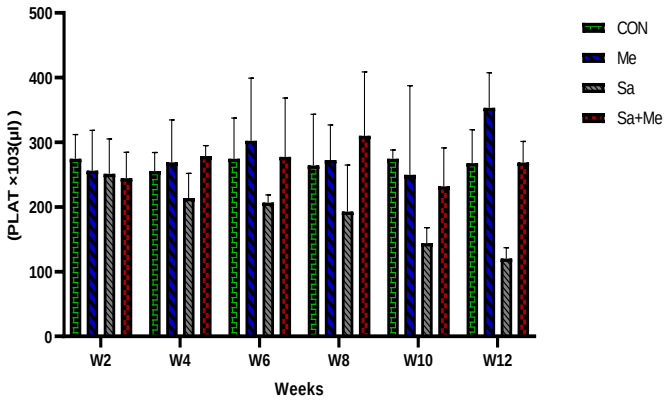

Figure 4: Change in platelets, during treatment of male rabbits with melatonin, (Sa) and/or their combination.

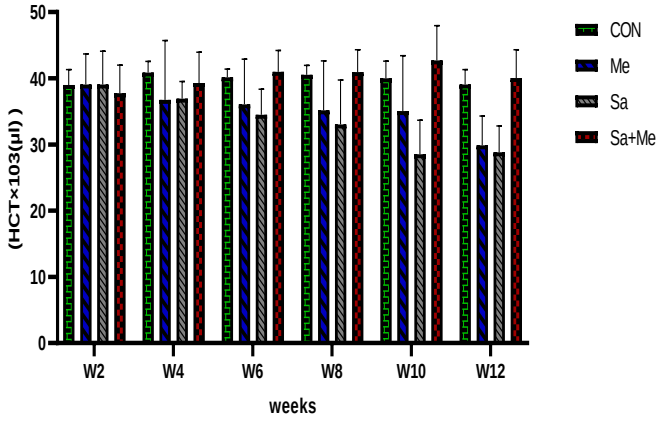

Figure5: Change in packed cell volume PCV during treatment of male rabbits with melatonin, (Sa) and/or their combination.

Discussion

Introduction to natural toxins constitutes a major danger to creature and human survival within the ever expanding industrialized world. Arsenic

defilement of drinking water from different sources has been detailed in numerous parts of the world counting created and creating countries(Smith and Smith, 2004).The assurance of hematological and serum biochemical parameters gives vital data on the modifications that influence the physiology of the blood in infection states or exposures to harmful poisons. Investigation of blood parameters is accepted to be important in chance assessment and reaction to therapy as changes within the hematological framework have tall prescient value (Olson *et al.,* 2000). In this consider, pretreatment of rabbits with melatonin altogether expanded the PCV concentration, and ruddy cell checks all of which were altogether diminished upon sodium arsenite introduction, (Table 1). The diminishment in PCV, Hb, and RBC tallies could be a result of hindrance of porphyrin or heme amalgamation. Arsenic is known to cause hindrance of aminolevulinic corrosive dehydratase activity, thereby changing the heme blend pathway (Gupta, 2005). It is obvious from the display ponder that the ruddy blood cell (RBCs) number, pressed cell volume (PCV) and hemoglobin (Hb) concentration were altogether expanded in melatonin treated bunches. Anwar et al. (1998) found that melatonin treatment in rats numerically expanded RBCs, Hb and PCV. On the other hand Durotoye and Rodway (1996) demonstrated that subcutaneous inserts of melatonin in ewes decreased RBCs tally and PCV. It showed up from the ponder of Karimungi *et al.* (1996) that the impact of melatonin treatment on RBCs tally depends on the time of melatonin infusion and the time of blood collection. Besides, The increment in RBCs number, PCV and Hb concentration gotten within the show consider may be credited either to its coordinate stimulatory impact on bone marrow, which was already detailed by Anwar *et al.* (1998) or in a roundabout way through incitement of a few cytokins, which was found to have effective stimulatory impact on bone marrow cells expansion (Lissoni *et al.,* 1993).

In conclusion, arsenic driven to harmfulness in rabbits. A critical increment was watched within the platelets checks and add up to lymphocyte check of the melatonin treated rabbits when compared to the controls. We conclude that whereas melatonin treatment may not be of any partner to an iron deficient quiet, it'll certainly be of colossal advantage for those who require platelet and lymphocyte substitution.

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Some Biological and Fisheries Indicators of the Golden Gray Mullet, *Liza aurata* (Mugilidae) in the Southern Mediterranean Coast (Libya)

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Abstract:

The objective of the present study was to establish some biological traits and fishery indicators of *L. aurata* (mullet) in Umm Hufayn lagoon in eastern Libya's southern Mediterranean Sea coast to help in constructing management plans for the fishery. Eighty *L. aurata* collected randomly from the artisanal catch of the lagoon during January and February 2018 were used in the study. The fish were aged individually by reading annuli on opercula and scales and by the length-frequency distribution of the sample. The mean length of the fish was 21.33 cm, corresponding to a weight of 89.01 g. The instantaneous rate of total mortality, Z, was 0.665 to 0.798, natural mortality, M, was 0.251 to 0.487, and fishing mortality, F, was 0.262 to 0.507. Survival, S, ranged from 0.450 to 0.514. The exploitation ratio (E) was 0.360 to 0.669 with a mean of 0.468, length at first maturity, L_m, was 10.360 to 14.434 cm with a mean of 11.836 cm, length at optimum yield, L_{OPt}, was 19.633 to 25.864 cm with a mean of 22.32 cm, and longevity, T_{max}, was from 18.237 to 42.857 years, with a mean of 23.112 years. These values indicated that the *L. aurata* fishery was healthy, and ran close to the maximum sustainable yield. However, certain causes of concern must be taken into consideration in future planning.

Keywords: *Liza aurata*, golden mullet, mortality, E, L_m, L_{OPt}, T_{max}, Libya, southern Mediterranean.

Introduction:

Fisheries management generally attempts to achieve the following points: Sustainable conservation of the fisheries resources and the aquatic environment that encompasses them. This is achieved by avoiding overexploitation and reduced recruitment, the damage caused to the environment by fisheries waste (Bi-catch, illegal fishing, litter, destruction of bottom habitats, etc.), and pollution, including dumping and excavation, etc. Maximizing the sustainable benefits, e.g., by operating the fisheries at the level of the Maximum Sustainable Yield (MSY) or Maximum Economic Yield (MEY) at the smallest possible effort, cost, and time. Minimizing the time, effort, and money spent on management. Equitable sharing of the resources among current beneficiaries (e.g., fishermen and other coastal communities) and those of future generations. And alleviate pressure on heavily exploited capture fisheries by aquaculture.

Availing baseline biological and fisheries indicators is crucial for setting up sound management policies that will realize these goals. Most of these indicators function interdependently. For example, the size and composition of the fish population (the stock) are affected by recruitment, growth rate, mortalities (natural and fishing), longevity, and the bio-physio-chemical traits of the habitat (Temperature, availability of food, competition, pollution, etc.). Recruitment is a function of fecundity, age and size at first maturity (Lm, L50), exploitation rate (E), length at optimum yield (Lopt), and longevity. These indicators are also dependent on the fishing effort and selectivity of the gear, which, in addition to the stock, determine the yield. The golden gray mullet**,** *Liza aurata*, family Mugilidae, is a medium-sized fish of significant importance in the artisanal fisheries of the southern Mediterranean coast. It's morphological traits and growth pattern on the southern Mediterranean coast, as exemplified by Um Hufayan lagoon, Libya, were established by Elshakh *et al*. (2021) and Elshakh *et al*. (2023) consecutively. The objective of the present study was to complement these studies by contributing to the availability of some of the other bio-fishery indicators needed for planning the *Liza aurata* fishery on the southern Mediterranean coast in general and in Um Hufayan lagoon in particular. Um Hufayan is a brackish lagoon typical of those found scattered along the entire southern coast of the Mediterranean.

The established indicators included fish size, composition of the population, natural and fishing mortalities, E, L_{opt}, L_m, L_{50}, and longevity.

Materials and Methods:

The Study Site

Um Hufayan (32° 33' 13.5" N, 23° 05' 57.2" E), from which the study fish were collected, is a 2 Km2 shallow (0.5 to 3m deep), brackish (11) lagoon located within the Gulf of Bomba on the eastern coast of Libya in the southern Mediterranean Sea (Fig. 1). This lagoon is a principal artisanal fishing ground, a natural nursery and feeding ground for several commercial fish, and an important wetland and resting site for migratory birds (Reynolds, *et al*., 1995; Mohamed, 2019; and Elshakh *et al*., 2021).

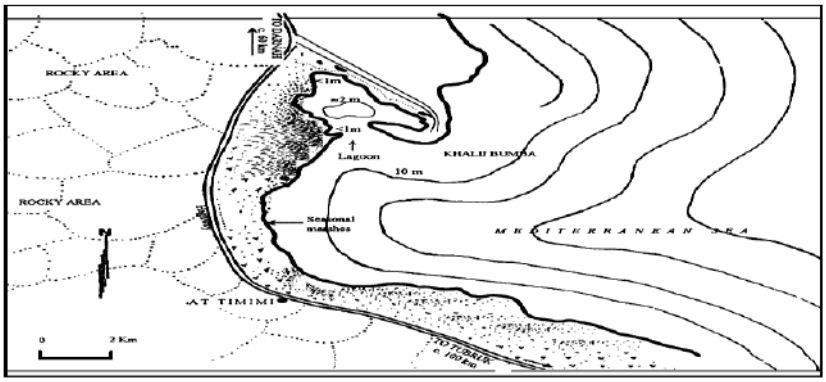

Fig. 1. Um Hufayan Lagoon (32° 33' 13.5" N, 23° 05' 57.2" E) within the Gulf of Bumba, eastern Libya, Mediterranean Sea (Reynolds *et al*., 1995).

Sample collection

Eighty *Liza aurata* random samples were collected from the artisanal catch of Umm Hufayn lagoon during January and February 2018 and used in the present study.

Size and aging of *Liza aurata*

The length and the corresponding weight of individual fish were measured by a digital Vernier calibre and a digitally sensitive balance. The fish were aged by:

1. Individually reading annuli on:

- Opercula according to Elzey *et al*. (2015), Table 1.

- Scales following Hile (1941) and Tesch (1968), Table 2.

- (sagittal annuli were not distinct).

2. Establishing the length frequency distribution at age according to Sparrer and Venema (1998), Table 3, Fig. 2 and Table 4.
3. Averaging fish length at age data obtained from the above two methods, Table 5.

Table 1. Fish length at age based on the number of annuli on the opercula Elzey et al. (2015)

Sample population length classes (cm)	Age in years						
	1	2	3	4	5	6	7
15-16.9	1						
17-18.9	17	1					
19-20.9	11	11					
21-22.9	1	11	7				
23-24.9			10				
25-26.9				3	1		
27-28.9				2	1		
29-30.9						1	
31-32.9							1
33-34.9							
Mean length	18.8	20.87	23.18	26.86	28.4	30	32
n	30	23	17	5	2	1	1

Table 2. Fish length at age based on the number of the scales.(Tesch 1968)

Sample population length classes (cm)	Age in years						
	1	2	3	4	5	6	7
15-16.9	1						
17-18.9	18						
19-20.9	1	8					
21-22.9		21	2				
23-24.9		11	6				
25-26.9			2	4			
27-28.9				1	3	2	
29-30.9							
31-32.9							
33-34.9							
Mean length	18.2	21.57	24	26.06	28.1	29.9	
N	20	40	10	5	3	2	

Table 3. Fish-sample length frequency distribution Hile (1941)

Length class (cm)	Frequency
16 - 17	1
17 - 18	5
18 - 19	13
19 - 20	11
20 - 21	11
21 - 22	10
22 - 23	9
23 - 24	3
24 - 25	7
25 - 26	3
26 - 27	1
27 - 28	1
28 - 29	2
29 - 30	
30 - 31	1
31 - 32	1

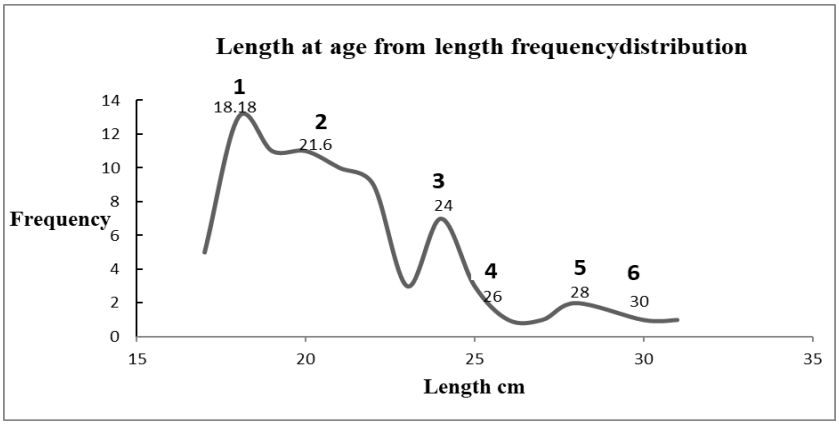

Fig. 2. The fish length at age deduced from the plot of the frequency at length

Table. 4. Fish length at age obtained from the length frequency distribution

Age	Opercula	Scales	Length frequency	Average
1	18.8	18.2	18.18	18.39
2	20.87	21.58	21.6	21.35
3	23.18	24	24	23.73
4	26.86	26.06	26	26.31
5	28.4	28.1	28	28.17
6	30	29.9	30	29.97
7	32			32

Table 5. Fish length (cm) at age (years) estimated from the opercula, scales, length frequency distribution, and their average.

Age in years	1	2	3	4	5	6	7
Length (cm)	18.18	21.6	24	26	28	30	

Estimation of mortality rates of *L. aurata*

The mortality rates of *L. aurata* were estimated according to Pauly (1980), Ricker (1975), and Gulland (1985), as described in Sparre and Venema (1998):

Total mortality (Z) was estimated by the linearized catch curve method based on age composition data:

Ln C (t_1, t_2) = q-z*t, or Ln C (t_1, $t_{+\Delta t}$) = q-z*t… the linearized catch equation with constant time intervals, where b = -Z, where: C: catch (or frequency) at t_1, t_2; t: age in years; q: constant; Z: total mortality.

i. Lengths (L_t) at age (t in years), obtained by the opercula method as an example, and their frequency of occurrence are tabulated in Table 6.

ii. A linear regression plot of t in the-X axis and Ln frequency in the-Y axis was made (Fig. 3).

iii. Judging from the plot, the data points that were not consistent with the linearized catch curve due to net selectivity or low frequency of occurrence were omitted, and a new table (Table 7) and a new plot (Fig. 4) were made.

iv. Total mortality (Z) was obtained from the regression equation as:

$$Z = -b$$

where -b is the slope of the regression line.
The steps for obtaining length at age based on the scales are shown in Table 8, Fig. 5, Table 9 and, Fig. 6. Those based on the length frequency distribution are shown in Table 10, Fig. 7, Table 11, and Fig. 8, while those based on the "average" in Table 12, Fig. 9, Table 13, and Fig. 10.

Table 6. The preliminary linearized catch method data for estimating the total mortality Z based on opercula; t: fish age in years, L_t: corresponding fish length in cm, X and Y are the regression axes.

t	L_t	Frequency	Ln frequency
X			Y
1	18.8	17	2.8332
2	20.87	25	3.2188
3	23.18	20	2.9957
4	26.86	13	2.5649
5	28.4	3	1.0986
6	30	1	0
7	32	1	0

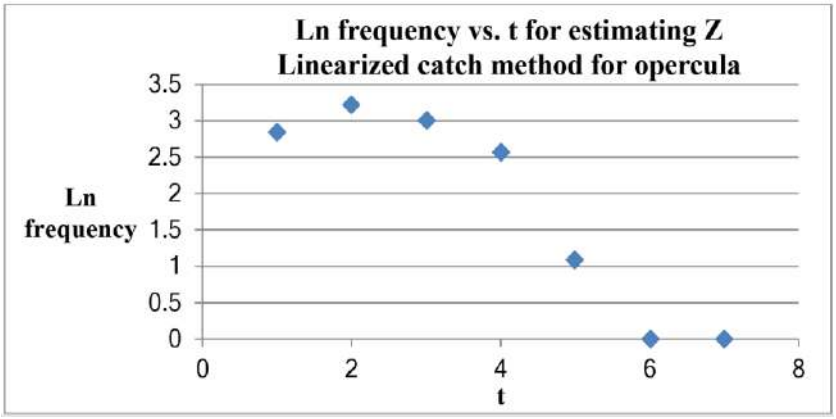

Fig. 3. The preliminary linearized catch plot for estimating the total mortality Z based on the opercula

Table 7. The final linearized catch data used for estimating the total mortality Z based on the opercula.

t	L_t	Frequency	Ln frequency
X			Y
2	20.87	25	3.2188
3	23.18	20	2.9957
4	26.86	13	2.5649
5	28.4	3	1.0986
6	30	1	0
7	32	1	0

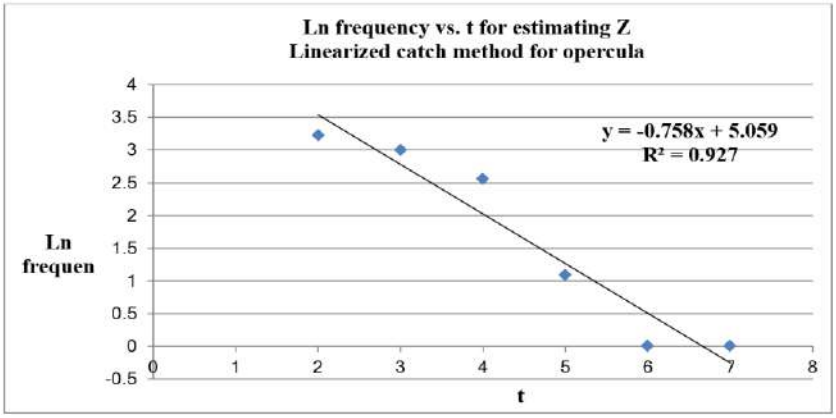

Fig. 4. The final linearized catch data used for estimating total mortality Z based on the opercula (plot of Table 7). Z = -b = 0.758.

Table 8. The preliminary linearized catch method data for estimating the total mortality Z based on scales; t: fish age in years, L_t: corresponding fish length in cm, X and Y are the regression axes.

t	L_t	Frequency	Ln frequency
X			Y
1	18.2	9	2.197225
2	21.58	41	3.7136
3	24	14	2.6391
4	26.06	10	2.3026
5	28.1	2	0.6931
6	29.9	2	0.6931
7			

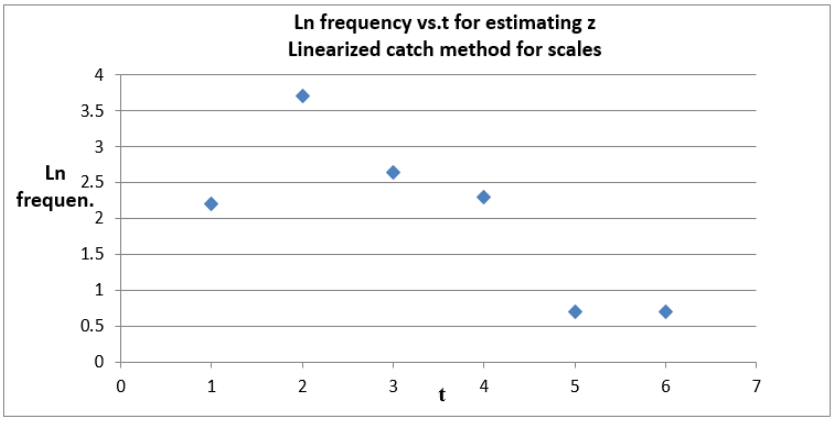

Fig. 5. The preliminary linearized catch plot for estimating the total mortality Z based on the scales (plot of Table 8).

Table 9. The final linearized catch data used for estimating the total mortality Z based on the scales.

t	Length	Frequency	Ln frequency
X			Y
2	21.58	41	3.7136
3	24	14	2.6391
4	26.06	10	2.3026
5	28.1	2	0.6931
6	29.9	2	0.6931
7			

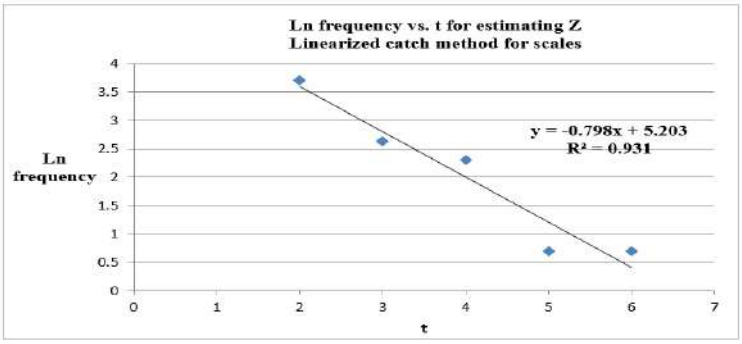

Fig. 6. The final linearized catch data used for estimating the total mortality Z based on the scales (plot of Table 9). Z = -b = 0.798

Table 10. The preliminary linearized catch method data for estimating the total mortality Z based on the length frequency distribution; t: fish age in years, L_t: corresponding fish length in cm, X and Y are the regression axes.

t	L_t	Frequency	Ln frequency
X			Y
1	18.18	7	1.94591
2	21.6	43	3.7612
3	24	14	2.639057
4	26	10	2.302585
5	28	2	0.693147
6	30	3	1.098612
7			

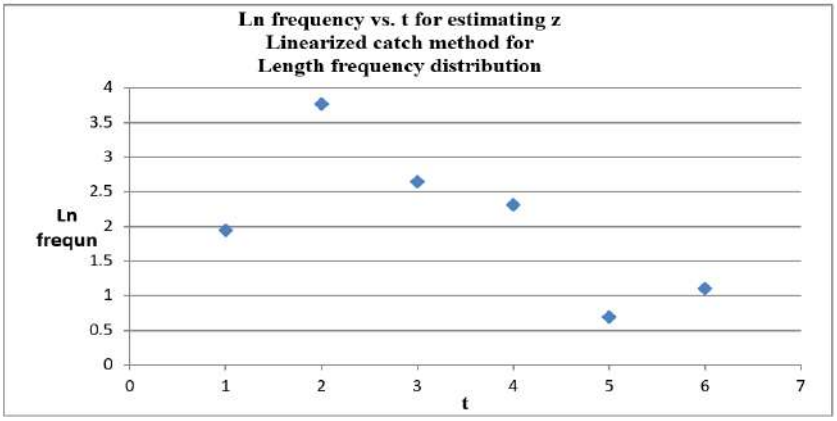

Fig. 7. The preliminary linearized catch plot for estimating the total mortality Z based on the length frequency distribution (plot of Table 10).

Table 11. The final linearized catch data used for estimating the total mortality Z based on the length frequency distribution.

T	L_t	Frequency	Ln frequency
X			Y
2	21.6	43	3.7612
3	24	14	2.639057
4	26	10	2.302585
5	28	2	0.693147
6	30	3	1.098612
7			

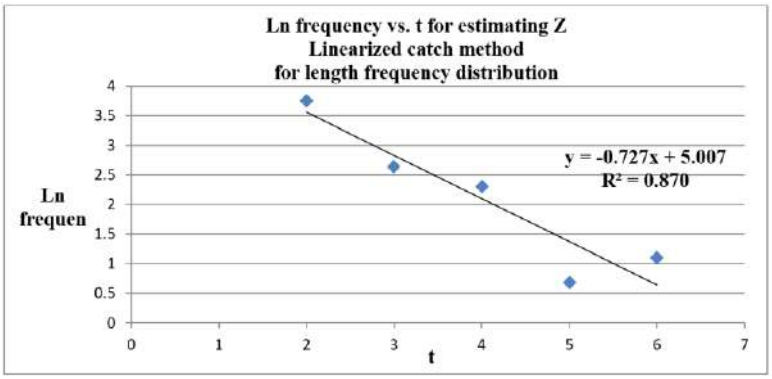

Fig. 8. The final linearized catch data used for estimating the total mortality Z based on the length frequency distribution (plot of Table 11). Z = -b = 0.727.

Table 12. The preliminary linearized catch data for estimating the total mortality Z based on the "average"; t: fish age in years, L_t: corresponding fish length in cm, X and Y are the regression axes.

t	Length	Frequency	Ln frequency
X			Y
1	18.3933	10	2.302585
2	21.35	39	3.663562
3	23.7266	15	2.70805
4	26.3066	11	2.397895
5	28.1667	1	0
6	29.9667	2	0.693147
7	32	2	0.693147

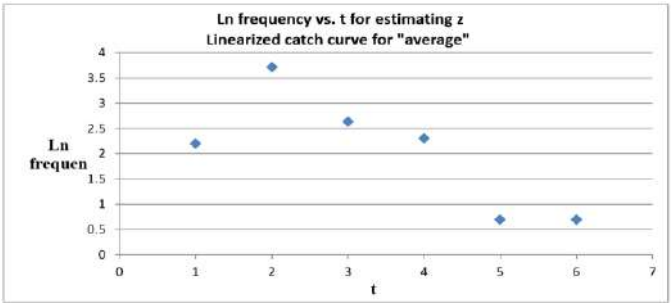

Fig. 9. The preliminary linearized catch plot for estimating the total mortality Z based on the "average" (plot of Table 12).

Table 13. The final linearized catch data used for estimating the total mortality Z based on the "average".

T	L_t	Frequency	Ln frequency
X			Y
2	21.35	39	3.663562
3	23.7266	15	2.70805
4	26.3066	11	2.397895
5	28.1667	1	0
6	29.9667	2	0.693147
7	32	2	0.693147

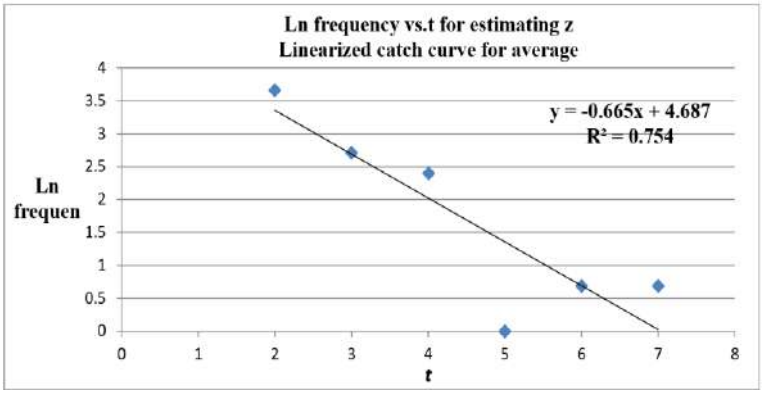

Fig. 10. The final linearized catch data used for estimating the total mortality Z based on the "average" (plot of Table 13). Z = -b = 0.665.

The natural mortality (M) was estimated by Pauly's (1980) empirical formula. The values of L_∞ and K based on the opercula, scales, and "average" were obtained from Elshakh (2019), and Elshakh *et al*. (2021). T was estimated as 25.50C for Umm Hufayn lagoon from the CNR MD UHD (Mediterranean Sea High Resolution and Ultra High-Resolution Sea Surface Temperature satellite imagery, Center: Italy):

$$\ln M = - 0.0152 - 0.279*\ln L_\infty + 0.6543*\ln K + 0.463*\ln T$$

Fishing mortality (F): F = Z-M.

Survival rate (S): $S= e^{-z}$ Ricker (1975).

Exploitation rate (E): E: F/Z, Gulland (1985).

Fish length at first maturity (Lm): Anti-Log Lm = 0.8776 Log L_∞ -0.38 (Froese and Binohlam, 2000).

Fish length at optimum yield (L_{opt}): $L_{opt} = L\infty$ (3/ (3+ M/K), (Beverton, 1992).

Longevity (T_{max}): $T_{max} = 3/K$ (Beverton, 1992).

Results:

The size of the fish sample

The mean length (± SD) of mixed male/female *L. aurata* was 21.33± 3.01 cm corresponding to a mean weight of 89.01±52.72g.

Estimates of instantaneous mortality and survival rates

The instantaneous rate of total mortality, Z, ranged from 0.665 to 0.798 (Table 14), that of natural mortality, M, ranged from 0.2509 to 0.4873, and fishing mortality, F, from 0.2618 to 0.5071. The survival, S, ranged from 0.4502 to 0.5143.

Table 14. Estimates of instantaneous rates of total mortality (Z), natural mortality (M), fishing mortality (F) and survival (S).

Parameters	Opercula	Scales	Length frequency	Average	Mean of parameters
Z	0.758	0.798	0.727	0.665	0.753
M	0.2509	0.4873	0.4652	0.3178	0.4004
F	0.5071	0.3107	0.2618	0.3472	0.3526
S	0.4686	0.4502	0.4834	0.5143	0.4709

Fisheries management indicators for *L. aurata*:

The exploitation ratio (E) ranged from 0.3601 to 0.669 with a mean of 0.4683 (Table 15), Length at first maturity, Lm, ranged from 10.3601 to 14.4338 cm with a mean of 11.8359 cm, Length at optimum yield, L_{Opt} (length at maximum possible yield per recruit), ranged from 19.6333 to 25.8638 cm with a mean of 22.3199 cm, and Longevity, T_{max}, was 18.2371 to 42.857 years with a mean of 23.1125 years.

Table 15. Exploitation ratio (E), Length at first maturity (Lm), Length at optimum yield (L_{Opt}), and Longevity (T_{max}).

Parameters	Opercula	Scales	Length frequency	Average	Mean of parameters
E	0.669	0.3893	0.3601	0.5221	0.4683
L_m cm	14.4338	10.3875	10.6243	12.8469	11.8359
L_{Opt} cm	25.8638	19.6333	20.0048	23.4871	22.3199
T_{max} years	42.8571	18.2371	19.3673	31.6122	23.1125

Discussion

In the present study, attempts to read annuli on whole sagittae failed; however, previous literature had shown that annuli may be read on whole (stained or unstained) or sectioned sagittae. The problem of indistinct annual growth rings on hard parts of tropical fish is well known and is generally attributed to the fact that differences in magnitudes of summer and winter environmental parameters, such as temperature and availability of food, are not extreme as compared with those of temperate and cold regions. The more the variations are extreme, the more the annual rings caused by them are distinct. Sparre and Venema (1998) and Abd El Rahman and Moghraby (1984) suggested the use of more than one method for aging fish in tropical regions. In the present study, *L. aurata* total length ranged from 16.9 to 31.2 cm, with an average of 21.33±3.01 cm, corresponding to a total weight of 39.59 to 316.25 g, with an average of 89.01±52.72 g. The ratio of "head to fish length", which is important in aquaculture as fish with a smaller ratio are preferred, was 19.66±2.75%. The size of *L. aurata* in the present study was somewhat smaller than some of those reported in the literature. For example, the total length and weight of *Liza aurata* from Guilan, Mazandaran, and Golestan in the southern Caspian Sea ranged from 29.6 to 57.4 cm and 165.3 to 1285.4 g (Khayyami *et al*., 2014). *L. aurata* specimens from the Messolonghi-Etoliko Lagoon system and the neighboring coastal waters of the Gulf of Patraikos, Western Greece, ranged from 9.7 to 59 cm (Hotos and Katselis, 2011). The fork lengths of *L. aurata* caught in Iranian waters of the Caspian Sea (Fazli *et al*., 2008) were 22.1 to 51.9 cm long. *L. aurata* from the Algarve, Portugal, was 20.1 to 40.5cm (Borges *et al*., 2003). In Croatia's Eastern Adriatic Sea, the fish's length ranged from 21.5 to 44.2cm (Dulcic and Glamuzina, 2006), but 16.2 to 44.0 cm and 10.00 to 917.00 g in the Middle Black Sea (Bilgin *et al.*, 2006). However, the smaller size of *L. aurata* in the

present study seemed to be an expression of the Um Hufayan lagoon brackish habitat rather than an expression of overexploitation. The exploitation ratio, E, ranged from 0.36 to 0.67 with a mean of 0.47, which is close to the suggested theoretical optimum value of 0.5. From the length at first maturity, L_m, of 10.3601 to 14.4338 cm, with a mean of 11.8359 cm, one can deduce that most fish in the lagoon had the chance to breed at least three times (three consecutive years) before being caught or dying naturally, which is within the acceptable limit. However, there were reasons for concern:

1- Although the mean longevity, T_{max}, of *L. aurata* was estimated at 23.11 (18.24 to 42.86) years, only seven age cohorts (1 to 7 years) were encountered in the present study. However, many studies have also reported 7 and 8 age groups for this fish, e.g., Thong (1969), Alexandrova (1964), Kraljević and Dulčić (1996), and Nikolskii (1954). The upper limit of T_{max} (42.86) seems to be high. The estimates obtained by Hotos (2019) for *L. aurata* in the Lagoon of Klisova-Messolonghi (W. Greece) were 16.75, 18.1, and 20.85 years for males, females, and combined sexes, in order.
2- Most of the fish constituting the study sample were within the range of 18 to 23 cm in total length (young cohorts: one to three years old).
3- The fish length at optimum yield, L_{OPt}, calculated in the present study ranged from 19.63 to 25.86 cm with a mean of 22.32 cm; both the upper limit and the mean are above the mean length of *L. aurata* (21.33 cm) of the present study.
4- Further, it must be cautioned that E and L_m are only two measures, among many others, for testing the efficiency of a fishery. Other measures, for example, include the maximum sustainable yield and the yield per recruit. Also, some studies reported much higher length at first maturity, Abdallah *et al*. (2013) reported 23.73, 23.84, and 23.79 cm total length for female, male, and combined sexes, respectively, for the same fish in the Gulf of Gabes, Tunisia. Ghaninejad *et al*. (2010) estimated 28.4 cm for the same species in the Iranian waters of the Caspian Sea. The smaller size and length at first maturity of *L. aurata* in the present study are probably a consequence of the brackish habitat of the lagoon. Further, in the present study, L_m was established according to Froese and Binohlam (2000); in most of the other studies, Sendecor's (1956) method was used.

5- The meagre presence of large fish in the lagoon must be taken into consideration. Nets with larger mesh (openings, eyes) must be used instead of the set one to allow for the escape of some of the large fish (a study must be carried out to decide the size of the mesh to be adopted). Alternatively, a small reserve area within the lagoon (no fishing area) should be set aside as a haven for old fish. In developed countries, a limit for the minimum size of fish allowed in the market can be set, but this is not likely to be enforced in Libya. The fishers must be alerted that adopting such measures, though might result in reduced yield in the first or second year, is expected to boost the yield in the years after, sometimes at reduced effort, cost, and time, and that the larger fish to be caught will score better prices.

The natural mortality rate (M) for *L. aurata* in the present study ranged from 0.25 to 0.49 yr^{-1}, and the survival rate, S, from 0.45 to 0.51. For the same fish in the lagoon of Klisova-Messolonghi, W. Greece (Hotos, 2019), M was 0.33, 0.367, and 0.39 yr^{-1} for all fish, females and males consecutively, and S was 0.238–0.571, 0.443–0.592, and 0.497–0.579, respectively. S is dependent on both natural and fishing mortality, i.e., on total mortality.

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The Influence of *Moringa oleifera* on Liver Enzyme and Glucose in Male Rabbits

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Abstract

Moringa oleifera (Moment) are presently so well known that there appears to be small question of the significant wellbeing advantage, to be realized by utilization of *Moringa* leaf powder in circumstances where starvation is found. The phytochemical components of *Moringa oleifera* (Moment) have been detailed to have antimicrobial parts and antioxidant exercises and in this way, (Moment) is commonly utilized in various restorative applications to control different infections such as stomach related unsettling influence, asthma, provocative malady, and cancers. It has been recorded that (Moment) might conceivably maintain a strategic distance from oxidation harm, improve the safe reaction, display antioxidant exercises that can prevail free radicals and responsive oxygen species synthesis. This work investigated the impact of *Moringa oleifera* on biochemical parameters in male rabbits. Rabbits were orally given of *Moringa oleifera* (400 mg/kg BW).The tried dosages were given to rabbits each other day for 12 weeks. Results appeared that *Moringa oleifera* caused a critical (PB/0.05) diminish within the levels of plasma aspartate aminotransferase (AST), alanineaminotransferase (ALT), soluble phosphatas (ALP) and glucose.

Keywords: Rabbits; *Moringa oleifera*; Biochemical parameters,

Introduction:

Moringa family Moringaceaeis (Leoneetal., 2015). It is develop best in dry sandy soil and endures destitute soil. It may be a sun and heat-loving plant (Rajangam,2001). It could be a source of restorative compounds and has

components of tall nutritive esteem such as protein, amino acids, carbohydrate minerals, vitamin and natural acids (Raja *et al.,* 2013). Moringa clears out are anti-bacterial and anti-inflammatory. Leaf tea treats gastric ulcers and diarrhea. Moringa clears out are great nourishment sources for those enduring from lack of healthy sustenance due to the tall protein and fiber substance. Takes off treat with fevers, bronchitis, eye and ear contaminations, and aggravation of the bodily fluid layer. The press substance of the takes off is tall and they are supposedly endorsed for anemia is utilized within the treatment of scurvy skin illnesses. The clears out are the foremost nutritious portion of the plant, being a critical source B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein, among other basic supplements (Arise *et al.,* 2014). Moringa are presently so well known that there appears to be small question of the significant wellbeing advantage, to be realized by utilization of Moringa leaf powder in circumstances where starvation is found (Atawodi *et al.,* 2010). Clears out rubbed against the sanctuary can calm cerebral pains (Kumar, 2004). The phytochemical components ofmoringaoleifera (MO) have been detailed to have antimicrobial parts and antioxidant exercises and in this way, (Moment) is commonly utilized in various therapeutic applications to control different maladies such as stomach related unsettling influence, asthma, provocative illness, and cancers (Osman *et al.,* 2012). It has been recorded that (Moment) might conceivably maintain a strategic distance from oxidationdamage (Osman *et al.,* 2012), improve the resistant response (jaiswal *et al.,* 2009). Display antioxidant exercises that can prevail free radicals and responsive oxygen species synthesis (Ogbunugafor *et al.,* 2011) ,and favorably balancing lipid metabolism (leone *et al.,* 2015) in rats. Moringa are presently so well known that there appears to be small question of the significant wellbeing advantage, to be realized by utilization of Moringa leaf powder in circumstances where starvation is found (Atawodi *et al.,* 2010). Clears out rubbed against the sanctuary can calm cerebral pains (Kumar, 2004). The phytochemical components ofmoringaoleifera (MO) have been detailed to have antimicrobial parts and antioxidant exercises and in this way, (Moment) is commonly utilized in various therapeutic applications to control different maladies such as stomach related unsettling influence, asthma, provocative illness, and cancers (Osman *et al.,* 2012). It has been recorded that (Moment) might conceivably maintain a strategic distance from oxidation damage (Osman *et al.,* 2012), improve the resistant response (Jaiswal *et al.,* 2009),

Materials and Methods:

In this study, the effect of *Moringa oleifera* on the biochemical indices of male rabbits was investigated. The leaves of *Moringa oleifera* were collected from a home garden in Samno, Sabha, Libya. Mature male New Zealand White rabbits aged 6 months were used. Animals were individually housed in cages and weighed weekly throughout a 12-weeks experimental period. Ten mature male rabbits were randomly divided into two groups (every five rabbits) as follows: Group I: Rabbits were used as control daily for 12 successive weeks. Group II: Rabbits were treated with *Moringa oleifera* given daily by gavage at a dose of 400 mg/kg BW (Khalifa *e tal*.,2016) for 12 successive weeks. The other part of the parted blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 860 xg for 20 min, and was stored at -20°C until used for analyses. Stored plasma samples were analyzed for total. Plasma glucose concentrations were measured by the method of (Patton and Crouch,1977), respectively. Plasma total bilirubin was measured using the method of (Pearlman and Lee, 1974).The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of (Reitman and Frankel, 1957). Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of (Principato*et al*., 1985).Statistical analysis. The data obtained were expressed as mean ±SEM. The significant differences were assessed by one-way ANOVA and Tukey test. After the detection of the normal distribution of the data and appropriate P-values, less than 0.05 is considered significant.

Results:

Tables 1 and Figures 1 to 4 represented the mean values of activities of aspartate transaminase (AST),alanine transaminase (ALT), alkaline phosphatase (AlP) and bilirubin in plasma of male rabbits treated with *Moringa oleifera*. Treatment with *Moringa oleifera* caused a significant ($p<0.05$) decrease in plasma bilirubin, glucose, AST, ALT, and AlP activities.

Table1. The activities of plasma enzymes of male rabbits treated with ***Moringa oleifera*** .

Parameter	***Experimental groups***	
	G1	***G2***
AST (U/L)	42.22 ± 0.768[a]	28.95 ± 2.251[b]
ALT (U/L)	43.30 ±1.109[a]	33.64 ±2.292[b]
ALP (IU/L)	51.18 ±5.434[a]	42.32 ± 2.705[a]
Bilirubin (mg/dL)	1.45 ± 0.027[a]	1.26 ± 0.058[b]
Glucose(mg\dl)	115.40± 0.424[a]	89.47±3.714[b]

Values are expressed as means ± SE; n = 5 for each treatment group.Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, p<0.05.

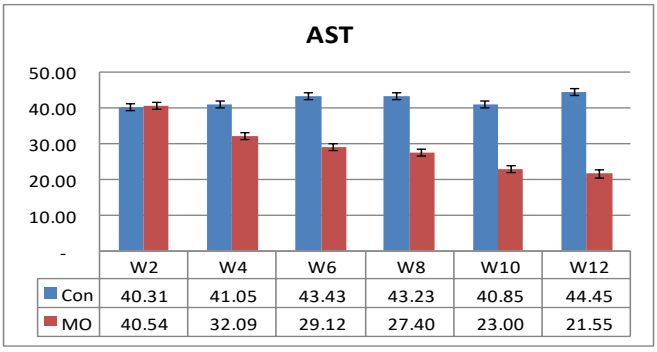


Figure. 1. Changes in aspartate transaminase (AST) during treatment of male rabbits *with Moringa oleifera*.

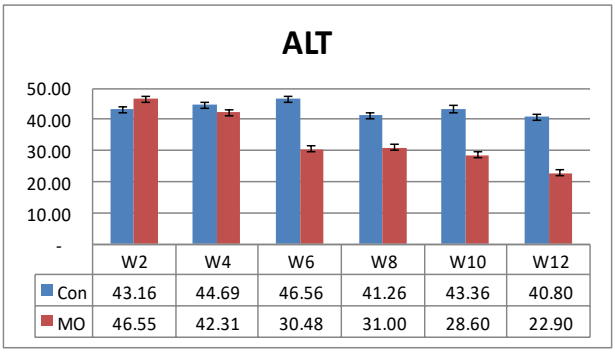
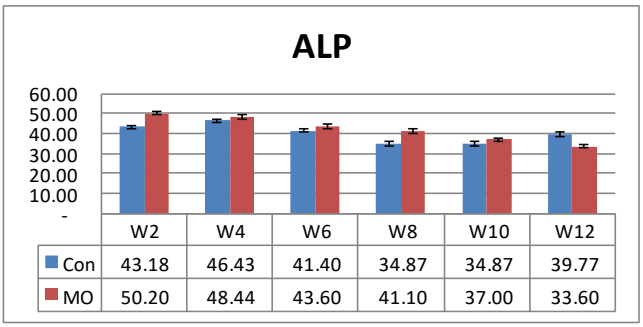
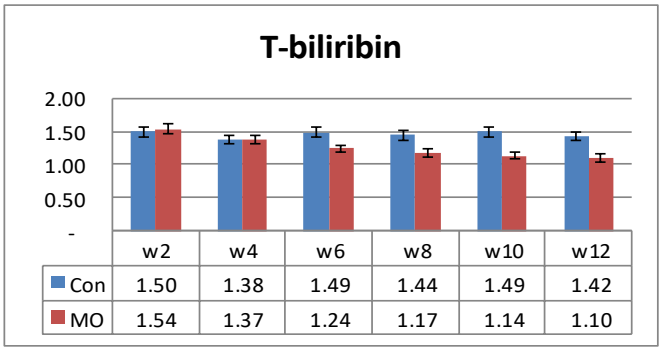


Figure.2.Changes in alanine transaminase (ALT) during treatment of male rabbits with *Moringa oleifera.*



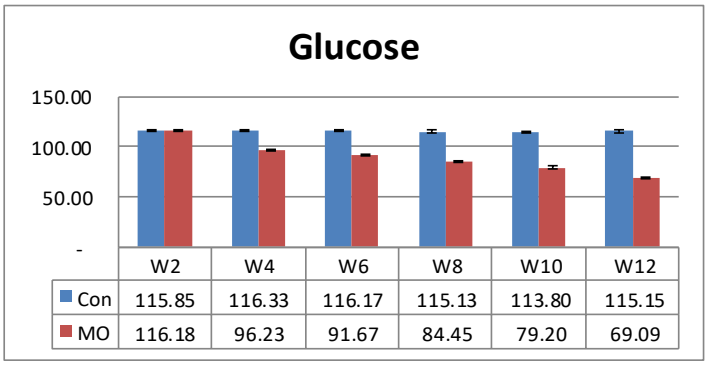
	W2	W4	W6	W8	W10	W12
Con	43.18	46.43	41.40	34.87	34.87	39.77
MO	50.20	48.44	43.60	41.10	37.00	33.60

Figure.3.Changes in alkaline phosphatase (ALP) during treatment of male rabbits with *Moringa oleifera*.



	w2	w4	w6	w8	w10	w12
Con	1.50	1.38	1.49	1.44	1.49	1.42
MO	1.54	1.37	1.24	1.17	1.14	1.10

Figure.4. Changes in T-biliriubin during treatment of male rabbits with *Moringa oleifera.*



	W2	W4	W6	W8	W10	W12
Con	115.85	116.33	116.17	115.13	113.80	115.15
MO	116.18	96.23	91.67	84.45	79.20	69.09

*Figure.*5.Changes in values of plasma glucose during treatment ofmale rabbits with *Moringa oleifera.*

Discussion:

The decreasein plasma biochemical (Table 1) is in assention with (Olatunji *et al.,* 2013) demonstrated that AST and ALT exercises in serum of rabbits were not altogether influenced (P>0.05) by the dietary consideration of Moringa leaf feast (Moment) over the treatment. The cruel values of albumin and globulin gotten within the ponder were moreover not altogether (p>0.05) impacted by dietary medicines. Since add up to protein, egg whites and globulin are by and large affected by the quality and amount of protein intake the values gotten within the ponder show wholesome ampleness of the dietary protein. No critical (p>0.05) impact of slim down was watched on serum glucose, cholesterol, urea and creatinine. This was a great sign that the dietary protein of Moringaoleifera leaf feast was well utilized by the rabbits. These comes about concur with (El-kashef, 2022) who appeared a diminish in ALT and AST values when they utilized ***Moringa*** takes off in nourishing buck rabbits beneath heat-stress conditions. Too, (Abdel-Latif *et al.,* 2018) appeared the same comes about by utilizing *Moringa oleifera* extricate in bolstering rabbits beneath heat-stress conditions. Rabbits that gotten moringa take off extricate appeared inconsequential changes in AST, ALT, High mountain, urea and creatinine. These minor changes in liver chemicals may be due to the antioxidant and immunological properties of moringa takes off (IsituaandIbeh,2013). These clears out have a hepatorenal defensive impacts actuating immaterial changes in liver proteins, urea and creatinine levels (Igbinaduwa and Ebhotemhem,2016). *Moringa oleifera* (Moment) lam on blood sugar of pale skinned person rats. Hyperglycemia was actuated in rats utilizing alloxan (120 mg/kg body weight, intraperitioneally) . Normoglycemia and hyperglycemic rats were treated with three diverse measurements of the watery extricates, tolbutamide (positive control) and typical saline (negative control). The glucose level of the pulled back blood tests was decided by 0-toluidine spectrophotometric strategy. The classes of chemical components of the watery extricate of the plant were decided. Proteins, settled oils and fats and carbohydrates were found to be present. The considers appeared that the fluid extricate of *Moringa oleifera* takes off do possess a noteworthy, dose-dependent hypoglycemic action in normoglvcemic and alloxan-induced diabetic rats and nearly as viable as the standard sedate. This too underpins its utilize in folkloric administration of diabetes (Edoga *et al.,* 2013). Watery extricate of Moringa clears out.

In conclusion, ***Moringa oleifera*** in the meals of rabbits appears to have a beneficial impact on the animals' growth performance, as well as their liver enzyme and glucose.

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Physical Properties of Bi_2O_3–PbO–P_2O_5 Glasses

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Abstract

Six glass samples in the system of xBi_2O_3–$(25–x)$ PbO–$75P_2O_5$ ($0 \leq x \leq 25$; x in mol %) were prepared using the conventional melt quenching technique. The physical properties investigated in this research are glass density; ρ, molar volume; V_M, oxygen molar volume; V_O, oxygen packing density; OPD Poisson's ratio; σ and oxygen ion concentration, $N_{O\text{-ions}}$. The glass density was evaluated experimentally using the Archimedes method and calculated theoretically using an empirical formula. The comparison result for determining density by the two methods was reasonable but not accurate enough. The results showed that density and molar volume increased with increasing Bi_2O_3 content. For oxygen molar volume (V_O) and oxygen packing density (OPD), their behavior was inconsistent, as a slight increase in V_O was observed, similarly offset by a decrease in OPD. The Poisson's ratio values indicate the tightening of the bonds in the glass matrix. The number of oxygen ions increases as the bismuth content in the glass increases. Overall, the findings demonstrated that adding bismuth improved the physical properties of the glass samples.

Keywords: phosphate glass, density, molar volume, oxygen molar volume, oxygen packing density, Poisson's ratio.

Introduction

In recent years, researchers have become more interested in glass research because of the material's many uses in various aspects of life. It has a wide uses in the construction industry, medical facilities, energy conversion, communications, research laboratories, and other fields (Bagheri *et al.*, 2020; Shams *et al.*, 2021). This is due to the various advantages that distinguish it from other materials, such as: high permeability, good mechanical strength, chemical stability, high durability, good water resistance, transparency, high

homogeneity, being friendly to the environment, ease of manufacture and processing in many ways, and low cost (Almuqrin *et al.*, 2022). To meet the requirements for a certain application, it is essential to investigate the physical properties of an appropriate type of glass with an appropriate composition. Studying the physical properties of glasses can help in predicting and reproducing densities, which is useful for radiation shielding purposes (Kaewjaeng *et al.*, 2021). The structural properties of glasses, such as molar volume and metallization parameter, provide insights into their behavior and functionality (Shahboub *et al.*, 2021). Additionally, studying the physical properties of glasses enables researchers to understand, manipulate, and optimize their characteristics for various industrial and scientific purposes (Al-Buriahi *et al.*, 2021).Glass containing Bi_2O_3 has useful optical and electrical properties such as a high refractive index, extended permeability in the mid-infrared region, and a high dielectric constant (Kumar *et al.*, 2018). Bi_2O_3 is not considered one of the basic glass components but belongs to the category of "conditional glass former due to the low field strength (0.53) of the bi^{3+} ion (Kaundal *et al.*, 2010). However, when mixed with other glass former such as SiO_2, B_2O_3, P_2O_5 and GeO_2, it forms glass and enhances its physical and mechanical properties (Barebita *et al.*, 2020). Due to the interesting properties of phosphate glass, a system of phosphate glass containing bismuth and lead was selected in this research to study its physical properties, like the density of the glass (ρ), molar volume (V_M), oxygen molar volume (V_O), oxygen packing density (OPD), Poisson's ratio (σ), and oxygen-ions density (N_O-ions).

Materials and methods

An alumina crucible containing the proper weights of the analytical reagent grades of Bi_2O_3 (99.99%), PbO (99.99%), and P_2O_5 (99.99%) was carefully mixed and heated to 300 °C for a duration of one hour. The purpose of this first heating was to prevent material volatilization. Subsequently, the crucible was put inside a melting furnace and swung continuously for over an hour at a temperature that ranged from 900 to 1100°C, depending on the composition. The homogenized melts were promptly poured into a 400°C preheated steel plate mold. This process reduced the likelihood of heat stress-induced glass fracture. The resulting glasses were placed in an annealing furnace and heated to 400°C for one hour before being allowed to cool gradually. The finished

glass has a disk-like shape and measures 2.5 cm in diameter and 2 mm in thickness, as shown in Table 1.

Table 1: Glass compositions and their photos for prepared samples.

Sample code	Chemical composition (mol %)	Prepared samples
Bi0Pb25P75	$0Bi_2O_3$-25PbO-$75P_2O_5$	
Bi5Pb20P75	$5Bi_2O_3$-20PbO-$75P_2O_5$	
Bi10Pb15P75	$10Bi_2O_3$-15PbO-$75P_2O_5$	
Bi15Pb10P75	$15Bi_2O_3$-10PbO-$75P_2O_5$	
Bi20Pb5P75	$20Bi_2O_3$-5PbO-$75P_2O_5$	
Bi25Pb0P75	$25Bi_2O_3$-0PbO-$75P_2O_5$	

The density of the manufactured samples was determined at room temperature by applying the Archimedes method using xylene as an immersion liquid. The glass samples were weighed on an electronic scale with an accuracy of 0.1 mg, and the experimental density; ρ_{exp} were calculated using the formula given below (Singh *et al.*, 2002)

$$\rho_{exp} = \frac{w_a \rho_x}{w_a - w_x} \qquad (1)$$

Where w_a is the sample weight in air, w_x is sample weight in liquid and ρ_x is the xylene density (ρ_x = 0.861 g cm^{-3}).

The empirical density the glasses (ρ_{emp}) can be found theoretically using the following formula (Singh *et al.*, 2021)**:**

$$\rho_{emp} = 0.53 \frac{\sum M_i X_i}{\sum V_i X_i} \qquad (2)$$

Where M_i is the molecular weight for reach component, X_i is the mole fraction for each component and V_i is the packing density parameter. For an oxide M_XO_Y, the Vi parameter can be determined by:

$$V_i = \frac{4}{3}\pi N_A (X.r_M^3 + Y.r_O^3) \quad (3)$$

Where r_M and r_O are the ionic radii of metal and oxygen, N_A is the Avogadro number, and X and Y are the number of metal and oxygen atoms, respectively.

The molar volume (V_M) of the glass offers useful information about the changes in its structure and is given by (Stalin *et al.*, 2020):

$$V_M = \frac{M}{\rho_{exp}} \quad (4)$$

Where M is the molecular weight of the glass sample, and x_i is the molar fraction of every constituent.

The volume of glass containing one mole of oxygen is known as the oxygen molar volume (V_O), and it can be represented as follows:

$$V_o = \frac{V_M}{\sum x_i n_i} \quad (5)$$

Where n_i is the number of oxygen atoms in each component oxide.

The oxygen packing density (OPD), which is a crucial factor in understanding the structure of glass and provides details on how tightly the glass network is packed, is determined by (Abd-Allah *et al.*, 2019):

$$OPD = 1000C\left(\frac{\rho_{exp}}{M}\right) \quad (6)$$

Where C is total number of oxygen atoms.

The packing density (V_t) is defined as the ratio of occupied space by atoms to the total available space and can be computed by (Zaid *et al.*, 2021):

$$V_t = \frac{\sum V_i X_i}{V_M} \quad (7)$$

Poisson's ratio (σ) is the ratio of lateral to longitudinal strain caused by a tensile force and can be expressed as follows (Kaur *et al.*, 2019):

$$\sigma = 0.5 - \left(\frac{1}{7.2\,V_t}\right) \quad (8)$$

The density of oxygen ions ($N_{O\text{-ions}}$) can be calculated by using the following formula (Altaf *et al.*, 2010):

$$N_{O-ions} = \frac{n_i\, X_i\, \rho_{exp} N_A}{M} \quad (9)$$

Results and Discussion

The physical parameters of the studied glasses such as: ρ_{exp}, ρ_{emp}, V_M, V_o, OPD, σ and N_{O-ions} are calculated using equations (1–9) and presented in Table 2. It is noted that from this table, Fig. 1 and Fig. 2, the ρ_{exp} and ρ_{emp} are linearly increasing as Bi_2O_3 increases, and there is a noticeable deviation between the ρ_{emp} calculated by equation (2) and ρ_{exp}, with the deviation (Dev.%) ranging from 10.6 to 12.18%. This deviation is not a close agreement but is reasonable compared to previous published results for different glass compositions calculated by several methods. (Aziz *et al.*, 2010; Mostafa *et al.*, 2015; Inaba & Fujino, 2010). This could be because Inaba and Fujino neglected to include the lead and bismuth data with the phosphate glass when computing the theoretical density using the fitting graph approach (Inaba & Fujino, 2010). The increase in density as Bi_2O_3 increases may be due to the fact that Bi_2O_3 (M=465.959 g mol^{-1}) was being replaced by PbO (M=223.1994 g mol^{-1}) in the synthesis, resulting in more dense glass samples. The values of the molar volume increase from 48.8272 cm^3 to 54.6141 cm^3 with the increase of Bi_2O_3 content in the glass matrix. The indicating a possible expansion in the glass network and is a sign of more open glass structure of the glass matrix (Singh *et al.*, 2021). This may be due to the fact that Bi_2O_3 forms more non-bridging oxygen in the glass matrix than the PbO (Sathiyapriya *et al.*, 2019). In principle, the molar volume of the glass matrix increases with the increase in the number of oxygen atoms, cation radius, coordination number and decrease in the field intensity of cations (Singh *et al.*, 2021).

Table 2: Physical Parameters of the glass system xBi_2O_3–$(25 - x)PbO$–$75P_2O_5$.

Sample code	Denisty ρ (g cm^{-3})			V_M	V_0	OPD	σ	N_{O-ions}
	ρ_{exp}	ρ_{emp}	Dev%	(cm^3 mol^{-1})	(cm^3 mol^{-1})	(mol L^{-1})		($x10^{22}$ cm^{-3})
Bi0Pb25P75	3.3231	2.9698	10.63	48.8272	12.2068	81.9216	0.2658	5.51
Bi5Pb20P75	3.5422	3.1108	12.18	49.2338	12.0082	83.2761	0.2699	5.90
Bi10Pb15P75	3.6896	3.2449	12.05	50.5572	12.0374	83.0743	0.2695	6.16
Bi15Pb10P75	3.8010	3.3724	11.28	52.2685	12.1555	82.2675	0.2675	6.37
Bi20Pb5P75	3.9272	3.4939	11.03	53.6796	12.1999	81.9678	0.2669	6.61
Bi25Pb0P75	4.0823	3.6098	11.57	54.6141	12.1365	82.3963	0.2683	6.89

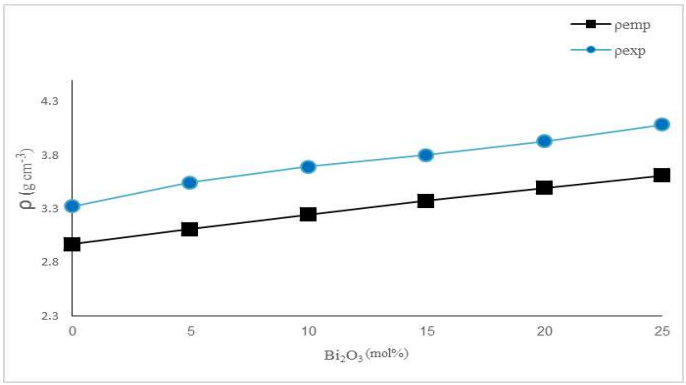

Figure 1: The experimental and empirical densities as a function of Bi_2O_3 mol % content.

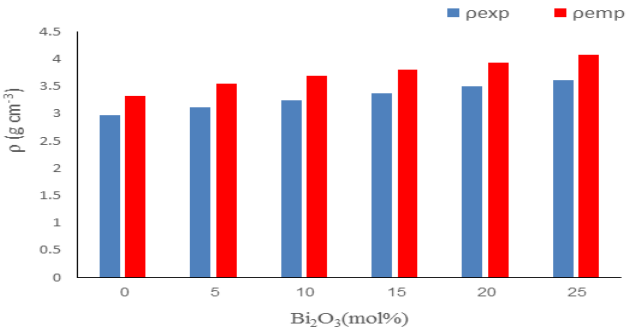

Figure 2: A histogram of the experimental and the empirical densities.

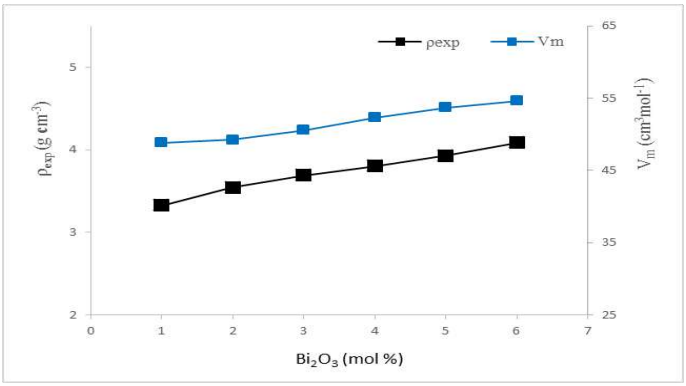

Figure 3: Variation of ρ_{exp} and V_M with Bi_2O_3 mol % content.

The stiffness of oxygen packing in the glass network has been observed by evaluating the OPD values of glass samples. As listed in Table 2 and depicted in Figure 3, the general behavior is that V_O increases with increasing Bi_2O_3 content while OPD decreases. This suggests that as there are fewer linkages in the matrix, the glass structure gets tighter (Sayyed *et al.*, 2019).

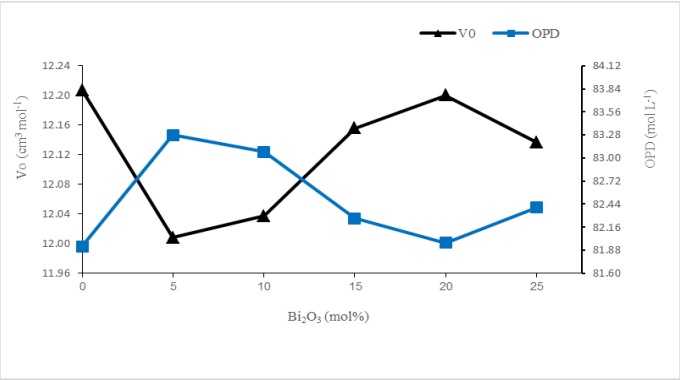

Figure 4: Variation of V_O and OPD with Bi_2O_3 mol% content.

Usually, the change in the cross-link density of glassy complexes resulting from structural modification has an impact on their Poisson ration (Saddeek *et al.*, 2004; Mhareb *et al.*, 2020; Sayyed *et al.*, 2021). Moreover, σ provides an indication of the stiffness of the glass system; for the majority of materials, Poisson's ratio normally ranges from 0 to 0.5. Materials with Poisson's ratio below 0.3 have a high cross-linking density, while those above 0.3 have a low cross-linking density (Gowda *et al.*, 2005; Kaur *et al.*, 2019). Table 1 and Figure 5 demonstrate that the prepared glass samples have a high cross-link density, with values of Poisson's ratio obtained within the range of 0.2658-0.2683. This indicates a better cross-link density of the tiled glasses, which is indicative of abetter application of shielding performance (Sayyed *et al.*, 2021).

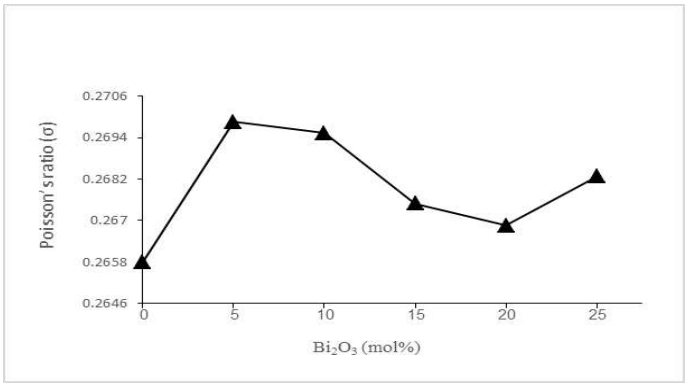

Figure 5: Poisson's ratio as a function of Bi_2O_3 mol% content.

The concentration of oxygen anion (Ni) in the glass networks is one of the key variables in the study of glass structure. Figure 6 illustrates how the N_{O-ions} increases as the Bi_2O_3 content rises, supporting the oxygen network's progressive extension and subsequent molar volume growth (Mostafa *et al.*, 2015).

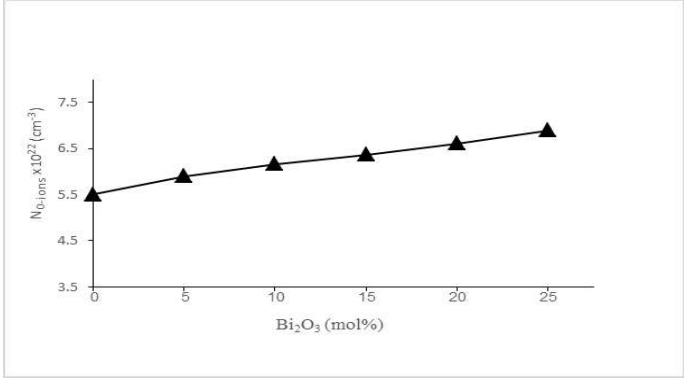

Figure 6: Variation in the number of N_{O-ions} versus Bi_2O_3 mol% content.

Conclusion

In this study, the effect of Bi_2O_3 on the physical properties of PbO-P_2O_5 glasses was presented. The comparison between the theoretical and experimental approaches for estimating the density of glass samples produced a result that was satisfactory. The finding showed that a rise in Bi_2O_3 was accompanied by increases in density and molar volume, suggesting a potential expansion of the glass network and a more open glass structure inside the glass matrix. The response of the oxygen molar volume (V_O) and

oxygen packing density (OPD) was contradictory; a little increase in V_O was noted, but this was likewise countered by a fall in OPD. This implies that the glass structure becomes tighter as the number of links in the matrix decreases. The Poisson's ratio readings indicate a tightening of the bonds within the glass matrix. The progressive extension of the oxygen network and the subsequent growth of the molar volume are supported by the presence of additional oxygen ions in glass that is caused by bismuth. Overall, the findings demonstrated that the inclusion of bismuth enhanced the glass samples' physical characteristics, enabling its usage in a variety of applications, particularly radiation shielding.

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Effects of chromium(VI) on body weight and organ weights in male rabbits: the protective role of *Moringa oleifera* leaf

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Abstract:

Chromium (VI) is one of the major causes of intense illnesses in people due to its mutagenicity, harmfulness, and carcinogenicity *Moringa oleifera* (Moment) is wealthy source of phytochemicals such as myricetin, phenolic substances, phenolic acids, flavonoids, isothiocyanates, tannins and saponins, quercetin, zeatin and kaempferol flavonoids which are successful cancer prevention agents that have a few restorative benefits. This work investigated the cautious impacts of *Moringa oleifera* against chromium(VI) on body and organs weight in rabbits. Five rabbits per gather were doled out to 1 of 4 treatment bunches: mg *Moringa oleifera* and mg Cr (VI)/kg BW (control); 400 mg MO/kg BW; 5 mg Cr (VI)/kg BW; 400mg MO/kg BW also 5 mg Cr(VI). Comes about demonstrated that treatment with Cr(VI) alone caused noteworthy ($P<0.05$) diminish in BW and relative weight of liver, kidney, spleen and heart compared to control creatures. On the other hand the BW and relative weight of liver, kidney, lung, spleen, and heart were altogether ($P<0.05$) expanded in rabbits treated with Moment alone as compared to control creatures.

Keywords: Rabbits; Chromium (VI); ***Moringa oleifera*** ; Weight

Introduction:

Moringa oleifera L. (Family: Moringaceae) is an inconceivably valuable therapeutic herb, have altogether tall wholesome esteem. It is an outstandingly sound herb which is consumable, and its tree may effectively and cheaply be developed and developed in Pakistan. It is additionally known as super nourishment because it contains inborn premise of exceedingly edible protein,

press, calcium, potassium, Vitamins A, C, E and polyphenols. Moringa is wealthy source of phytochemicals such as myricetin, phenolic substances, phenolic acids, flavonoids, isothiocyanates, tannins and saponins, quercetin, zeatin and kaempferol flavonoids which are viable cancer prevention agents that have a few helpful benefits. It is utilized as a restorative herb having different wellbeing benefits. Additionally, diverse parcels of moringa such a seed, roots, buds, takes off, blossoms and bark, have different shapes of organic activities, such as anti-inflammatory, antimicrobial, anti-carcinogenic, antihypertensive, anti-hyperlipidemic, antidiabetichepatoprotective and neuroprotective exercises, that makes a difference within the treatment of distinctive aliments. The current audit highlights the therapeutic, restorative properties of, and instruments of compounds extricated from *Moringa oleifera* moreover picking up unused points of view for advance inquires about and advancement (Babar *et al.*, 2022). Cr(VI), chromium 6) is chromium in any chemical compound that contains the component within the +6 oxidation state (in this way hexavalent). Essentially all chromium metal is handled by means of hexavalent chromium, particularly the salt sodium dichromate. Hexavalent chromium is key to all materials made from chromium. Approximately 136,000 tonnes (150,000 tons) of hexavalent chromium were created in 1985 (Jasim *et al.*, 2017).Cr (VI) may be a solid oxidant that's decreased to Cr (III) within the body, producing responsive oxygen species (ROS), which is destructive to the tissues. Cr (VI) can cross cell films. Past considers have appeared that dichromate, a hexavalent chromium compound, increments ROS concentration and causes lipid peroxidation and oxidative harm in hepatocytes and renal cells (Kotyzova *et al.*, 2015). Besides, long-term introduction to expansive dosages of Cr (VI) decreases the serum level of apolipoprotein-A1 and increments fasting blood glucose, triglycerides, add up to cholesterol, and other apolipoproteins (Feng *et al.*, 2018).

Materials and Methods:

Tested compounds

Cr(VI) was brought from the chemistry department, faculty of science, Omar Al-Mukhtar University (5 mg/ml body weight every other day). ***Moringa oleifera*** were collected from a home garden in Samno, Sabha, Libya, with a dose of 400 mg/ml body weight every other day for 12 weeks. twenty male New Zealand white rabbits (6 months old) were individually housed in cages and weighed weekly throughout 12 weeks' experimental period.

Study design

Twenty mature male rabbits were randomly divided into four equal groups (n=5 for each group) as follows:

Group I: Rabbits were used as control for 12 successive weeks.

Group II: In this group rabbits were given oral administration of MO with a dose of 400 mg/kg/day (Mohlala*et al.,* 2023 and Khalifa*et al.,* 2016)

Group III: These rabbits received orally with Cr(VI)5 mg/kg/day (El-Demerdash *et al.*, 2006).

. Group IV: These rabbits were administered orally with a combination of Cr(VI) and MO. At the end of the experimental period body weight of rabbits were recorded. Animals were sacrificed by decapitation and liver, lung, spleen, kidney and heart were immediately removed and weighed then the organs weight ratio was calculated. The relative weight of organs (%) was calculated as g/100 g body weight.

Statistical analysis

The data obtained were expressed as mean ±SEM. The significant differences were assessed by one-way ANOVA and Tukey test. After the detection of the normal distribution of the data and appropriate P-values, less than 0.05 is considered significant.

Results:

The changes in body weight (BW) and the relative weights of liver, kidney, lung, spleen and heart of male rabbits. The relative organ weights (%) were calculated as g/100 g body weight throughout the 12-week experimental period of rabbits treated with MO, Cr(VI) and their combination were summarized in (Table 1 and Figurers1 to 6). Overall means indicated that treatment with Cr(VI) caused significant ($P<0.05$) decrease in BW and relative weight of liver, kidney, lung, spleen, and heart compared to control animals. On the other hand the BW and relative weight of liver, kidney, lung, spleen and heart were significantly ($P<0.05$) increased in rabbits treated with MO alone as compared to control animals. The combination between MO and Cr(VI) caused significant increase in the reduction of BW and improvement in relative organ weights due to treatment with Cr(VI), and this means that MO alleviated its toxicity.

Table 1. Body weight (BW) and relative weight of kidney, liver, lung, heart, and spleen of male rabbits treated with MO, Cr(VI) and **their** combination

Parameter	Experimental groups			
	CON means ± SE	***MO means ± SE***	***Cr(VI) means ± SE***	***MO+Cr(VI) means ± SE***
BW (gm)	2008.03±49.21^{b}	2241.90±45.33^{a}	1694.07±68.81^{c}	1896.90±31.23^{b}
Kidney (g/100gm)	10.840± 0.652^{a}	12.847±0.581^{a}	10.076±0.644^{a}	12.126 ± 1.694^{a}
Liver (g/100gm)	45.47 ± 0.599^{a}	49.578±3.944^{a}	38.412 ±4.167^{a}	35.341 ± 6.811^{a}
Lung (g/100gm)	8.910 ± 0605^{ab}	10.516±1.316^{a}	7.296 ±0.630^{b}	7.648 ± 0.644^{b}
Heart (g/100gm)	5.333 ± 0.587^{b}	7.870±0.899^{a}	5.166±0.422^{b}	5.473 ± 0.171^{b}

Values are expressed as means ± SE; n=5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, $p<0.05$.

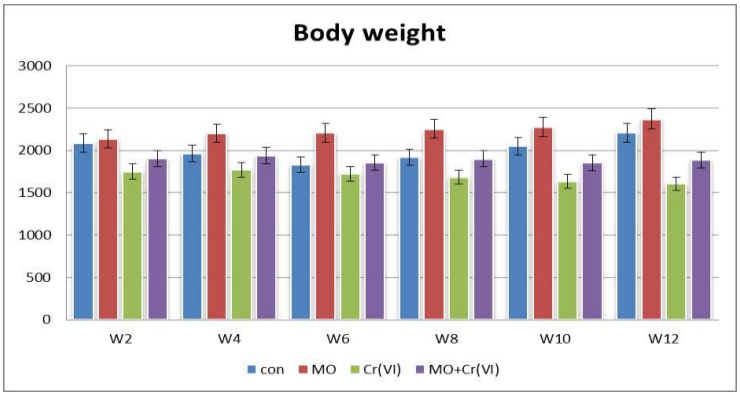

Figure 1. Changes in body weight treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

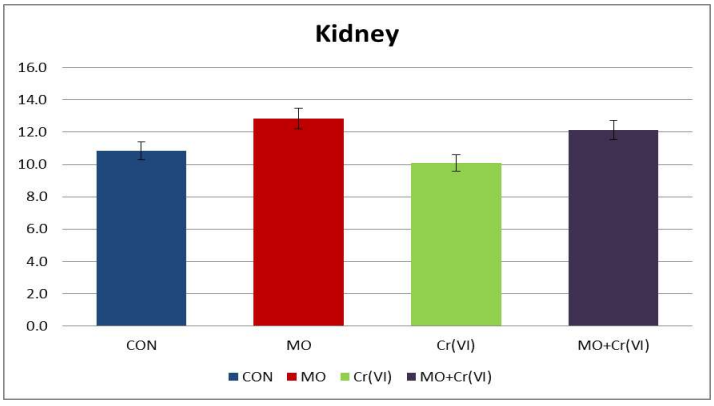

Figure2. changes weight of kidney treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

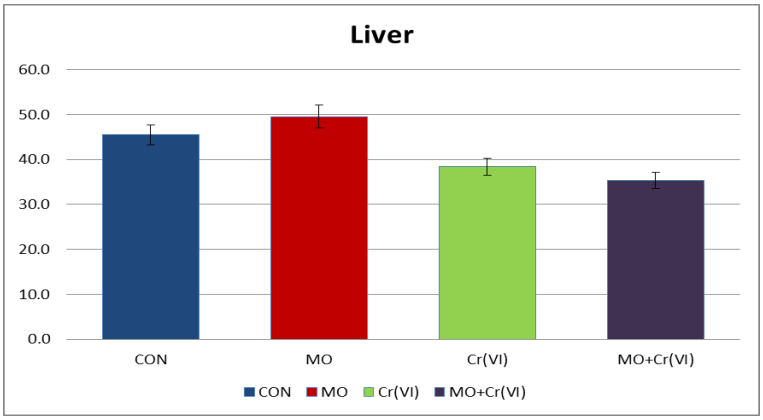

Figure 3. Changes weight of liver treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

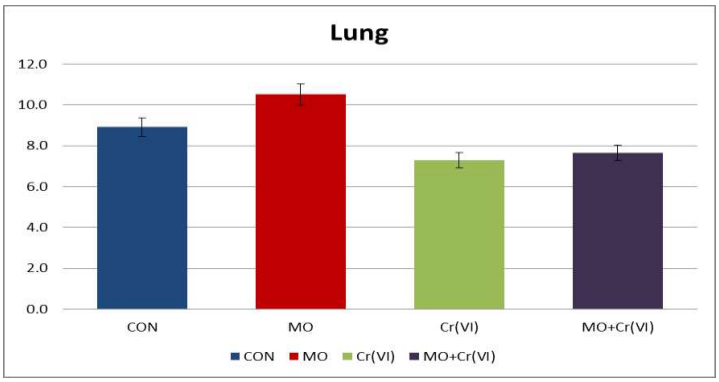

Figure 4. Changes weight of lung treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

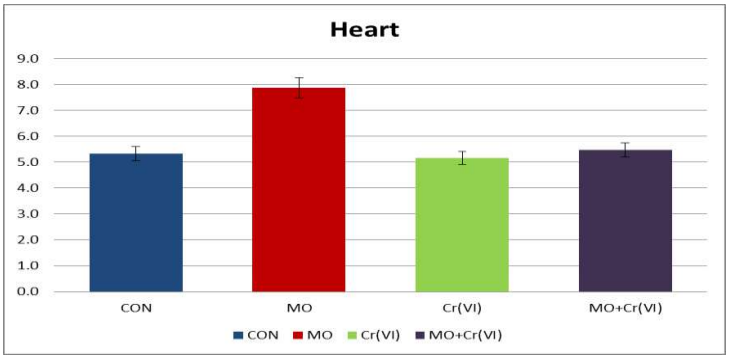

Figure 5. Changes weight of heart treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

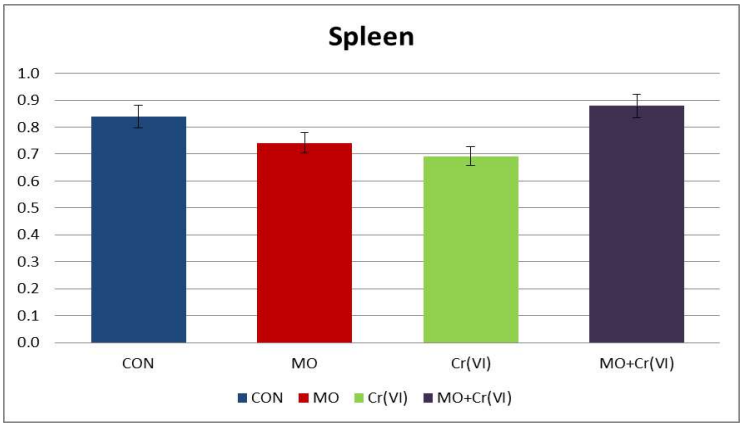

Figure 6. Changes in weight of spleen treatment of male rabbits with ***Moringa oleifera*** , chromium(VI) and/or combination.

Discussion:

The display comes about demonstrate that treatment with Cr (VI) caused critical decreases in body weight (BW) and relative organs weight (Push) (Table 1 Figure 1 to 6). The lessening in BW and ROW of the Cr (VI) treated rabbits concurs with that gotten by (El-Demerdash *et al.,* 2006; Bataineh *et al.*, 1997; Elbetieha and Al-Hamood, 1997 and Quinteros *et al.*, 2007) in rats uncovered to an airborne of sodium dichromate for 30 or 90 days or for 90 days taken after by an extra 30 days of non-exposure. Body weight pick up was essentially diminished at 0.2 and 0.4 mg chromium(VI)/m3 for 30 days ($p<0.001$), at 0.4 mg chromium(VI)/m3 for 90 days ($p<0.05$), and at 0.2 ($p<0.01$) and 0.4 mg chromium(VI)/m3 ($p<0.05$).The presentation of creatures to overwhelming metals can lead to antagonistic impacts on their wellbeing in common and on their generation in specific. Kidney and liver are organs exceptionally imperative within the assessment of the harmful potential of a substance (Oloyede *et al.*,2011). They are related with the digestion system and excretion of harmful substances (Moss *et al.*,2015) such as overwhelming metals. Within the current think about, live body weight diminished altogether with expanding dosages of potassium dichromate all through the test period. This result is in agreement with those of (Garcia *et al.*,2013). This may be since of potassium dichromate on female rabbits. The weight and volume of kidney and liver were comparable among medications. This perception may mean that the measurements of potassium dichromate managed did not have a articulated impact on the life systems of those organs. These comes about don't concur with (Saha *et al.*,2017), who

recorded a critical diminishment within the weight of the regenerative organs along side an increment within the weight of the liver and kidney. However, it ought to be famous that tall concentrations of chromium in drinking water diminish tastefulness of water, coming about in diminished water utilization; in this way, diminished body weight may, in portion, be due to diminished water utilization, in expansion to other causes. In male rats uncovered to 73 mg chromium (VI)/kg/day as potassium dichromate in drinking water for 30 days, body weight was diminished by 11.6% (Quinteros *et al.,* 2007). A 19% diminish in body weight pick up was watched in male rats uncovered to 42 mg chromium (VI)/kg/day (Bataineh *et al.,* 1997) and a 10% diminish was detailed in male mice uncovered to 6 mg chromium (VI)/kg/day (Elbetiehaand and Al-Hamood, 1997) as potassium dichromate in drinking water for 12 weeks. In differentiate, Chromium (VI) could be a noxious metal commonly utilized in industrial field. It produces harmful impacts within the liver and other diverse organs (Sánchez *et al.*,2015).The increment in body weight of rabbits treated with *Moringa oleifera* may be due to the androgenic properties of Moringa takes off which have anabolic movement. The clears out contain tall concentrations of rough protein, fundamental vitamins, calcium and press (Makkar and Beckar, 1997; Gidamis *et al.,* 2003 and Odeyinka *et al.,* 2007). These comes about may speak to the useful impact of *Moringa oleifera* takes off dinners in diets on the rabbits' digestion system and resistant. This perception concurs with (Ologhobo *et al.*,2014) who appeared that fowls encouraged diets containing *Moringa oleifera* leaf had more prominent butcher weights. On the other hand, the reason may relate to a change in weight and length within the stomach related tract of rabbits encouraged Moringa, which leads to a alter within the intestinal absorptive zone, as demonstrated by (El-Badawi *et al.*,2017) when the creators taken note that the utilize of Moringa clears out driven to progress hot and cold carcass weight and dressing rate. On the same slant, (Nuhu, 2010) and Dougnon *et al.* (2012) specified that rabbits nourished Moringa leaf supper had way better butcher weight and dressed weight compared to bolstered control slim down, and the values were expanded with expanding Moringa level in diets. In differentiate, (Abubakar *et al.,* 2015) found that Moringa takes off can be utilized in rabbit diets at up to 45% without any antagonistic side impacts on carcass and organs. Moreover, (El-Desoky *et al.*,2018) detailed that using Moringa leaves at rates of 3, 6, and 9% did not influence carcass weight and Inner organs, but the dressing rate, have made strides with utilizing Moringa within the rabbit diet. Hashem *et al.* (2017)

and Soltan *et al.,* (2018) state that Moringa leaves contain organosulfur compounds and amides/alkaloids. These compounds have a solid impact on pathogenic microbes, such as *E. coli* (Makkar *et al.,* 2007). In expansion to anti-inflammatory and immunomodulatory properties (Khatab *et al.,* 2016 and Hashem *et al.,* 2017). These chemicals may progress the intestinal microbial biological system, improving rabbits' stomach related proficiency and safe condition. This progresses the digestibility of rough protein and fiber and increments body weight. Comparative comes about were detailed by a few analysts (Omara *et al.,* 2018; Sun *et al.,* 2018; Jiwuba and Ogbuewu, 2019). In expansion, the tall substance of Moringa leaves from vitamins C and A (Ferreira *et al*., 2008; Konmy *et al*., 2016) and flavonoids act as capable cancer prevention agents and have the capacity to kill the un-favorable impacts of overabundance free radicals coming about from warm stretch.

In conclusion, the comes about of the display ponder convincingly illustrated that Cr(VI) presentation brought about in shifting degree in organs weight. Moment treatment advancement the body and organs weight.

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Analysis of the Main Chemical Components of the Remains Marble Sculptures at the Cyrene Museum, Libya

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Abstract:

Fluorescence X-ray (XRF), atomic absorption spectrometry (AAS) and flamephotometerwere used to determine the percentages of broken samples that cannot be restored from sculptures in the city of Cyrene, Libya. The results showed fairly close ratios between fluorescence and atomic absorption of samples. The proportions of calcium oxide and magnesium oxide showed that the marble samples are made of coarse grains that have the composition of dolomite, as they contain a magnesium carbonate component, which is likely to have an origin from the Thassos quarry in Turkey.

Keywords: XRF; AAS; Flamephotometer; Dolomite Composition.

Introduction:

Libya is a country in northern Africa is located on the Mediterranean Sea's southern shore. Geomorphosites are geomorphological landforms that humans have utilized or observed and assigned scientific, cultural/historical, aesthetic, or social/economic significance (Abdel-Maksoud *et al.,* 2022). The Shahhat area (previously known as Cyrene, Al-Jabal Al-Akhdar, Libya) is located in the country's northeast, east of the Sidra Gulf and near the Mediterranean Sea (Salvini *et al.,* 2004) see figure (1). In 1982, UNESCO listed Shahat City as a World Heritage Site. The Temple of Apollo, which was built in the early seventh century BC, is one of the city's most outstanding attractions (AbdelMaksoud *et al.,* 2022). Al-Jabal Al-Akhdar is classified into 14 rock units and is categorised as thick sequences of upper Cretaceous to upper Miocene carbonate rocks. These are listed in the following order, from bottom to top: Al-Majahir Formation (Campanian) overlain by Wadi Dukhan Formation (Maastrichtian) (both are the lateral equivalents of Al-Hilal Shale and the lower part of Al-Athrun Formation in the coastal area); Qasr Al-Abid

Formation (late Cenomanian) overlain by Al-Baniyah Formation (both are the lateral equivalents of Al-Hilal Shale and the lower part of Al-Athrun Formation in the coastal area); Tertiary, which begins with the Paleocene Uwayliah Formation and is overlain by the Eocene Apollonia and Darna Formations in an interfingering relationship; Oligocene, which begins with the early Oligocene Bayda Formation and is overlain by the late Oligocene Al-Abraq Formation. The early Miocene Al-Faidiyah Formation is overlain by the middle Miocene Benghazi Formation, which is overlain by the late Miocene Wadi Al-Qattarah Formation (Abdel-Maksoud *et al.*, 2022). In antiquity, marble was one of the most expensive stones. Marble was widely utilized in Roman and Byzantine architecture and monuments. They moved vast amounts and blocks of marble from ancient Mediterranean quarries to the majority of Near Eastern archaeological sites (Capedri *et al.*, 2004). On the other hand, archaeological evidence has provided important information about a great catastrophe that gripped the city, damaging the North Stoa of the Agora of Cyrene in its penultimate period; Stucchi said that "the major earthquake, which hit and devastated Crete in 251 AD", also hit Cyrene. This information is derived from a Hagiography of the "Holy Ten" (Wilkes, 1976) as shown in figure (2). In this research, we will try to identify the chemical compositions of some archaeological species located in the Shahat Museum using different techniques, knowing the presence of four semi-destroyed samples, from which approximately 2 grams were sampled to conduct measurements.

Figure 1: shows the location of the ancient city of Cyrene in Libya and the quarry in Thassos in Greece.

Figure 2: General map of the archaeological site of Cyrene (Kenrick & Buzaian, 2013).

Materials and Methods:

The measurements were made using X-Ray Fluorescence, atomic absorption spectroscopy and flam photometer techniques on damaged samples of archaeological sculptures that cannot be restored. The proportion of metal oxides and other chemical components in the sand samples was ascertained by measuring the marble samples using X-ray fluorescence. The detection and counting of X-rays from the impact of photoelectric events is the basis of the X-ray fluorescence measurement. The target atom's (sample's) electrons are exposed to high-energy beams (gamma radiation, X-rays), which causes the electric photo effect. If the light energy is greater than the electron-binding energy in the target atom's K, L or M orbits, the electron will leave the orbit. As a result, the target atom will feel the electron vacuum. If the light energy is greater than the electron binding energy in the target atom's K, L or M orbits, the electron will leave the orbit. As a result, the target atom will feel the electron vacuum. The outer orbital electrons will fill this electron vacuum, and then X-ray energy will be released (Sari, 2019). To estimate metals and compare some ratios, atomic absorption spectroscopy was used to estimate magnesium and iron according to the procedures followed by the device and standard methods (Chmilenko *et al.*, 2002; Bufarwa *et al.*, 2022; Elmanfe and Abbas, 2022).

Results and Discussion:

According to the previously mentioned criteria, the results for the destroyer sculptures can be clarified in Table 1.

Table 1: showing the percentages of the chemical components under study for marble samples of sculptures

Sample	Na_2O%	MgO%	CaO%	Fe_2O_3%	SiO_2%	MnO%
	Falme photometer	XRF	XRF	XRF	XRF	XRF
		AAS		AAS		AAS
S1	0.009	22.6	36.4	0.46	0.098	0.01
		18.4		0.39		0.007
S2	0.014	21.8	33.8	0.44	0.084	0.01
		17.3		0.35		0.008
S3	0.011	22.3	31.6	0.54	0.086	0.01
		20.9		0.48		0.006
S4	0.017	18.5	35.8	0.61	0.088	0.01
		21.4		0.53		0.008

Figures 3 and 4: showing intact and damaged specimens in the museum.

Analysis was conducted on four destroyed marble remains from the sculptures of the city of Shahat (Cyrene). The cause of the destruction of the marble samples, which could be carved funerary pieces, can be attributed to random excavation, looting and theft of antiquities (Wanis, 1978; Al Khabour, 2022). The measurements obtained by the flamephotometer method (Carmo Freitas *et al.*, 1988; Prochaska and Grillo, 2010) indicate that the level of minerals such as Na_2O for the remains of marble samples ranges between 0.009 and 0.017. The percentages of silicon oxide for the four samples according to the X-Ray Fluorescence technique ranged from 0.084 to 0.098. Magnesium oxide results for samples using two methods, X-Ray Fluorescence and Atomic Absorption, ranged from (22.6-18.5%) and (20.9-17.3 %) respectively. The values obtained for magnesium oxide for the four samples are close to the results obtained by the non-destructive X-ray method for samples in the analysis of marble sculptures of Greek origin

(Calliago *et al.*, 2013). With the same methods used in analyzes with magnesium oxide, the results of iron(III) oxide for X-ray fluorescence and atomic absorption were: (0.61-0.44 %) and (0.53-0.35 %), respectively (Prochaska *et al.*, 2018). Analyzing calcium oxide and comparing the results using the fluorescence X-ray method with the gravimetric method. The X-ray results for calcium oxide were between (31.6-36.4 %). Although the methods used for analysis are only suitable for destroyed samples of sculptures that cannot be re-thrown, the results were very similar to the results obtained in analyzing marble samples of sculptures, such as non-destructive X-rays of the samples (Calliago *et al.*, 2013; Prochaska *et al.*, 2018). According to the percentages obtained for the samples studied, they were very close to the marble samples of the Thassos type, which the Greeks are believed to have brought with them to establish the city of Cyrene in Libya.

Conclusion:

The analytical methods used to determine the proportions of the components of sodium oxide, magnesium oxide, calcium oxide, iron (III) oxide, manganese oxide, and calcium oxide showed that the proportions are somewhat close in two ways. These percentages are found in the case of dolomite marble, which we can say was imported in the Greek era from the city to Cyrene, Libya from one of the quarries, perhaps the Thassos mine in Turkey.

Recommendations:

- Due to the presence of statues and sculptures that are still in good condition in the museum, we recommend and hope that they be analyzed using non-destructive X-rays of the sample, which can be used to determine the places from which the marble was brought and the foundations and history on which the ancient engineers built the city.
- The oxygen and carbon isotopic ratios, $\delta^{18}O$ and $\delta^{13}C$; It is very important to know the exact time history for extracting marble samples.
- The techniques that were used in the research are only used to identify the main components of the sculptures, which were destroyed and cannot be restored.

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Accurate and Rapid Methods for Determinate Anti-Oxidant and Total Phenol Contents in Two Different Types of Pomegranate Peels

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Abstract

Two different peels samples of were collated from two different sources including Libyan Tunisian pomegranate pees. Sensitive, rapid and accurate methods were used to estimate the contents of anti-oxidant capacity and total phenols in the studied samples. The contents were measured by spectrophotometric methods, the values were calculated from standard curves of Tannic acid as reference material. The results showed that the contents of anti-oxidant and total phenols were increased in pomegranate peels compared with the Tunisian ones.

Keywords: Anti-oxidant, T. P., spectrophotometric methods.

Introduction

The search for cheap and abundant sources of natural antioxidants is attracting worldwide interest. Due to their lower costs many research is needed in order to select raw materials; those of residual origin are especially promising. Antioxidants containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT), have restricted use in foods as these synthetic antioxidants are years suspected to be carcinogenic (Madhavi *et al*., 1995). The pomegranate (*Punica granatum* L.) is one of the oldest edible fruits. It is an important commercial fruit in Iran with a total production of 665,000 tons in 2003 (Anonymous, 2003). It is a significant fruit found in tropical and subtropical areas that was first domesticated in India and the Middle East. Ancient cultures have long utilized it for medical purposes. Pomegranates are known to have antibacterial, antioxidant, anticancer, and ant proliferative

properties (Faria *et al*., 2006). Pomegranate is consumed fresh and in processed form as juice, wines, flavors, and extracts. Commercial pomegranate juice is currently a high-value commodity on the agricultural market and has the highest antioxidant activity when compared to other fruit juices, red wine, and green tea. Significant concentrations of polyphenols, including gallic acid, ellagic acid, and tannins, can be found in pomegranate juice and peel (Loren *et al.,* 2005). It has been used in the preparation of tinctures, cosmetic, therapeutic formula and food recipes (Finkel, and Holbrook, 2000) and in this regard pomegranate peel is a good source of antioxidants (Singh *et al.,* 2001). Pomegranate (*Punica granatum* L.) has gained commercial importance in food and health industries due to increasing scientific evidence linking its consumption to better health outcomes (Sarkhosh *et al*., 2007). The peel makes up ~ 50% of the fruit (Viuda-Martos *et al*., 2010). Pomegranate marc is typically used as low-value cow fodder or disposed of immediately in the field, which may constitute an environmental risk. On the other hand, due to its high antioxidant concentration, pomegranate marc may make a useful raw material for making natural antioxidants (Que *et al.,* 2009). Compounds known as antioxidants can extend the shelf life of food goods by delaying the lipid peroxidation process. This is especially true for lipids and lipid-containing systems. In addition to causing chemical food spoiling, lipid peroxidation in fats and fatty foods generates free radicals like hydroxyl and peroxyl radicals, which are allegedly linked to aging, mutagenesis, and carcinogenesis (Nasr *et al*., 1996). On the other hand, the most widely used synthetic antioxidants, butylated hydroxyanisole and butylated hydroxytoluene, which have been used as antioxidants since the beginning of this century, and have been restricted recently, mainly because of their possible carcinogenicity (Mahdavi and Salunkhe, 1995) causing liver swelling and changing liver enzyme activities (Martin and Gilbert,1968). However, in recent years, many attemps have been made to study natural antioxidants, particularly those of plant origin (Zainol *et al.,* 2003) Great interest has recently been focused on the addition of polyphenols to foods and biological systems, due to their well-known abilities to scavenge free radicals, i. e. antioxidant power. The generation of free radicals plays an important role in the progression of numerous pathological disturbances, such as atherosclerosis (Steinberg, 1992), brain disfunction (Gordon, 1996) and cancer (Ames, 1983). Extraction is a key step for obtaining antioxidants with

an acceptable yield. Solvent extraction is more frequently used for the isolation of antioxidants and the extraction yield and economic viability is dependent on the type of solvent and method of extraction, mostly due to the differing polarity of these compounds. Several extraction techniques have been reported for the extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether (Cheung *et al.,* 2003). Furthermore, supercritical CO_2 (Persson *et al.,* 2002) and solvent extraction along sonication have been applied for this purpose (Bicchi *et al.,* 2000). The pomegranate antioxidant activity is typically higher in commercial juices extracted from whole pomegranates than in experimental juices obtained from the arils only. This can be attributed to its high content of polyphenols in peel, such as condensed tannins and anthocyanin's. The processing of pomegranate juice involves squeezing juice from the fruit with the seeds and the peels together. The resulting marc on a weight basis consists of approximately 73 % peels and 27% seeds and has a high potential for value addition as a source of phenolic , pronto cyanides and flavonoids which are herein also referred to as antioxidants. Natural antioxidants have become very popular for medical and food applications and are preferred by consumers than synthesized antioxidants, such as BHA and BHT. For instance, the use of agricultural wastes such as wine-making wastes as alternative low-cost sources of phenolic compounds has been on the increase (Spigno and De Faveri, 2007). The process of commercially isolating these antioxidant components from pomegranates begins with extraction. Nevertheless, effective techniques for removing antioxidants found in pomegranate peels, such as flavonoids, proanthocyanidins, and phenolic acids, as well as determining the kinetic parameters necessary to create an effective extraction process for producing these compounds from peels, have not been researched. As a result, the goal of this study was to assess the effectiveness of solid-solvent extraction of antioxidants from pomegranate marc peel (PMP) and to clarify the ways in which various solvents, temperature ranges, and solvent-to-solid ratios impact the antioxidant chemicals' extraction. Polyphenols have demonstrated beneficial effects (Leja *et al.,* 2003), having free radical scavenging capacity and antioxidant ability (Kahkonen *et al.,* 2001). Pomegranate Peel exhibited high antioxidant activity in various in vitro models (Li *et al*., 2006). The peel of the pomegranate has been extensively used in folk medicine (Ahmad and

Beg, 2001). Although several studies have shown that pomegranate peels proved to be important source of phenolic compounds, with several health benefits, its use remained very limited (Cai *et al.,* 2004). This study aimed to determine the phenolic compound and anti-oxidant capacity in some pomegranate peels types including (Libyan and Tunisian).

Materials and methods

Samples:

Two different peels samples of were collated from two different sources including Libyan pomegranate peel and Tunisian pomegranate peel.

Samples preparation:

The peels were washed several times with distilling water then dried at overnight conditions, then grinded in mortar.

Determination of antioxidant power by Prussian Blue method:

One gram of powder was defatted with petroleum ether.

The defatted powder was then extracted sequentially by stirring with 10 ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol (v/v). The three combined extracts were evaporated under vacuum and the residue was dissolved in 10 ml methanol. Half ml of the solution was diluted with 3 ml distilled water, 3 ml 0.008 M $K_3Fe(CN)_6$ was added, 3 ml 0.1 M HCl, and 1 ml 1% $FeCl_3$. The blue color is allowed to develop for 5 min and the absorbance is measured at 720 nm against the blank. Construct a calibration curve within 1-10 µg/ml tannic acid.

Determination of total phenols by Folin Ciacalteu Method

Procedure:

Aliquots of the extracts were taken in a 10 ml flask and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of tannic acid from 4 to 20 µg/ml.

Results and discussion

The results of the antioxidant activity were recorded in the Table (1) and representative in the Figure (1): The data showed that the high contents of phenolic compounds were recorded in Libyan pomegranate peel (29.95 ppm) compared with Tunisian pomegranate peel (25.04 ppm). The contents of anti-oxidant values were correlated with the values of phenolic compounds, it was

reported that the phenolic compounds are the most compounds which give the anti- oxidant activity, on the hand the variations of capacity of the antioxidant are related to the presence other compounds which may be gave antioxidant activity and some acids.

Table 1: The contents of the studied parameters

Sample Type	Total phenolic (ppm)	Anti-Oxidant Activity (ppm)
Libyan pomegranate peel	29.95	7.30
Tunisian pomegranate peel	25.04	3.09

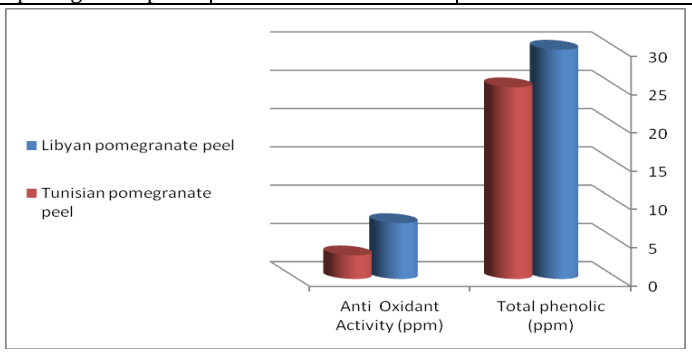

Figure 1: The distribution of phenolic compound and anti-oxidant in the studied peels.

Singh *et al.* (2002) also reported that extracts of Punica granatum peel in different concentrations were effective against *S. epidermidis*, *S. aureus*, *S. mutans*, *S. sanguinis* and *S. salivarius*. McCarrell *et al.* (2008) also demonstrated antibacterial activity of autoclaved pomegranate peel extract against Staphylococcus aureus and *B. subtilis*. The antioxidant properties of pomegranate peel methanol extract that were reported in this study may be due to the polyphenols (ellagic acid and gallic acid) that are present (Gil *et al.*, 2000). Hence, in order to investigate phenolic compounds from the PPE, the HPLC analysis of methanolic extract was performed. These compounds included 3 hydroxybenzoic acids (vanillic, gallic and ellagic acids), 2 hydroxycinnamic acids (caffeic and p-coumaric acids), and one flavonol (quercetin). In literature, many authors proved the presence of quercetin and vanillic acid in pomegranate peel (Artik, 1998; Cai *et al.*, 2004; Van Elswijk *et al.*, 2004). For p-coumaric acid, as analyzed by HPLC, these results are greater than those reported by Ben Nasr *et al.* (1996) in Tunisian pomegranate peel, who reported 11.7±0.1 mg 100 DW-1 ellagic acid and 3.0±0.1 mg 100 DW-1 gallic acid. However, in current results corroborate those obtained by Elfalleh *et al.* (2011) who reported that gallic acid as the major phenolic compound (123.8±9.6 mg 100 g-1), followed by ellagic acid (35.9±2.4 mg

100 g-1), caffeic acid (20.6±1.5 mg 100 g-1 and pcoumaric acid (4.5±0.4 mg 100 g-1).

Conclusion

The study showed that the peels from pomegranate marc are a potential resource for phenolic. The antioxidant activity of pomegranate peel was attributed to the total phenolic compounds, also the Libyan pomegranate pees have high contents of phenolic compound comparing with the Tunisian ones.

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Comparative Study between Vitamin E and Graviola on Hematological Parameters in Male Rabbits

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Abstract:

Vitamin E may have different parts as a vitamin. Numerous organic capacities have been hypothesized, counting a part as a fat-soluble antioxidant. Graviola (Annunonamuricata L.), a plant developing in tropical districts, has numerous names and a run of ethno medicinal employments. The key dynamic components are accepted to be annonaceous acetogenins, with more than 100 such compounds having been separated from A. muricata. Five rabbits per gather were relegated to three bunches: mg A. vitamin E and mg graviola BW (control); 100 mg of Vit E /kg BW; 100 mg Graviola/kg BW. Rabbits were orally managed the individual dosages each day for 12 weeks. Treatment with graviola did not influence red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelet count (PLT), hemoglobin (Hb), mean cell volume (MCV), cruel cell hemoglobin (MCH) and cruel cell hemoglobin concentration (MCHC). On the other hand comes about shown that treatment with vitamin E critical increment in ruddy blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelet count (PLT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Point of the think about comparative ponder between vitamin E and graviola on hematological parameters in male rabbits.

Keywords: Rabbits; Vitamin E; Graviola; Hematological parameters

Introduction:

Vitamin E could be a bunch of eight fat solvent compounds that incorporate four tocopherols and four tocotrienols (Marriott *et al.,* 2020). Vitamin E insufficiency, which is uncommon and as a rule due to an underlying issue with processing dietary fat instead of from a slim down moo in vitamin E

(McDermott, 2000), can cause nerve issues (Traber *et al.,* 2019). Vitamin E could be a fat-soluble antioxidant which may offer assistance secure cell layers from responsive oxygen species (Traber *et al.,* 2019). As of 2017, vitamin E proceeds to be a point of dynamic clinical investigate (Galli et al., 2017). There's no clinical prove that utilize of vitamin E skincare items are successful (Sidgwick *et al.,* 2015). Both characteristic and manufactured tocopherols are subject to oxidation, and so in dietary supplements are esterified, making tocopheryl acetic acid derivation for solidness purposes (Marriott *et al.,* 2020). Both the tocopherols and tocotrienols occur in α (alpha), β (beta), γ (gamma) and δ (delta) forms, as determined by the number and position of methyl groups on the chromanol ring. Vitamin E may have various roles as a vitamin (Traber *et al.,* 2019). Numerous organic capacities have been hypothesized, counting a part as a fat-soluble anti-oxidant (Traber *et al.,* 2019). In this part, vitamin E acts as a radical forager, conveying a hydrogen (H) particle to free radicals. At 323 kJ/mol, the O-H bond in tocopherols is around 10% weaker than in most other phenols (Lide *et al.,* 2004). Alpha-tocopherol, either actually extricated from plant oils or, most commonly, as the manufactured tocopheryl acetic acid derivation, is sold as a prevalent dietary supplement, either by itself or consolidated into a multivitamin item, and in oils or moisturizers for utilize on skin (Marriott *et al.,* 2020). *Annona muricata* Linn, which has a place to the Annonaceae family, is commonly known as soursop, graviola or guanabana. It is local to sub-Saharan nations (Gavamukulya *et al.,* 2014). In spite of the fact that it is presently broadly developed in numerous tropical nations within the world such as India, Malaysia and Nigeria. Frequently, this plant is looked for for its helpful impacts. Each portion of the tree i.e. the root, stem-bark, takes off, natural product and indeed the seed is utilized in conventional drugs around the world (Onyechi *et al.,* 2012). The assumed helpful benefits of the soursop has pulled in seriously inquire about on the chemical composition of the takes off and seeds that has driven to the finding of acetogenin compounds (Moghadamtousi *et al.,* 2015). This atomic structure may be exceptionally strong compound against cancer because it denies the high-energy requesting cancer cells of adenosine triphosphate (ATP) supply by means of the disturbance of the mitochondrial electron transport framework, coming about in apoptosis (McLaughlin, 2008). These disconnected compounds, which are auxiliary metabolites/antioxidants, reply the potential of the soursop for having anti-cancer, insecticidal, steadying as well as torment and immune-suppressing properties (Bermejo *et al.,* 2005). Within the past, a few

considers focused on antioxidant action of extricates from pulps, clears out and peel of *Annona muricata* Linn. Akomolafe and Ajayi (2015) alluded to a comparative consider on antioxidant properties of the peel and mash of ready *Annona muricata* Linn that reported that the antioxidant potential in soursop peel was found to be essentially higher than within the mash, as decided by ferric decreasing antioxidant control (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Fe2+ chelation and hydroxyl rummaging tests. Furthermore, *Annona muricata* Linn leaf fluid extricate was found to reduce the pancreatic B-cells of Streptozotocin treated diabetic rats by straightforwardly extinguishing lipid peroxides and in a roundabout way improving generation of endogenous cancer prevention agents, in this way tending to its antioxidant potential (Adewole and Caxton-Martins, 2006). In any case, past considers have appeared that there are distinctive levels of antioxidant/phenolic substance among plants of comparable species (Lim and Quah, 2006).

Materials and Methods:

Vitamin E was purchased from pharmacy in El -Bayda-Libya. Graviola was purchased from maximum international company, Brasil. Each capsule contains 3 g powder and the content of each capsule was dissolved in corn oil just before use. Mature male New Zealand White rabbits (age of 6 months and initial weight of (1892±50.79 g) were used. All animals were used following appropriate approval from the University of Omar Al-Mukhtar. Fifteen mature male rabbits were randomly divided into three equal groups: Group I: The control group up received an equivalent of 1 ml of the vehicle (corn oil) alone by oral for 12 weeks. Group II: Rabbits were treated with graviola was given daily by gavage at a dose of 100 mg/kg B.W, (Ibrahim *et al.,* 2021),which dissolved in corn oil for three month. Group III: Rabbits were treated with vitamin E 100 mg/kg BW (Minardi *et al*., 2020). Hematological parameters: Blood samples were collected from the ear vein of all animals every week throughout the 12-week experimental period. Blood samples were obtained in the morning before accesses to feed and water. Values derived from complete blood count (CBC). All CBC tests were performed by automatic blood cell analyzer (XP-300). Automated Hematology Analyzer, Sysmex American,Inc (Bain, 2001).

Statistical analysis: Where applicable, statistical analysis was carried out in Minitab software (version17) /statistical significance was assessed using

ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the data and appropriate $P < 0.05$ consider significant.

Results:

Values derived from complete blood counts (CBC), including differential cell counts were recorded for each group and presented in Table 1. Treatment with graviola was caused non-significant effect on RBCs, PCV, HB concentration, MCH and MCHC. Only MCV was significantly decreased with graviola. On the other hand treatment with vitamin E significant increase in red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), platelet count (PLT), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC).

Table 1. Mean values of hematological parameters in male rabbits treated with graviola and vitamin E.

Parameter	Groups		
	Control	Vitamin E	Graviola
RBC $\times 10^6$ (µl)	6.04 ± 0.110^a	6.10 ± 0.136^a	6.26 ± 0.084^a
WBC $\times 10^3$(µl)	8.5 ± 0.18^b	10.17 ± 0.44^a	9.00 ± 0.22^b
PCV$\times 10^3$(µl)	40.01 ± 0.428^a	44.19 ± 0.552^a	40.48 ± 0.465^a
PLT $\times 10^3$(µl)	288.03 ± 6.35^b	289.63 ± 9.948^a	441.81 ± 25.66^a
Hb (g/dl)	12.44 ± 0.14^a	13.56 ± 0.19^a	13.31 ± 0.19^a
MCV (fl)	68.16 ± 0.40^a	69.53 ± 2.20^b	64.0 ± 1.11^a
MCH (pg)	22.4 ± 0.11^a	24.37 ± 0.89^b	21.2 ± 0.30^a
MCHC (dl)	33.49 ± 0.23^a	27.73 ± 0.705^a	32.27 ± 0.44^a

Values are expressed as means ± SE; n=5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b)were significantly different, $p<0.05$

Discussion:

Table 1 speak to the hematological parameters of male rabbits treated with graviola and vitamin E. Comparative to the comes about of this consider, Cerda *et al*. (2003) also did not watched any noteworthy distinction in blood parameters analyzed. From Table 1, in spite of the fact that platelet appeared a critical increment with expanding levels for all parameters were inside the ordinary extend as detailed by Petterino and Argentini-Storino (2006). Graviola extricate contains tall add up to antioxidant which is nice in promoting health. Blood hematology comes about within the display consider did not appear any anomalies (Farag *et al.*, 2006). The discoveries from display considers proposed that the organization of graviola extract did not cause any toxicological impact since the values were within the ordinary extend as detailed by Chengelis *et al.* (2008) and Petterino and Argentini-

Storino (2006). In any case, pre-treatment with vitamin E caused a noteworthy height in WBC check compared to bunches. The leukocytosis recorded within the vitamin E pretreatment bunch was due to lymphocytosis. The reason for the leukocytosis isn't known and merits further investigation. In any case, we conjecture that vitamin E, being the foremost vital lipophilic antioxidant in cells, plays a crucial part within the upkeep of cellular layer judgment Bread-cook *et al*.(1986), particularly within the confront of oxidative attack by CPF (Ambali *et al.,* 2011). This may have contributed to the enhancement in leukocyte tally by stabilizing its film from the impact of free radical intervened cytotoxicity. Besides, antioxidants have been appeared to repress free radical actuated apoptosis (Knight, 2000). Vitamin E organization lead to rise in blood parameters, this concurred with (Rizvi *et al.,* 2014) which demonstrates that the primary line of security against the peroxidation of lipid and free radical assault of the cell film is the vitamin E. This vitamin includes a more prominent lipid peroxidation inhibitory impact since of its antioxidant impact which lead to ensure blood cells from harm.

In conclusion, treatment with vitamin E caused an improve within the hematological parameters of male rabbits more than graviola.

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A study of the association between Blood pressure and study stress, gender and ABO in a random sample of students from Al-Mahara Institute at AL-Bayda City –Libya

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Abstract:

The present study was undertaken to study the association between blood pressure (BP) levels, gender, blood group type and study stress. 40 students (28 females and 12 males) from al-Mahara Higher Institute for Health and Administrative Sciences in Al-Bayda City / Libya were included in this study in the period from 9-5-2023 to 30-5- 2023. BP measurement result shown 23 students had a drop in BP on the exam day compared to their BP measurement during the normal school day. Conversely 16 students had a rise in BP and there were no changes when measuring BP for one student between normal and exam day. Also the individuals with blood group (A+) had the highest number reaching 12 followed (O+) 11, and (B+) group recorded 10. Meanwhile the (AB+) recorded 4, the lowest number was O- recorded only 3students. Result shown there are non-significant decrease in BP on normal day and exam day. Meanwhile the results shows there are($P<0.05$) significant difference in BP between males and females in normal study day ,and non-significant differences in exam day. Also there no significant differences in BP measurement in different blood group types. The highest rate of BP recorded in the normal study day was (139/89) and the day of exam was (124/82), conversely the lowest pressure rate recorded in the normal school day (100/72) and the day of exam was (95/60).

Keywords: Blood pressure, study stress, blood group type, normal study day, exam day.

Introduction:

Blood pressure (BP) :is the pressure of blood pushing against the walls of the arteries this pushing creates pressure besides There are 2 types of BP measures Systolic BP (SBP) is the pressure in the arteries when the heart beats. On other hand the diastolic (DBP) is the pressure in the arteries when

the heart rests ,normal SBP is less than 120 millimeters of mercury (mm Hg), and DBP is less than 80 mm Hg, together described as 120/80 mm Hg (Desai, 2020). Furthermore According to Beevers *et al*. (2011), the majority of blood pressure monitoring devices rely on occluding the artery of an extremity, such as the arm, wrist, finger, or leg. Furthermore, BP disorder has been linked to a number of variables, including age, gender, blood group type, obesity, and stressors particularly those related to studying and exams. For psychologists, the subject of stress in college students is crucial (Pledge et al., 1998). Additionally, there are different ways that clinicians define high blood pressure (hypertension) among BP disorders. For example, some guidelines define high blood pressure as continuously exceeding 130/80 mm Hg, while others suggest exceeding 140/90 mm Hg (Desai, 2020). One of the main health issues with hypertension is that it has no known cure (Ghosh and Bandyopadhyay, 2007, Jafar *et al.,* 2006, Low *et al.,* 2009, Tassaduqe *et al.,* 2004). Conversely Lower BP (Hypotension): Most experts would consider SBP ≤90 mmHg and/or DBP ≤60 mmHg as hypotension (Kennelly and Collins, 2012). In the absence of a commonly definition of low BP geriatric studies defined BP <120/75 mm Hg as hypotensive (Barrett- Connor and Palinkas, 1994, Gilmore *et al.,* 1995, Jorm, 2001, Stroup –Benham *et al.,* 2000). Furthermore, a number of studies have demonstrated that aging reduces heart rate variability (HRV), indicating that age and gender have a significant impact on the risk of cardiovascular disease (Hellman and Stacy, 1976, Schwartz, 1991) .BP increases with age Both systolic and diastolic (Children, 1977, Roberts and Maurer, 1977), and in addition the sex effect on BP between ages 16 and 40 years, SBP of males was higher than that of females, whereas above age 60, females had higher systolic blood pressures.(Johnson *et al.,* 1965). Likewise Oparil and Miller (2005) results found that during the first six years of adulthood, women's SBP levels are lower than men's, but after the sixth, the opposite occurs. Furthermore, regardless of age, DBP is generally slightly lower in women than in men. Women are less likely than men to have hypertension in their early adult years, but after the age of fifty, women's incidence of hypertension rises more quickly. Additionally, a significant percentage of college students experience stress from student life, which may be a factor in the high rates of illness, risk-taking, and depression that are observed among student populations worldwide (Pledge *et al.,* 1998). Stressors that frequently surface in student studies include worries about grades and other issues (Furr *et al.,* 2001). Additionally the different blood groups have been shown to be particularly associated with different diseases (Aird *et al.,* 1953, Klein and Anstee, 2014). According to Chandra and Gupta (2012) the type of blood group effects on BP and hypertension moreover B blood group seen more in the hypertension and obesity followed by blood group O, A and AB observed that the B blood group seen more in hypertension. This study was aimed to study the

relationship between PB levels and the gender, blood group type and study stress. It also aimed to investigate the relationship between BP disorders and the exam stress.

Materials and Methods:

The study was conducted on a random sample of 40 students from Al-Mahara Higher Institute for Health and Administrative Sciences in Al-Bayda city / Libya in the period from 9-5-2023 to 30-5-2023. After obtaining consent from the students, the questionnaire was filled out and the BP of the students was measured by using Mercury sphygmomanometer. BP was measured twice: The first time during a normal school day. And the second time during the end-of-semester exams. **Statistical analysis** was performed using Minitab software two samples T test.

Results:

The results of study which was conducted on a random sample 40 students from Al-Mahara Higher Institute in the period from 9-5-2023 to 30-5- 2023 and with an air temperature range between 11 - 30 °C , to find out how BP is related to several factors (Gender , ABO and study stress). The average age ranges between 17 and 38 years old. The total number of the sample was 40 male and female students, divided into 12 males and 28 females. however the result show 23 students had a drop in BP levels on the exam day compared to their BP measurement during the normal school day conversely 16 students had a rise in BP and One student had no change in BP measurement between the normal school day and the exam day. Moreover the distribution of blood groups was as follows Table 1. The individuals with blood group (A+) had the highest number reaching 12 compared to the rest of the blood groups. The (O+) group recorded the second highest rate, reaching 11, and (B+) group recorded 10, while the (AB+) group recorded 4 , the lowest number of the random sample groups was O- recorded only 3, were (A-, B-, and AB-) groups not recorded any ratio.

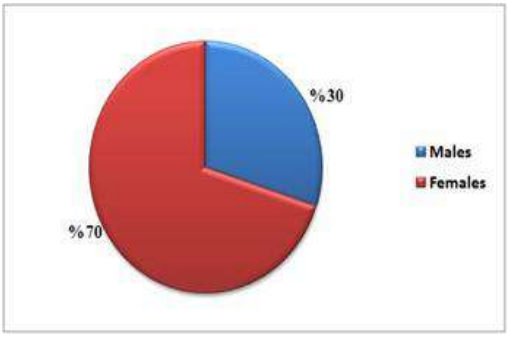

Figure 1. The percentage of males and females were recorded in the study**.**

Table 1. Shows the percentage and numbers of blood groups were recorded in the study.

Blood group	Number	Percentage
A+	12	30%
O+	11	27.5%
B+	10	25%
AB+	4	10%
O-	3	7.5%
Total 40		

PB differences between the normal school day and the exam day:

The highest rate of BP recorded on the normal study day was (139/89 Mm Hg) and (120/100 Mm Hg), on the day of exam was (124/82 Mm Hg), conversely the lowest BP rate recorded in the normal school day was (100/72 Mm Hg) and on the exam day was (95/60 Mm Hg). Twenty eight students recorded an increase in the BP rate on the exam day compared to the normal school day, and twelve others recorded a decrease in BP on the exam day. One student had no change in BP measurement. Table 2. represented the differences in PB between the normal study day and the exam day, there are non-significant (P>0.05) decrease in BP both SBP /DBP between normal day and exam day, Meanwhile were recorded personal decreases in BP like (139/89 Mm Hg) in normal day and (123/82 Mm Hg) in exam day, also there are increases recorded (112/79 Mm Hg) in normal day and (124/86 Mm Hg) in exam day.

Table 2. Illustrate the mean values of BP in normal study day compared to exam day.

	Normal day (Mean ±SEM)	Exam day (Mean ±SEM)
SBP mm Hg	117.4± 1.7a	114.8± 1.2a
P=0.221		
DBP mm Hg	79.31± 1.2a	78.67± 0.90a
P=0.66		

Values are expressed as means ± SEM; n = 40

PB differences between males and females:

The difference in PB between genders (female and male) were summarized in table 3. shows there significant (P<0.05) decreased in BP (SBP/DBP) between males and females in normal study day .And non-significant (P>0.05) differences in exam day. The percentage of male smokers reached 58.3% of the total males registered in the study and 17.5% of the total sample.

PB differences between ABO:

Table 4. shows the difference between the means of different blood groups and the result shown there non-significant decrease (P>0.05) in BP measurement in normal study compare to exam day in A+ ,B+ and AB+ blood groups and non-significant increase in BP in exam day compared to normal

study day in O+ and O- blood groups, in addition A+ Blood group recorded the highest levels of BP in the normal study day. **Note: A+, B+ and AB+** recorded high levels of PB in normal study day while O- and O+ recoded lowest levels, in exam day the opposite happened the PB was arises.

Table 3. Shows the different in means of BP between males and females on normal study day and exam day.

BP	Males Mean ±SEM	Females Mean ±SEM
Normal day (DBP)	125±2.5[a]	114.71± 2.0[b]
P=0.004		
SBP	84±2.1[a]	77±1.4[b]
P=0.019		
Exam day (DBP)	115±1.8[a]	114±1.5[a]
P=0.815		
SBP	77.10±1.5[a]	79.21±1.1[a]
P=0.278		

Values are expressed as means ± SEM; n = 40.

Table 4. Illustrate the mean values of PB between different blood groups in normal day compared to exam day.

	Blood groups				
PB mm Hg	A+ Mean±SEM	B+ Mean±SEM	AB+ Mean±SEM	O+ Mean±SEM	O- Mean±SEM
Normal day SBP	119.2±3.6[a]	118.38±2.9[a]	116.8±3.8[a]	114±5.9[a]	114±2.6[a]
Exam day SBP	114.73±2.6[a]	116.51± 2[a]	112.91±2.5[a]	115.5±2.3[a]	118.3±5.2[a]
P value	P=0.327	P=0.327	P=0.410	P=0.828	P=0.534
Normal day DBP	77.25± 2.8[a]	82.50± 2.4[a]	78.42± 2.3[a]	81.67±1.5[a]	80.3±0.88[a]
Exam day DBP	78.27± 2.2[a]	80.45±1.1[a]	78± 1.5[a]	76.50± 3[a]	78.3±3.2[a]
P value	P=0.776	P=0.458	P=0.882	P= 0.197	P=0.606

Values are expressed as means ± sem; n = 40.

Discussion:

BP disturbances are very common, and measurements may vary depending on the psychological and physical condition, and may vary within a single day. Also study pressures effect on BP which include the pressure of studying, preparing for the exam, fear of failure and frustration after difficult exams. Among the results we obtained in our study there is a decrease in BP measurement on the day of the exam (114/78 mm Hg) compared to the normal school day (117/79 mm Hg) this agreement with (Hildrum *et al.*, 2007) who

found that low BP was associated with increase prevalence anxiety and depression. Also there an association of low DBP with depression (Barrett-Connor and Palinkas, 1994, Jorm, 2001, Paterniti *et al.*, 2000) ,as well as another studies found there association between low SBP and DBP and depression.(Alexander, 1939, Barrett- Connor and Palinkas, 1994, Markovitz *et al.*, 1993, Paterniti *et al.*, 2000, Pilgrim *et al.*, 1992, Rosengren *et al.*, 1993, Rutledge and Hogan, 2001, Stroup -Benham *et al.*, 2000, Wessely *et al.*, 1990). Moreover A variety of studies have documented hypotension as an associated feature in subjects with fatigue (Onrot *et al.*, 1986) anergia, and emotional lability (Bengtsson *et al.*, 1987). Furthermore Hughes (2005) results found that students with high fear of failure manifested lower BP responses to stress than those with low fear of failure. And a main effect for 'exam' was found. Before their exams, the students' blood pressure was noticeably higher than it was afterwards. However some research has suggested that gender differences might be important, with men demonstrating some physiological reactivity increments in response to background stress to a greater extent than women (Matthews *et al.*, 2001). The current study's findings demonstrated that there are non-significant differences in blood pressure between the sexes on exam days and a significant difference in blood pressure between them on regular study days. for men but not for women, this agreement with (Matthews et al., 2001). Male participants in the high stress group of their study showed significantly higher baseline DBP than did male participants in the low stress group. Although some studies have suggested that the mechanism responsible for BP differences between the sexes is not sufficiently understood, but may be attributed to androgens and female hormones, (Reckelhoff, 2001), Furthermore, data point to the possibility that female sex hormones might actually prevent a salt-induced rise in blood pressure by increasing sodium excretion via the kidneys (Dahl *et al.*, 1975). Additional research has demonstrated that ovarian hormones, which are partially to blame for premenopausal women's lower blood pressure and postmenopausal women's higher blood pressure, are the cause of the variations in blood pressure readings between males and females (Chen and Meng, 1991, Masubuchi *et al.*, 1982, Reckelhoff *et al.*, 1998, Reckelhoff *et al.*, 2000), furthermore these differences sex-associated in BP also may be due to changes in testicular hormones.(Dubey *et al.*, 2002) .In addition among the differences in BP between the sexes, there is a contributing factor to high BP, which is cigarette smoking for males , the active smokers can display BP values which vary widely according to a great number of individual, smoker is actively smoking, transiently sympathetic responses, which acutely raise BP levels.(Leone, 2011). On other hand According to Garrison *et al.* (1976) study there association between ABO phenotype and the risk factor of many diseases such as cardiovascular diseases (CV), this association may explain the differential susceptibility of ABO phenotypes to CV disease. Additionally

another study indicate that ABO blood type O appears to have a small but statistically significant effect on SBP (Nance *et al.*, 1965). Also ABO blood phenotype may be a genetic risk factor for cardiovascular disease, but the impact of ABO blood groups on Coronary artery diseases in hypertensive patients with different BP control levels has not been determined (Zhou *et al.*, 2017). In our study A+ recorded the highest level of BP in normal day followed by B+, O+,AB+ and O- this almost agreement with (Chandra and Gupta, 2012) Found that the B blood group was more susceptible to hypertension as compared to blood group O and A. Anees *et al.* (2007) results found the O+ blood type is the most common type of blood group around the world and in various ethnic groups and AB less common this agreement with our study were O blood group type both (O+ and O-) recorded the highest percentage among different blood groups. Also the distribution of blood types in the African race in United States of America arrangement as follow O, A,B and AB. (Adeyemo and Soboyejo, 2006).

In conclusion despite the small sample studied There relationship between BP and study stress , gender factors. From the results of our study it became clear that BP disorders are prevalent among all ages and are not limited to the elderly. Raising awareness of the importance of measuring pressure for all ages, especially for students during examination period . Also, attention should be paid to following a healthy lifestyle, avoiding smoking, and donating blood in the case of male students.

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The Potential Protective Effect of Sidr Honey on Some Hematological Changes Caused by Exposure to Cigarette Smoke on Male Albino Rats

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Abstract:

The present study investigated the negative impact of smoking (CS) on some blood parameters in adult male rats and the protective effect of the sidr honey. Twenty four individual were divided into four groups: Normal control (NC), H Group: received Sidr honey orally (100 mg/kg b.w./d.) for 4 weeks, CS group: exposed to cigarette smoke (5 times/d.) for 4 weeks and protective (P) group: received Sidr honey orally (100 mg/kg b.w./d.) for 2 weeks then treated with cigarette smoke for 4 weeks. The results indicated that treatment with cigarette smoke caused significantly decreased ($P<0.05$) in red blood cells (RBCs), haemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV) and platelets (PLT). While, it was a significant increase in white blood cells (WBCs) count compared to control and honey animals. On the other hand, p group showed a slight increase in the mean value of HG, HCT, MCV and PLT, but showed a significant positive decline in WBCs as compared with the CS group. This study indicates that treatment with Sidr honey caused somewhat of an improvement against CS-induced hematological changes in male albino rats.

Keywords: Cigarette smoke, Sidr honey, Hematological changes, rats.

Introduction:

Smoking is known to be a significant risk factor for cardiovascular disease, high blood pressure, infections, stroke, thrombosis, and respiratory disease (de Heens *et al*., 2009). Moreover, many harmful substances, especially free radicals such as superoxide anions, hydroxyl radicals, H2O2, and HOCL, present in smoke can damage cellular components, leading to serious inflammation, high of white blood cells (WBC), these ROS can damage lipids, proteins and DNA, thus changing the structure and function of cells (Marnett *et al*., 2003). Folic acid is an essential vitamin. Folate levels have been

hypothesized to be important in the pathophysiology of many diseases, including neonatal neural tube defects (Butterworth and Bendich, 1996). Smoking addicts have lower levels of folic acid in their blood serum, red blood cells, and respiratory tract (Heimburger *et al*., 1992; Piyathilake *et al*., 1994; Giles *et al*., 1998). Another study also provides evidence that lower folate levels associated with smoke exposure may be important in studies linking smoking to diseases such as breast cancer, colon cancer, and birth defects (Chao *et al*., 2000). Honey is a natural product with very complex chemical composition, it contains more than 180 substances (Bogdanov *et al*., 2008; AL-Waili *et al*., 2012), including, proteins, phenolic, phytochemicals, peroxidase, flavonoids, ascorbic acid minerals, moisture; sugars; enzymes; trace essential elements; vitamins as well as some flavonoids and phenolic acid (Martos *et al*., 2000; Cooper *et al*., 2002). And because, there are no scientific reports on the effectiveness of Libyan sidr honey to validate its traditional use on the cure and control of physiological changes in general. Therefore, the current study examined the positive effect of Sidr honey against hematological changes and stressors caused by exposure to smoking in male rats.

Materials and Methods:

Chemicals:

- Libyan Sidr honey. -Karelia red cigarettes.

Experimental animals:

24 adult male albino rats, 10 weeks old weighing 180-200 g were used. Rats were obtained from the animal house of the Zoology Department, Faculty Science, University of Omar Al-Mukhtar, El-Beyda, Libya.

Experimental design:

Rats were randomly assigned into four groups of 6 animals as follows: **Group 1:** The normal control group (NC), nothing was exposed. **Group 2:** (H) group, rats were given Sidr honey (100 mg/kg b.w./d.) **(Kolawole *et al*., 2015)** orally by gavage for 4 weeks. **Group 3:** (CS) group, were exposed cigarette smoke by a machine was designed locally in the Zoology Department, Faculty Science, University of Omar Al-Mukhtar, El-Beida, Libya (Figure1). As stated by Alshailabi *et al*. (2023). **Group 4:** (P) group, rats were given Sidr honey

(100mg/kg b.w./d.) orally for 2 weeks then treated with cigarette smoke after taking the Sidr honey for 4 weeks.

Figure 1: The glass box and smoking machine (Alshailabi *et al*., 2023).

Hematological analysis:

Blood samples will collect from the orbital sinus. EDTA will use an anticoagulant agent to determine the Red blood cells count (RBC), white blood cells count (WBC), platelet count (PLT), hematocrit value (HTC), hemoglobin level (HB) and mean corpuscular volume (MCV).

Statistical analysis:

Statistical analysis was performed using a computer run package (Graph Pad Prism 7). One way ANOVA followed by Tukey's HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean± standard error of the mean (SEM). P-value below 0.05 was considered to be statistically significant.

Results:

Red blood cells (RBCs) count:

RBC counts were obtainable in table (1) and figure (2). Statistically, a significant decrease ($P<0.05$) occurred in the mean value of RBCs in CS group (8.2±0.22) compare with NC group (9.09±0.17) and H group (9.98±0.18). On the other hand, no a significant changes between the mean value of p group (7.89±0.1) and CS group (8.2±0.22).

Haemoglobin (HB) level:

The mean values of the HB level were obtainable in table (1) and figure (3). A significant decrease ($P < 0.05$) occurred in HB level of CS group (19.71 ± 0.56) compare with NC group (22. 29±0.85) and H group (25±0.69). While, there is a slight improvement in p group (20±0.53) compared with CS group (19.71±0.56) with CS group in a percentage of increase (1.47%). As in all results, there were not significant changes between the mean values of H group (25±0.69) with NC group (22.29±0.85).

Hematocrit (HCT) level:

HCT levels were obtainable in table (1) and figure (4). Statistically, a significant decrease ($P < 0.05$) occurred in HCT level of CS group (28.16±1.14) compare with NC group (44.6±0.89) and H group (40.98±1.02). No significant differences between P group (30.26±0.46) and CS group (28.16±1.14). However, slight improvement was observed on the mean value of p group compared to CS group with a percentage of increase (7.46%).

Mean corpuscular volume (MCV) level:

The mean values of the MCV level were obtainable in table (1) and figure (5). There was a significant decrease ($P < 0.05$) occurred in MCV level of CS group (72.97±1.18) compare with NC group (94.31±0.79) in a percentage of decrease (-22.62%). On the other hand, a significant increase was recorded in P group (79.80±0.45) compared with CS group in a percentage of increase (8.56%).

White blood cells (WBCs) count:

WBC levels were obtainable in table (1) and graphically represented by the figure (6). It has shown very high ($P < 0.05$) in the CS group (16.8±0.73). Whilst, the P group showed a significant positive decline ($P<0.05$) in the mean value (10.89±0.28) as compared with CS group, and it was non-significant ($P<0.05$) between the H group (5.42±0.53) and the NC group (6.73±0.53).

Platelets (PLT) count:

The mean values of the PLT level were obtainable in table (1) and figure (7). Statistically, a significant decrease ($P<0.05$) occurred in PLT level of CS group (98.71±2.85) compared with NC group (147.43±4.17), While, P groups showed noticeable improvement in PLT count with percentage of increase (28.80%) compared with CS group.

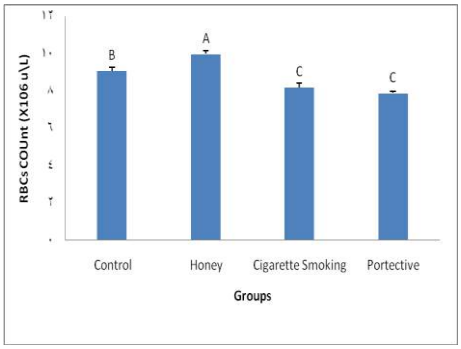

Figure 2: Averages of the mean value of the RBCs count ($X10^6$ µl/L).

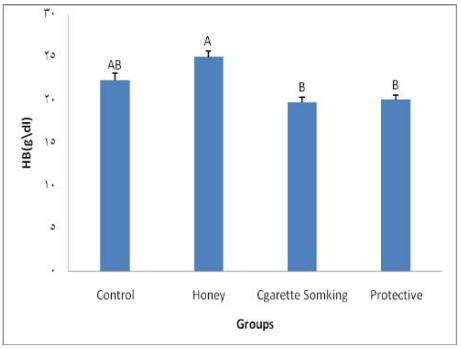

Figure 3: Averages of the mean value of the HG (g/d) level.

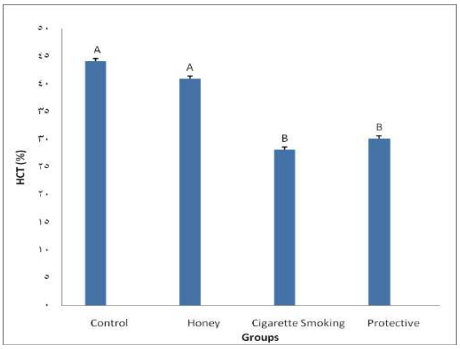

Figure 4: Averages of the mean value of HCT level (%).

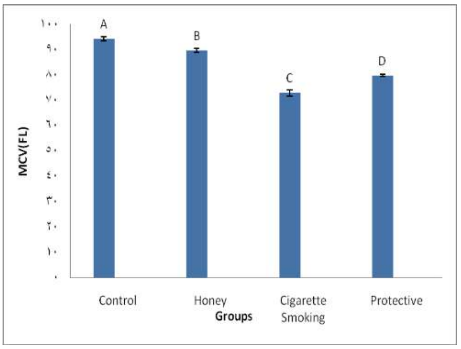

Figure 5: Averages of the mean value of MCV (FL) level.

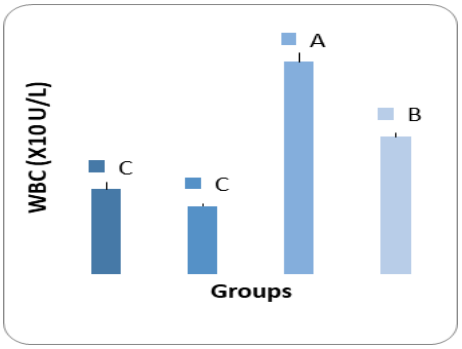

Figure 6: Averages of the mean value of WBCs count ($X10^3$ µl/L).

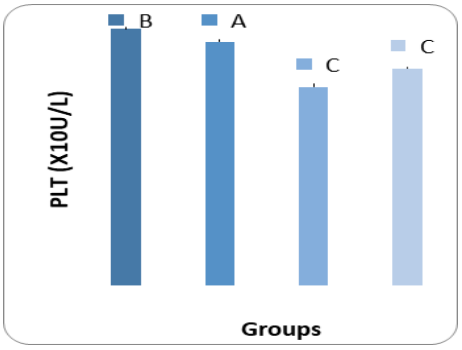

Figure 7: Averages of the mean value of PLT count ($X10^3$ µl/L).

Table 1: Average the mean values of RBC, HB, HCT, MCV, WBC and Platelets levels in control and experimental groups.

Parameter	NC	H	CS	P
RBC ($X10^6$ µl/L)	9.09 ± 0.172^{b}	9.98 ± 0.18^{a}	8.2 ± 0.22^{c}	7.89 ± 0.10^{c}
%Of change 1		9.79%	-9.79%	-13.20%
%Of change2				-37.8%
HB (g/dL)	22.29 ± 0.85^{ab}	25 ± 0.69^{a}	19.71 ± 0.56^{b}	20 ± 0.53^{b}
Of change1%		12.16%	-11.57%	-10.27%
Of change 2%				1.47%
HCT (%)	44.6 ± 0.89^{a}	40.98 ± 1.02^{a}	28.16 ± 1.14^{b}	30.26 ± 0.46^{b}
%Of change1		-8.11%	-36.9%	-32.26 %
%Of change 2				7.46%
MCV (FL)	94.31 ± 0.79^{a}	89.69 ± 0.81^{b}	72.97 ± 1.18^{c}	79.80 ± 0.45^{d}
%Of change1		-4.9%	-22.62%	-15.38%
%Of change2				8.56%
WBC ($X10^3$µl/L)	6.73 ± 0.53^{c}	5.42 ± 0.19^{c}	16.8 ± 0.73^{a}	10.89 ± 0.28^{b}
%Of change1		-19.5%	149.6%	61.81%
%Of change 2				-35.18%
Platelets($X10^3$µl/L)	147.43 ± 4.17^{b}	170.57 ± 3.97^{a}	98.71 ± 2.85^{c}	127.14 ± 2.48^{c}
%Of change1		15.69%	-33.04%	-13.8%
%Of change 2				28.80%

%of change1 = Percentage of change between NC and other groups.
%of change2 = Percentage of change between p group and CS group.

Discussion:

In the present study, results showed that cigarette smoke caused changes that vary between significant and non-significant ($P < 0.05$) in levels of RBC, HB, HCT, MCV PLT and WBC) compared to control and honey animals. This study demonstrates the rats exposed to CS for 4 weeks showed significant disturbances in the levels of hematological parameters which included significant decrease in RBCs, HB, HCT, MCV and PLT. These results were supported by Sherwin and Gastwirth (1990); Siana *et al*. (1992); Sharif *et al*. (2014); Alfourti *et al*. (2021) who presented that CS showed a significant decrease in RBC, HB, HCT, MCV and PLT. Also, this study showed that CS caused a significant increase ($P<0.05$) in WBC count compared to control and honey animals. These results are in agreement with Noble and Penny (1975); Schwartz and Weiss (1994); Freedman *et al*. (1996); Blann *et al*. (1998); Al-Awadhi *et al*. (2008); Aula and Qadir (2013); El- Sawi *et al*. (2020); Alfourti *et al* . (2021). The reason for this may be due to Cigarette smoke has a toxic effect on the bone marrow, and therefore it will have immune responses as a result of inflammation after smoking for many years, which can damage all blood cells leading decreases of RBCs and Hb (Salamzadeh, 2004). Cigarette

smoke has 4000 substances among which CO and tars are the main toxic substances. CO can diffuse rapidly across alveolar capillaries, bind firmly to Hb, which can cause a high risk factor for cardiovascular diseases, increase the risk of intravascular clotting, coronary vascular resistance, and decreased coronary blood flow in RBC, PLT, and a predisposition to thrombosis (Richter *et al*., 2008 ; Zhong *et al*., 2008; Ravala and Paula, 2010; Soldin *et al*., 2011), thus the increasing risk of cardiac disease in smokers may be associated with high fibrinogen levels through arterial wall infiltration and effects on blood viscosity, platelet aggregation, and fibrin formation (Wannamethee *et al*., 2005). On the other hand, this study demonstrates the rats in the p group showed a slight increase in the mean value of HG, HCT, MCV and PLT, but showed a significant positive decline in WBCs as compared with the CS group, which is in agreement with other studies (Yao *et al*., 2004; Michalkiewicz *et al*., 2008) who indicated that the sider honey which contains moisture, sugars such as glucose and fructose, enzymes such as catalase and glutathione reductase, trace essential elements such as iron, copper, zinc, and calcium, vitamins such as vitamin A, C, and E, and some flavonoids and phenolic acids, which leading increases RBC, HB And HCT. However, Sidr Honey has been suggested to protect against lipid peroxidation by reducing the production of lipid hydroperoxides, which leading decreases to inflammation, WBC (Alvarez-Suarez *et al*., 2012; Hegazi *et al*., 2017). Folic acid is an essential B vitamin. It is found naturally in Sidr honey and is important in DNA repair. Blood folate levels reflect short-term exposure, while red blood cell levels reflect long-term exposure (Snow, 1999).

Conclusion:

In conclusion, these present findings identify that exposure to cigarette smoke leads to imbalances in the normal range of blood parameters. Moreover, treatment with Sidr honey caused somewhat of an improvement hematological changes in male albino rats.

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Assessment of Heavy Metals in Water of Karaçomak River (Kastamonu), Turkey

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Abstract:

To ascertain the seasonal accumulation of heavy metals, eight stations along the Karaçomak River were chosen for this study, and water samples were collected. Using an ICP-OES instrument, the concentrations of Pb, Cr, Ni, Zn, Mn, and Cu were measured. Sewage, industrial effluent, fertilizers, pesticides, and herbicides were identified as the sources of heavy metal pollution in the Karaçomak River. Our study's objective was to look into how variations in sampling period and station affect the amounts of metals in river water. It was observed that the metal concentration means per annum were in the water Mn> Cu> Zn> Pb> Ni> Cr. Seasonal highest values of heavy metals were observed as follows; Pb (14.018 µg/L) and Ni (2.469 µg/L) in summer, Cr (2.079 µg/L), Zn (20.789 µg/L), Mn (58.296 µg/L) and Cu (19.072 µg/L) in winter. In general, the values of heavy metals in the water recorded in this study did not exceed permissible limits for international standards of drinking water according to the reference values except lead metal that exceeded permissible limits. The findings of this study may be useful for further biomonitoring studies.

Keywords: Seasonal Variability, Karaçomak creek, heavy metal, water.

Introduction:

One of the most significant problems the world is currently dealing with is the existence of pollutants in the environment as a result of human and natural activity. As a result of the expansion of the economy and industry, as well as the creation of various chemical groups and compounds that pose risks to the environment and human health (Shanbehzadeh *et al.*, 2014). The metals in the environment are usually found as a result of natural activities, such as erosion and rocks weathering, volcanic activity, earthquakes and floods (Espinoza-Quinones *et al.*, 2005). Also, metals enter the environment as a result of

human activities by municipal, industrial and agricultural waste (Shanbehzadeh *et al.,* 2014). Heavy metal pollution is a global issue that has caused concern in the majority of the world's cities, particularly the major industrial cities. The problem dates back to the nineteenth century, when the industrial revolution got underway (Nriagu, 1979). Heavy metals estimation studies have been the main environmental focus in recent decades, particularly in rivers, lakes, sediments and aquatic biota (Özmen *et al.,* 2004; Begum *et al.,* 2005; Fernandes *et al.,* 2008; Öztürk *et al.,* 2008; Poté *et al.,* 2008; Praveena *et al.,* 2008). The pollution of the aquatic environment by heavy metals is a global problem of the biggest environmental problems that cause global concern because heavy metals are not biodegradable and have negative and toxic effects on flora and fauna organisms (MacFarlane and Burchett, 2000; Censi *et al.,* 2006; Oronsaye *et al.,* 2010). Heavy metals enter the aquatic environment such as rivers, streams, lakes and ponds from different sources such as erosion and rock weathering, which directly touch the surface water in addition to discharge contaminated fluids directly into these water bodies without treatment (Eaton *et al.,* 2005; Osman and Kloas, 2010). Water resources are among the most important natural resources, especially rivers water, which meet the human, animal and industrial needs of water, which require the essential need to protect them from the pollution by municipal, agricultural and industrial waste that cause water pollution with biological and chemical inputs, including heavy metals (Shanbehzadeh *et al.,* 2014). Despite some metals are important micronutrients, but then their high concentrations in the food chain system lead to negative effects that can cause toxicity and endanger the aquatic ecosystem and its users (Prabu, 2009; Kane *et al.,* 2012). Most of the heavy metals find their way into the aqueous phase and where the deposition of heavy metals in rivers and lakes ecosystems, which are the main basin of heavy metals (Samecka-Cymerman and Kempers, 2001; Désy *et al.,* 2002; Morillo *et al.,* 2002). Environmental contamination by heavy metals can cause what is known as bioaccumulation and biomagnifications, thereby increasing the toxic effect on aquatic ecosystems (Ward, 1995). Aquatic organisms have the ability to pick up small effects of heavy metals from the water in which they live and thus the concentration of these metals and storage in their bodies (Censi *et al.,* 2006). Levels of heavy metals are generally monitored in the aquatic environment by measuring their concentrations in each of water, sediments, and organisms (Camusso *et al.,* 1995). The presence of heavy metals in aquatic environments has led to

serious apprehension about their impact on biota. Nutritional requirement of some metals such as copper, zinc vary substantially between species or elements, and optimum range of their concentration is low, but intense imbalances in their attribution due to exposure to high concentrations can cause even death for biota (Mohiuddin *et al.*, 2012). Heavy metals such as lead, cadmium, Arsenic show extremist toxicity even at trace concentrations (Nicolau *et al.*, 2006). Examining seasonal variations in Pb, Cr, Ni, Zn, Mn, Zn, and Cu concentrations as well as determining heavy metal contamination of Karaçomak River water were the goals of this study. These results are critical for both the development of sensible management strategies and for elucidating the river's current levels of heavy metal pollution.

Materials and Methods:

Study area

This study was conducted on samples taken from the river Karaçomak which passes through the center of Kastamonu city, it is a major source of drinking water for the region of Kastamonu, which rises above sea level about 904 m. It has a population of about 110,000 inhabitants. The water is collected by Karaçomak dam. The volume of the dam is 1.100.000 m^3, the height of the river bed is 49 m, the volume of the lake is 23,10 hm^3, and the water area is 1,43 km^2. It provides irrigation services to an area of 2.569 ha and provides 3 hm^3 of drinking water every year to the inhabitants of the Kastamonu city (URL-1). Figure (1) shows the map of the city of Kastamonu, indicating a river and dam Karaçomak as well as sampling sites.

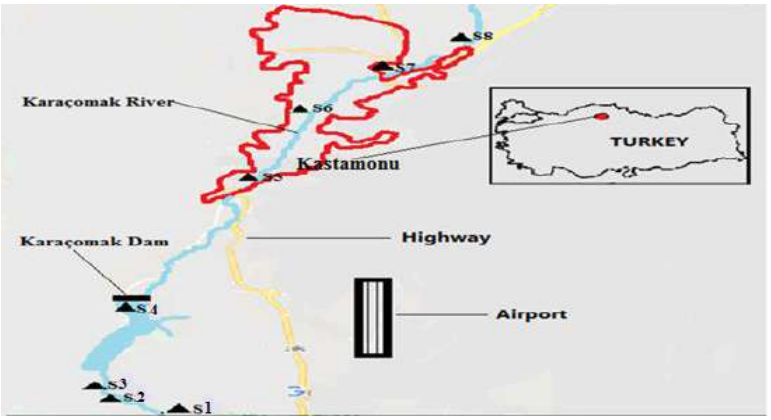

Figure 1: Map of the study location and sampling sites.

Sampling

In this study, eight sampling sites for surface sediment samples were selected along the shore. Coordinates were determined by Global Positioning System (GPS). The surface sediment samples were collected in October 2016 and February, May, July in 2017 for covering any seasonal differences that may occur during the study period.

Water samples preparation

All water samples from the sites were filtered by a 0.45 µm filter and then were taken 100 ml of water required for analysis and then transferred into acid cleaned 100 mL polypropylene bottles. It was added to each sample 6 ml of concentrated HNO_3 (Merck, 65%) then put them in the fridge at 4°C for preservation until preparation and analysis (Salem *et al.*, 2014).

Metal analysis

Standard stock solutions containing 1,000 µg/ml were prepared from nitrate salts of lead nitrate, chromium nitrate, nickel nitrate, zinc nitrate, manganese nitrate, and copper nitrate in 1% of HNO_3 into 1 L calibrated flasks. Diluted standard solutions were prepared from the standard stock solutions. The concentrations of Lead (Pb), Chromium (Cr), Nickel (Ni), Zinc (Zn), Manganese (Mn) and Copper (Cu) were estimated by using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) ((Duman *et al.*, 2007).

Statistical analysis

Statistical analysis was used through the program statistical package for social sciences (SPSS) version 24, to calculate some descriptive statistics such as means, standard deviation, maximum values and minimum values. As well the indicative statistics analysis was also examined through a test of significant values (p) which results of the research were considered significant if were the calculated significant values $p \leq 0.05$ (α was chosen to be 0.05). For comparison of means, ANOVA test and Post Hoc were done.

Results and Discussion:

Lead (Pb)

In (Table 1) statistically, the highest seasonal mean lead (Pb) of water samples from Karaçomak River was observed in the summer season (14.018±2.357

µg/L) and the lowest in the spring season (10.707±1.545 µg/L) and the annual mean lead (Pb) was found to be (12.546 µg/L). As high temperatures lead to increase evaporation and associated decrease in water level to increase the concentration of these metals in the aquatic environment and this is what they reached (Gbaruko and Friday, 2007). As high temperatures in summer lead to an increase in vital actives, as well as change the physical and chemical factors of the study stations, an important role in seasonal variation in concentrations of heavy metal in the aquatic environment may happen (Hatje *et al.*, 2003). At the level of sites (Figure 2), we found that the highest values recorded at the site (6) with an annual average concentration (15.388 µg/L). This is due to contaminating this site by sewage coming from residential communities located on the riverbank and corrosion of household plumbing systems as a result of the use of Polyvinyl chloride (PVC) pipes leading to increase the amount of lead in water due to the addition of lead nitrate during the process of manufacturing pipes to raise the hardness and resistance of these pipes as well as car wash position and special workshops for the replacement oils and maintenance of vehicles which are located on the riverbank near the study station (6). The low concentrations of heavy metal in the water of the Karaçomak River in some stations may be due to the tendency of these metal to accumulate in the bodies of Phytoplankton, plants and other aquatic organisms or may be due to the tendency of heavy metals to adsorption on the sediments or configure complexes with organic matter and studies have shown the ability of this Phytoplankton to remove heavy metals from the water body (Kwon & Lee, 2002). ANOVA and post hoc testing in (Table 1) show that there were significant differences with a statistical significance for the lead (Pb) with the other seasons ($p<0.05$) where the value of (Sig=.004). This indicates that there is a difference for the mean lead (Pb) between the four seasons, noting that there was no statistically significant differences between mean lead (Pb) between the seasons for each of fall and winter ($p>0.05$) where the value (Sig=.262) as a result of the rapprochement in the temperature of these seasons of each other resulting in rapprochement in the lead values between the two seasons which are affected by the increase and decrease in temperature. Also, fall and summer ($p>0.05$) where the value of (Sig=.354) and winter and spring ($p>0.05$) where the value of (Sig=0.082). In general, the values of lead recorded in this study were ranged (8.337-19.228 µg/L) which exceeded permissible limits for international standards of

drinking water for each of the WHO (10 µg/L) (WHO, 2017) and EU (10µg/L) (EU, 2011).

Chromium (Cr)

In (Table 1) statistically, the highest seasonal mean chromium (Cr) of water samples from Karaçomak River was observed in the winter season (2.079±0.574 µg/L) and the lowest in the summer season (1.919±1.710 µg/L). And the annual mean chromium (Cr) was found to be (2.011 µg/L). The increase in rates during the winter season (2.097±0.574 µg/L) may be due to the abundance of rain and snow water in this season, which carried contaminants to rivers, which are the final destination of these contaminants (Duman *et al.*, 2013). At the level of sites (Table 4.14) and (Figure 3), we found that the highest values recorded at the site (6) with an annual average concentration (3.33 µg/L). This is due to the contamination of this site by sewage coming from residential communities located on the riverbank as well as car wash position and special workshops for the replacement oils and maintenance of vehicles which use electroplating for their maintenance which is located on the riverbank near the study station (6). ANOVA and post hoc testing (Table 4.15) show that there were no significant differences with a statistical significance for the chromium (Cr) with the other seasons ($p>0.05$) where the value of (Sig=.996). This indicates that there is no difference for the mean chromium (Cr) between the four seasons, as a result of the lack of high concentrations of chromium metal in the study sites until there is an effect of temperature and then there are significant differences between seasons. In general, the chromium values recorded in this study were ranged (0.565-5.696 µg/L) where they did not exceed permissible limits for international standards of drinking water for each of the WHO (50 µg/L) (WHO, 2017) and EU (50µg/L) (EU, 2011).

Nickel (Ni)

In (Table 1) statistically, the highest seasonal mean Nickel (Ni) of water samples from Karaçomak River was observed in the summer season (2.469±2.176 µg/L) and the lowest in the spring season (1.073±1.016 µg/L). And the annual mean Nickel (Ni) was found to be (2.090 µg/L). The increase in rates during the summer (2.469±2.176 µg/L) may be due to an increase in temperature. As high temperatures lead and increase evaporation and associated decrease in water level to increase the concentration of these metals

in the aquatic environment and this is supported by Gbaruko and Friday (2007). As high temperatures in summer lead to an increase in vital actives, as well as changing the physical and chemical factors of the study stations, thus having an important role in seasonal variation in the concentrations of heavy metal in the aquatic environment (Hatje *et al.,* 2003). At the level of sites and (Figure 3), we found that the highest values recorded at site (6) with an annual average concentration (5.099 µg/L). This is attributed to the anthropogenic pollution sources by sewage coming from residential communities located on the riverbank and burning fossil fuels and old batteries wastes as well as car wash position and special workshops for the replacement oils and maintenance of vehicles which is located on the riverbank near the study station (6) ANOVA and post hoc testing in (Table 4.19) show that there were no significant differences with a statistical significance for the Nickel (Ni) with the other seasons ($p>0.05$) where the value of (Sig=.239). This indicates that there was no difference for the mean Nickel (Ni) between the four seasons, as a result of the lack of high concentrations of Nickel metal in the study sites until there was effect to temperature and then there were significant differences between seasons. In general, the Nickel values recorded in this study were ranged (0.150-7.627 µg/L) where they did not exceed permissible limits for international standards of drinking water for each of the WHO (70 µg /L) (WHO, 2017) and EU (20 µg/L) (EU, 2011).

Zinc (Zn)

In (Table 1) statistically, the highest seasonal mean zinc (Zn) of water samples from Karaçomak River was observed in the winter season (20.789±11.84974 µg/L) and the lowest in the fall season (11.450±4.860 µg/L). And the annual mean zinc (Zn) was found to be (14.324 µg/L). The increase in rates during the winter season (20.789±11.849 µg/L) may be due to the abundance of rain and snow water in this season, which carried contaminants to rivers, which are the final destination of these contaminants (Duman *et al.,* 2013). At the level of sites (Figure 5), we found that the highest values recorded at the site (7) with an annual average concentration (27.612µg/L). It may be due to the agricultural runoff, domestic activities, wastewater discharges, on the riverbank which is located on near the study station (7). ANOVA and post hoc testing (Table 4.23) show that there were no significant differences with a statistical significance for the zinc (Zn) with the other seasons ($p>0.05$) where the value of (Sig=0.330). This indicates that there was no difference for the

mean zinc (Zn) between the four seasons, as a result of the lack of high concentrations of Zinc metal in the study sites until there is effect to temperature and then there are significant differences between seasons. In general, the Zinc values recorded in this study were ranged (0.538-52.639 µg/L) where they did not exceed permissible limits for international standards of drinking water for each of the WHO (3000 µg/L) (WHO, 2017) and EU (30-2000 µg/L) (EU, 2011).

Manganese (Mn)

In (Table 1) statistically, the highest seasonal mean Manganese (Mn) of water samples from Karaçomak River was observed in the winter season (58.296±60.717 µg/L) and the lowest in the spring season (13.547±23.038 µg/L). And the annual mean Manganese (Mn) was found to be (35.705 µg/L). The increase in rates it during the winter season (58.296±60.717 µg/L) may be due to the abundance of rain and snow water in this season, which carried contaminants to rivers, which are the final destination of these contaminants (Duman *et al.*, 2013). At the level of sites (Figure 6), we found that the highest values recorded at the site (6) with an annual average concentration (48.542 µg/L). This is attributed to the anthropogenic pollution sources by sewage coming from residential communities located on the riverbank and burning fossil fuels and industrial wastes such as wastes old batteries and the atmospheric inflow of dust which is located on the riverbank near the study station (6). We also found an increase in the concentration of manganese at the site (3) with an annual average concentration (47.856 µg/L) that may be due to dissolution of impending rocks as the rocks and soils directly exposed to surface water which is the largest natural sources (Osman and Kloas, 2010), and the cause may also be due to the discharge of agricultural waste containing fertilizers used by farmers near this site. ANOVA and post hoc testing (Table 1) show that there were no significant differences with a statistical significance for the Manganese (Mn) with the other seasons ($p>0.05$) where the value of (Sig=0.196). This indicates that there was no difference for the mean Manganese (Mn) between the four seasons, as a result of the lack of high concentrations of Manganese metal in the study sites until there is an effect of the temperature and then there are significant differences between seasons. In general, the Manganese values recorded in this study were ranged (0.491-178.382 µg/L) where they did not exceed permissible limits for international standards of drinking water for WHO (500 µg/L)

(WHO, 2017) and they exceed permissible limits for international standards of drinking water for EU (50 µg/L) (EU, 2011).

Copper (Cu)

In (Table 1) statistically, the highest seasonal mean Copper (Cu) of water samples from Karaçomak River was observed in the winter season (11.582±3.452 µg/L) and the lowest in the fall season (10.449±2.171 µg /L). And the annual mean Copper (Cu) was found to be (10.924 µg/L). The increase in rates it during the winter season (11.582±3.452 µg/L) may be due to the abundance of rain and snow water in this season, which carried contaminants to rivers, which are the final destination of these contaminants (Duman *et al.*, 2013). At the level of sites (Figure 7), we found that the highest values recorded at the site (1) with an annual average concentration (16.252 µg/L). This is attributed to the anthropogenic pollution sources caused by the discharge of agricultural wastes containing residues of fertilizers and pesticides containing copper compounds which are used by farmers near this site indicates that there was difference in the mean Copper (Cu) between the eight sites. ANOVA and post hoc testing (Table 1) show that there were no significant differences with a statistical significance for the Copper (Cu) with the other seasons ($p>0.05$) where the value of (Sig=0.868). This indicates that there was no difference for the mean Copper (Cu) between the four seasons, as a result of the lack of high concentrations of Copper metal in the study sites until there is an effect of the temperature and then there are significant differences between seasons. In general, the values Copper recorded in this study were ranged (5.811-19.072 µg/L) where they did not exceed permissible limits for international standards of drinking water for WHO (2000 µg/L) (WHO, 2017) and EU (2000 µg/L) (EU, 2011).

Table 1: Seasonal mean concentrations of heavy metals (µg/L) in water samples together with standard deviation values.

	Pb	Cr	Ni	Zn	Mn	Cu
Fall (October)	13.216^{b}±1.357	1.973^{a}±1.739	2.414^{a}±1.380	11.450^{a}±4.860	24.474^{a}± 21.125	10.449^{a}±2.171
Winter (February)	$12.243a^{b}$±1.339	2.079^{a}±0.574	2.403^{a}±1.487	20.789^{a}±11.849	58.296^{a}±60.717	11.582^{a}±3.452
Spring (May)	10.707^{a}±1.545	2.072^{a}±1.821	1.073^{a}±1.016	12.310^{a}±2.824	13.547^{a}±23.038	10.620^{a}±2.903
Summer (July)	14.018^{b}±2.357	1.919^{a}±1.710	2.469^{a}±2.176	12.747^{a}±18.272	46.504^{a}±46.999	11.043^{a}±2.993
Annual	12.546	2.011	2.090	14.324	35.705	10.924

For a given metal, mean concentrations followed by the same letter are not significantly different. ($p<0.05$).

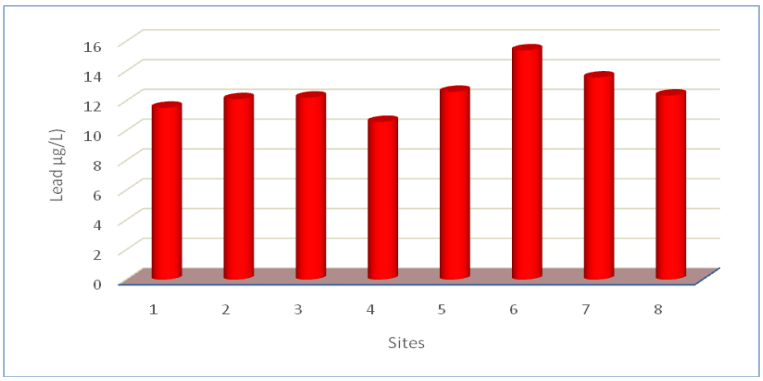

Figure 2: The relationship between sites and Lead (µg/L) during the annual mean

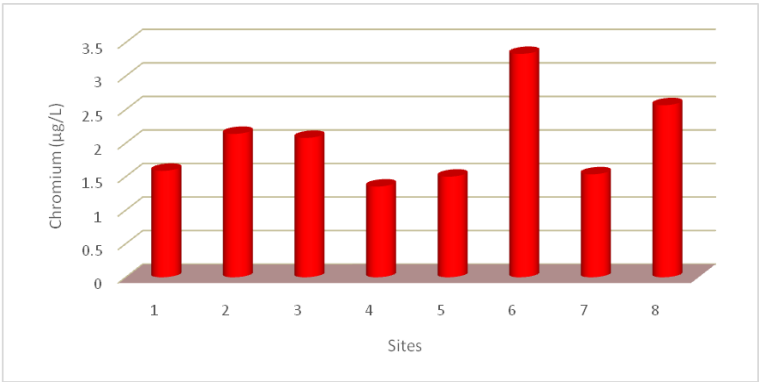

Figure 3: The relationship between sites and Chromium (µg/L) during the annual mean.

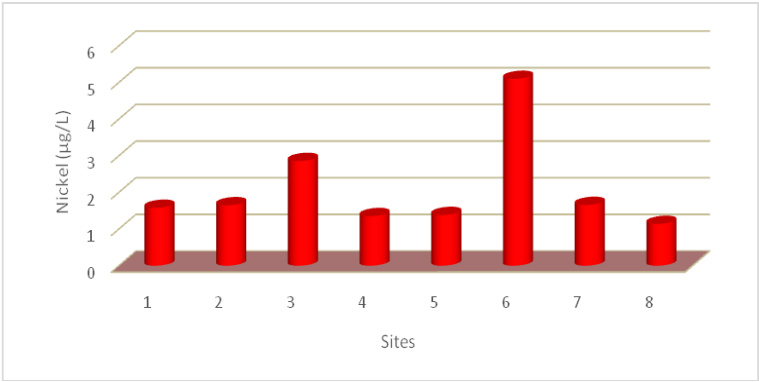

Figure 4: The relationship between sites and Nickel (µg/L) during the annual mean.

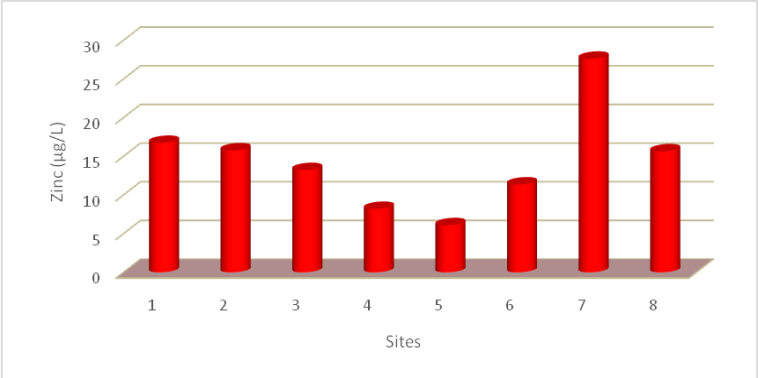

Figure 5: The relationship between sites and Zinc (µg/L) during the annual mean.

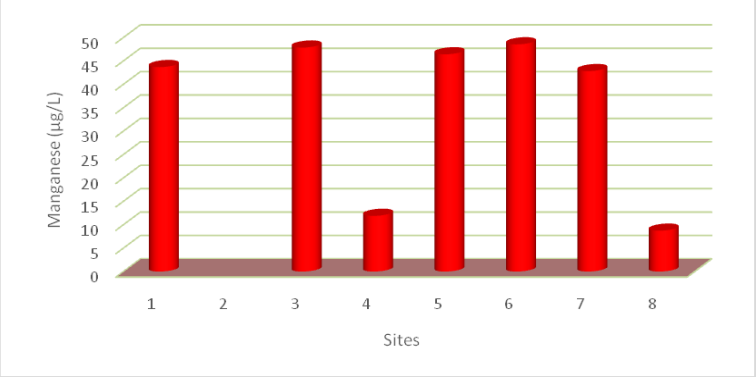

Figure 6: The relationship between sites and Manganese (µg/L) during the annual mean.

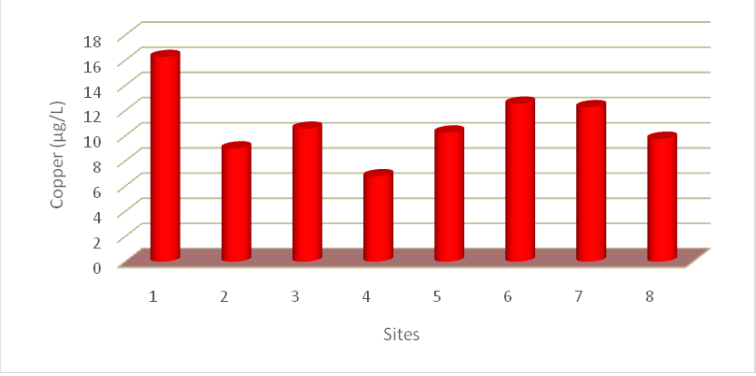

Figure 7: The relationship between sites and Copper (µg/L) during the annual mean.

Conclusion:

It can be concluded that the sediment of the Karaçomak River has a minor Ni and Cu contamination. It is evident that if appropriate measures are not taken,

the concentrations of metals in the water will surpass the critical levels. This study is the first to be conducted to determine metal accumulation in the sediment samples of Karaçomak River in spatial and temporal aspects. The study's findings suggest that, in biomonitoring investigations, the sampling period matters. Aquatic ecosystems can be impacted by multiple metal types that come from a single source. It is recognized that this case poses a significant challenge to heavy metal removal techniques. The findings of this study may be useful for future biomonitoring studies.

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Effect of Aqueous and Alcoholic Extracts of Frankincense Plant on the Growth of Bacteria

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Abstract:

This study is based on the evaluation of the antimicrobial activity of aqueous and alcoholic extracts of normal frankincense and *Boswellia sacra* against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Salmonella enterica*, and different concentrations of extracts (25%, 50%, 75% and 100%) were used in an agar well diffusion method. The results indicated that there were significant differences between the ethanolic extract of normal frankincense and *Boswellia sacra* (p 0.005) for *E. coli*, (p 0.023) for *S. enterica*, and (p 0.004) for *S. aureus*. However, there were no significant differences between the ethanolic extract of normal frankincense and *Boswellia sacra* used against *S. pyogenes*. Moreover, the current results showed that there are no statistically significant differences between the effect of the aqueous extract of normal frankincense and *Boswellia sacra* on the bacteria species used in the study.

Keywords: frankincense, *Boswellia sacra*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli, Salmonella enterica*.

Introduction:

The health of people is significantly impacted by the medicinal plant. The resin used to make frankincense, a plant used in medicine, is extracted from trees in the *Boswellia* genus, family Burseraceae (Akpanabiatu, Umoh, Udosen, Udoh, & Edet, 2005). Elkichaoi, El-Hindi, Mosleh, & Elbashiti (2015) state that frankincense, an aromatic resin, is derived from dry fluids (also called Olibannum) that are obtained from *Boswellia* trees, particularly the Boswellia sacra. According to Dominicic et al. (2018), this material typically contains 5-9% essential oil, 65-85% alcohol-soluble resin, and long-lasting water-soluble gum (polysaccharide fraction). (Dominic *et al*., 2018). Frankincense is a popular folk remedy with several health benefits. In addition to having a significant amount of natural collagen, which is good for the body

and hair and helps with weight loss, it is also used as medicine and the treatment of many diseases, including tumors, ulcers, and chest diseases like coughing, asthma, and distressed breathing. It is also used as a tonic for the heart (Aldory, Ali, & Sultan, 2018; Bonjar, 2004). Frankincense contains cortisone of tall quality and much superior viability than manufactured cortisone, as considers have affirmed that gum cortisone has no side impacts, not at all like manufactured cortisone (Zerrouki *et al*., 2021). Furthermore, compared to the negative effects of some antibiotics used by humans and their expensive cost, this natural source is inexpensive and has very little side effects (Hamidpour, Hamidpour, Shahlari, & Hamidpour, 2015). Boswellia is a medium-to-large tree up to 18 m in height and 2.4 m in circumference. The bark is greyish green with outer flaking thin layers and a thick and leaves are alternate. There are many species and varieties of frankincense trees, in East Africa and China, India, Somalia, Sudan, yamen, and southern Egypt (Maloney, 1997). The variations in frankincense resin species are influenced by the soil and environment. Many Middle Eastern countries' economies rely heavily on frankincense trees (Schmiech *et al*., 2019). Frankincense resin is obtained from incisions made in the trunks of the trees to create exuded gum, which appears as resin that resembles milk. The resin hardens into orange-brown (Orwa, Mutua, Kindt, Jamnadass, & Simons, 2009). Medicinal plants are used as alternative antimicrobial sources due to the pathogenic microorganisms' recurring resistance to antibiotics as well as the side effects that medications present. Plants produce secondary Metabolites (phytochemicals), which have shown antibacterial activity when employed alone or as boosters for other antibacterial agents. In order to treat germs resistant to traditional antibiotics, phytochemicals frequently work through alternative methods (Abreu, McBain, & Simoes, 2012).

Materials and Methods:

Sample collection: Frankincense samples were obtained from Attar Shuaib.

Microorganisms: The extracts were tested against pathogenic bacteria as follows bacterial strains:

Gram-positive bacteria: (*Staphylococcus aureus* and *Streptococcus pyogenes*) were used and obtained from El-Beida Hospital.

Gram-negative bacteria: (*Escherichia coli*, and *Salmonella enterica*) Obtained from El-Beida Hospital.

Preparation extract:

Plant extracts were prepared by steeping method an appropriate 10 g of the resin of Frankincense was pulverized and soaked in 95 % ethanol. After 24 hours the filtered extract and the ethanol was evaporated in rotary equipment at 50°c. and 10g 0f Frankincense pulverized and soaked in distilled water after 24 hours The filtered extract was evaporated in rotary equipment at 70°C (Yassin *et al*., 2013). The determination of the concentration was done with different dose levels of (25, 50, 75, and 100 mg/ml) of extract. The procedure was repeated on all the test organisms (Orwa *et al.*, 2009).

Agar Well Diffusion Methods:

The agar well diffusion method was used to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed [sterile cotton swabs] and respective bacteria wells were made in each of these plates using a sterile borer. About 100 l of various extracts was added by sterile syringe into wells. Using a millimeter rule, the inhibition zone of bacterial pathogens was evaluated after the plates were incubated at 37°C for 18 to 24 hours as described by (Mahmoudi *et al*., 2011).

Results:

The results of the antimicrobial activity by diffusion against the tested microorganisms showed that for normal frankincense extract ethanol had the highest inhibition zone in *S. aureus* by aqueous extract of *Boswellia sacra* (20 mm) at 100 % concentration, and the lowest inhibition zone was (5 mm). In addition to *S. pyogenes*, the highest inhibition zone was achieved by the ethanolic extract of normal frankincense (15 mm) at a concentration of 100%, while the lowest inhibition zone was obtained by the ethanolic extract of normal frankincense (3 mm). As for *E. coli* bacteria, the highest inhibition zone was by the aqueous extract of normal frankincense (11 mm), at a concentration of 100 % and the lowest inhibition zone was by the ethanolic extract of normal frankincense (2 mm) at a concentration 25 %. While, the highest inhibition zone in *S. enterica* (20 mm) at a concentration of 100 % and it was a lower Zone of inhibition in *S. enterica* for *Boswellia sacra* extract Aqueous (3 mm) at a concentration of 25 %. Also, *S. pyogenes* showed resistance against the aqueous extract of frankincense while *S. enterica*, *E. Coli*, and *S. aureus* showed resistance against the aqueous extract of normal

frankincense. Static significance of the differences between extracts of normal frankincense and *Boswellia sacra*. Differences between normal frankincense and *Boswellia sacra* were taken as $p < 0.05$. The results indicated that there were significant differences between the ethanolic extract of normal frankincense and *Boswellia sacra* (p 0.005) for *E. coli*, (p 0.023) for *S. enterica*, and (p 0.004) for *S. aureus*. However, there were no significant differences between the ethanolic extract of normal frankincense and *Boswellia sacra* used against *S. pyogenes*. Moreover, the current results showed that there are no statistically significant differences between the effect of the aqueous extract of normal frankincense and *Boswellia sacra* on the bacteria species used in the study.

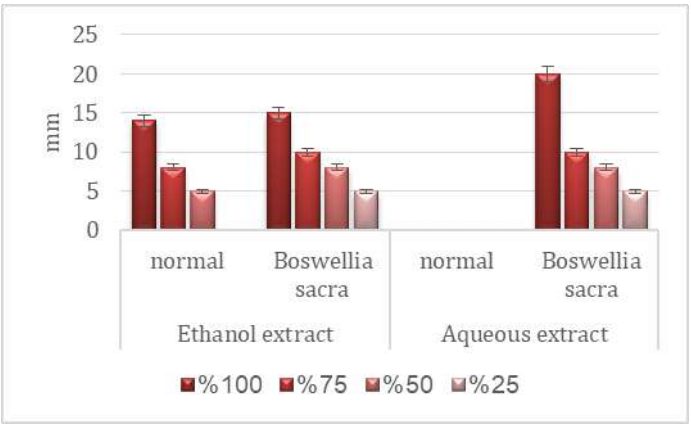

Figure1: The effect of aqueous and ethanol extract of frankincense on *S. aureus*

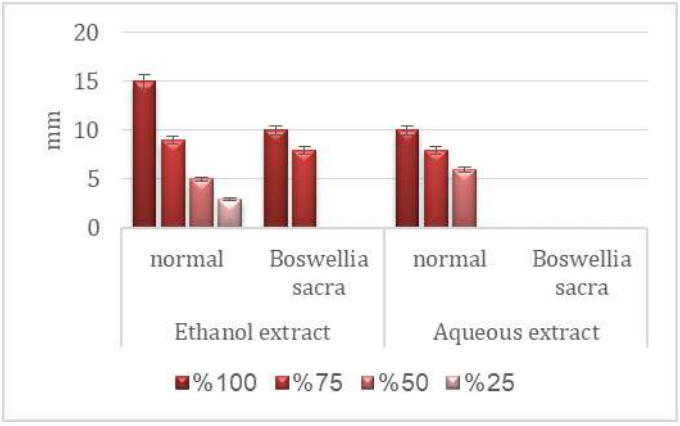

Fig.2: The effect of aqueous and ethanol extract of frankincense on *S. pyogenes*

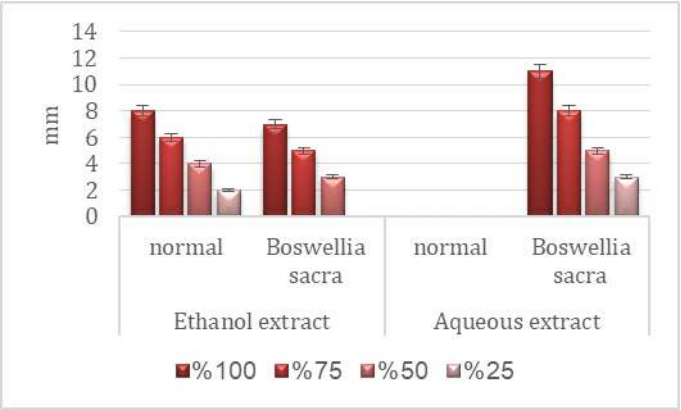

Fig.3: The effect of aqueous and ethanol extract of frankincense on *E. coli*

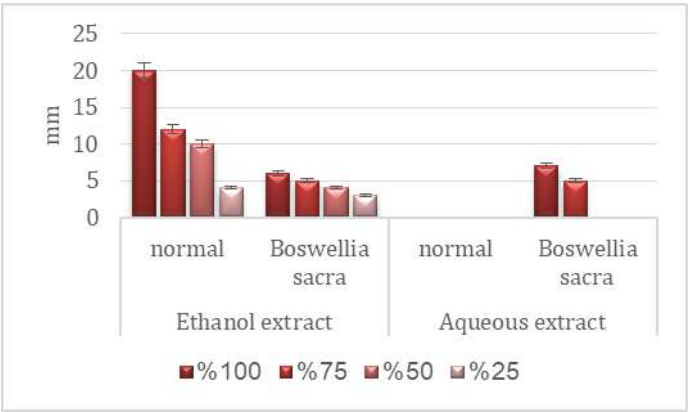

Fig.4: The effect of aqueous and ethanol extract of frankincense on *S. enterica*

Discussion:

This study was conducted to test the effect of the ethanol and aqueous extract of frankincense on bacteria, two types of positive bacteria *S. aureus, S. pyogenes***,** and two types of negative bacteria *E. coli***.** *S. enterica* was used, and the sensitivity of the extracts was tested by the disc diffusion method. There are a lot of different chemicals in the crud plant material that can inhibit bacteria by various mechanisms (Adwan & Mhanna, 2008). This study proved that frankincense extracts, whether with water or ethanol, were active against the used bacteria, and this is agree with a study (El Kichaoui, Abdelmoneim, Elbaba, & El Hindi, 2017). Despite the difference in the composition of the cell wall of negative and positive bacteria, the extracts used in this study affected both negative and positive bacteria as shown in the figure (1, 2, 3, 4).

This study disagrees with (Elbashiti, Elmanama, & Masad, 2011). As the result of this study was that the extracts affected the positive bacteria and did not affect the negative bacteria. This study also agreed with the study of (Mahmoudi *et al.*, 2011), which demonstrated the effect of frankincense extracts on bacteria S. *aureus*, *S. pyogenes*, and *E. coli*. Thus, this study may be useful in developing the pharmaceutical industry as a new antibacterial treatment from plant extracts.

Conclusion:

The results of the current study confirmed that the use of plant extracts such as frankincense may be an alternative solution to many problems of multidrug-resistant bacteria such as *S. aureus*, *S. pyogenes*, *E. coli*, and *S. enterica* confirmed by this study.

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Chromium (VI)-Induced Oxidative Stress and Biochemical Perturbations in Rabbits

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Abstract:

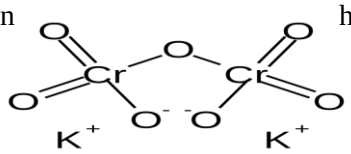
Pernicious impacts of chromium (VI) compounds are expanded influencing nearly all the organ frameworks in a wide assortment of creatures. Hence, the show ponder was carried out to decide the poisonous impact of chromium (VI) on certain biochemical parameters, lipid peroxidation, and protein exercises of male new zealand white rabbits. Six rabbits per bunch were relegated to one of two treatment bunches: 0 mg control and 5 mg Cr (VI)/kg BW, individually. Rabbits were orally managed their particular dosages each day for 12 weeks. Comes about gotten appeared that Cr (VI) essentially ($P < 0.05$) expanded the levels of free radicals and the exercises of AST and ALT were essentially expanded in plasma, whereas High mountain diminished. Chromium (VI) treatment caused a noteworthy diminish in plasma add up to protein (TP), and expanded cholesterol, glucose, urea, creatinine, and bilirubin concentrations.

Keywords: Rabbits; chromium (VI); enzyme activities; TBARS.

Introduction:

Anthropogenic exercises have ended up an essential inquire about concern due to their impacts on the environment and people (Lin *et al.,* 2021). Uncontrolled contamination due to anthropogenic exercises has altogether affected the air, causing biodiversity modification (Ke *et al.,* 2022). This wonder leads to the generation of pointless and destructive squanders. Anthropogenic exercises such as electroplating, mining, wood conservation, material, color and stainless steel fabricating and calfskin tanning produce harmful overwhelming metals, such as chromium within the hexavalent shape (Fig. 1) (Fernández *et al.,* 2018). Chromium is one of the major causes of intense infections in people due to its mutagenicity, harmfulness and carcinogenicity (Besharat *et al.,* 2021). It effectively penetrates the cell film with the help of the sulfate anion transport framework show within the film

and from that point diminished to other lower oxidation states, driving to collection in different organs and activating a multiplex of receptive oxygen species (ROS) and organ harm (Barhoma *et al.*, 2018).



Figuer1. Structure of potassium dichromate

In vivo tests in rodents report that ingested and (to a lesser degree) breathed in Cr (VI) can collect within the liver (Jin *et al*., 2016), illustrating the metal can reach the target tissue and assist supporting the organic credibility for Cr (VI)-induced liver poisonous quality. For chronic verbal introduction within the (National Toxicology Program, 2008) tissue dispersion consider (collection days 182 and 371, with a 2-day washout period), liver chromium concentrations were essentially raised at all dosage bunches compared to controls, demonstrating aggregation of chromium in this organ. A pharmacokinetic ponder by O'Flaherty and Radike (1991) illustrated that taking after inward breath or verbal presentation to about proportionate target ingested dosages of Cr (VI), verbal introduction brought about in liver concentrations that were 1-2 orders of size higher than those from inward breath introduction. As a result, the degree of hepatotoxicity would be anticipated to vary by course of presentation. An expansive body of robotic data exists to advise the potential hepatotoxicity of Cr (VI). In this manner, ponders which are more enlightening for inveterate human introduction were prioritized for assist examination and elucidation. These included mammalian thinks about that centered on introduction courses more significant to people (e.g., verbal and inward breath thinks about), as well as rehash measurements considers of longer lengths (≥28 days). Shorter term ponders utilizing verbal and inward breath courses of organization and in vitro considers in human cell lines moreover given knowledge into natural credibility and human significance of the watched instruments. Oral rehash measurements thinks about give back for oxidative stretch, mitochondrial harm, aggravation, and apoptosis as components of Cr (VI)-induced liver impacts. A 36-day dietary think about in male mice getting 1 and 4 mg/kg/$K_2Cr_2O_7$-day (0.35 and 1.41 mg/kg-d Cr[VI]) detailed noteworthy increments in

hepatic lipid peroxidation and other markers of ROS-related push (Jin *et al*., 2016), comparable to a 10-week gavage consider in rabbits getting 5 mg/kg-day (El-Demerdash *et al*., 2006). Rafael *et al*. (2007) depicted immunohistochemical prove for expanded expression of Caspase-3, a marker for apoptosis in Wistar rats uncovered to around 3 mg Cr (VI)/kg-day for 10 weeks. A 28-day think about in male rats accepting 30 mg/kg/$K_2Cr_2O_7$-day (10.6 mg/kg/d Cr [VI]) by gavage (Navya *et al.*, 2017), also detailed increments in lipid peroxidation and diminished Turf, CAT, and GST movement, concurrent with increments in serum markers of liver poisonous quality (ALT, AST, and High mountain) and histological changes within the liver. Cr (VI) could be a solid oxidant that's diminished to Cr (III) within the body, producing receptive oxygen species (ROS), which is hurtful to the tissues. Cr (VI) can cross cell layers. Past thinks about have appeared that dichromate, a hexavalent chromium compound, increments ROS concentration and causes lipid peroxidation and oxidative harm in hepatocytes and renal cells (Kotyzova *et al*., 2015). Besides, long-term presentation to expansive measurements of Cr (VI) decreases the serum level of apolipoprotein-A1 and increments fasting blood glucose, triglycerides, add up to cholesterol, and other apolipoproteins (Feng *et al*., 2018). The over discoveries propose that Cr (VI) presentation is related to glucose digestion system and oxidative stretch.

Materials and Methods:

Tested Compounds

In this consider the impact of chromium (VI) on biochemical lists, free radicals and protein exercises of male rabbits were examined. Chromium (VI) was brought from the chemistry department, Faculty of science, Omar Al-Mukhtar University (20 mg/ml body weight each day). Twelve male Unused Zealand white rabbits (6 months ancient) were separately housed in cages and weighed week by week all through 12 weeks' exploratory period.

Animals and Treatments

Twelve male rabbits weighing 1892±50.79g. Animals were housed 12 per cage and kept on commercial diet and tap water was provided ad libitum. All

animals received human care and our study complies with the instruction's guidelines. After 12 weeks of acclimation, animals were divided into two equal groups, 6 animals in each group. The first group was used as control. While, groups 2 was treated with $K_2Cr_2O_7$ daily at a dose of 5 mg/kg BW (El-Demerdash *et al*., 2006). Rabbits were orally administered their respective doses for three month.

Measured parameters

Plasma was obtained by centrifugation of samples at 860 xg for 20 min, and was stored at -20°C until used for measured blood parameters. Stored plasma samples were analyzed for total protein (TP) by the Biuret method according to Armstrong and Carr (1964). Albumin (A) concentration was determined by the method of Doumas *et al*., (1977). Plasma glucose, urea and creatinine concentrations were measured by the method of Trinder (1969), Patton and Crouch (1977) and Henry *et al*., (1974), respectively. Plasma total bilirubin was measured using the method of Pearlman and Lee (1974). Plasma cholesterol and triglycerides (TG) were determined according to the methods of Watson (1960) and Fossati and Principe (1982), respectively. High-density lipoprotein (HDL) was determined according to the methods of Warnick *et al*. (1983). Low-density lipoprotein (LDL) was determined by the calculation (cholesterol-(TG/5+HDL). The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of Reitman and Frankel (1975). Alkaline phosphatase (AlP; EC 3.1.3.1) activity was determined in plasma according to the method of (Principato *et al*., 1985). Plasma thiobarbituric acid-reactive substances (TBARS) were measured by the method of Tappel and Zalkin (1959).

Statistical Analysis:

The data obtained were expressed as mean ±SEM. The significant differences were assessed by one-way ANOVA and Tukey test. After the detection of the normal distribution of the data and appropriate P-values, less than 0.05 is considered significant.

Results:

Table 1. Results obtained showed that Cr (VI) significantly ($P < 0.05$) increased the levels of free radicals and the activities of AST and ALT were significantly increased in plasma, while AlP and HDL decreased. Chromium (VI) treatment caused a significant decrease in plasma total protein (TP), and

increased cholesterol, LDL, TG, glucose, urea, creatinine, and bilirubin concentrations.

Table 1. Changes in plasma total protein (TP), Albumin (A) , glucose, urea, creatinine, bilirubin, cholesterol, triglycerides (TG), High-density lipoprotein (HDL), Low-density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase (AlP), thiobarbituric acid-reactive substances (TBARS; nmol/ml) of male rats treated with chromium (VI).

Parameter	Experimental groups	
	Control	Cr(VI)
TP(g/dl)	7.11±0.112^{b}	6.33±0.168^{c}
AST(U/L)	42.22±0.768^{b}	47.82±2.230^{a}
ALT(U/L)	43.30±1.109^{b}	59.09±3.355^{a}
ALP (IU/L)	51.18±5.434^{a}	60.21±2.309^{a}
Albumin (mg/dl)	3.96±0.068^{bc}	3.69±0.156^{c}
Bilirubin (mg/dL)	1.45±0.027^{a}	1.70±0.038 ^{a}
Glucose (mg/dl)	115.40±0.306^{a}	122.27±1.551^{a}
Urea (mg/dl)	40.73±0.91^{b}	46.67±1.66^{a}
Creatinine (g/dl)	0.710±0.02^{b}	1.34±0.09^{a}
TBARS (nmol/ml)	2.673±0.025^{b}	3.073±0.081^{a}
Cholesterol (mg/dl)	113.34±1.78^{b}	120.81±1.704^{b}
TG (mg/dl)	62.37±1.48^{a}	44.53±1.77^{b}
HDL(mg/dl)	45.22±0.49^{b}	37.89±1.69^{a}
LDL (mg/dl)	64.39±0.80^{b}	67.46±1.33^{c}

Values are expressed as means±SE; n=6 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, $p<0.05$.

Discussion:

The display ponder appeared that Cr (VI) caused changes within the exercises of biochemical parameters in plasma. The increment in plasma AST and ALT exercises (Table 1) is in assention with the discoveries of (El-Demerdash *et al*., 2006). Serum transaminases (AST and ALT) and soluble phosphatase exhibited a common increment within the blood and liver tissue of Cr (VI) treated rats compared to control. The watched rise in proteins exercises in reaction to Cr (VI) organization is in assention with previous studied of Kim and Moon (1998). Chromium (VI) could be a noxious metal commonly utilized in mechanical field. It produces poisonous impacts within the liver and other diverse organs (Sánchez-Martín *et al*., 2015). This think about, demonstrated that Se enhanced Cr initiated hepatic harm. This was demonstrated by biochemical, quality expression, histopathological and morphometric ponders. A tall level of serum of chemicals alanine aminotransferase (ALT) and aspartate aminotransferase (AST) demonstrate liver harm, such as that due to viral hepatitis, as well as cardiac violation and muscle harm. Serum ALT catalyzes the conversion of alanine to pyruvate and

glutamate. Hence, serum ALT is more particular to the liver, and is hence distant better; a much better; a higher; a stronger; an improved" >a distant better parameter for recognizing liver harm (Williamson *et al*., 1996). Their increment within the current consider recommends that potassium dichromate may have produced responsive oxygen species, in this way oxidative push driving to hepatotoxicity and nephrotoxicity. This may be due to the impedance in their union or destitute liver work related to oxidative push (Soudani *et al*., 2013). Comparable comes about were watched by Zhu *et al*. (2014); Mehany *et al*. (2013); Mohamed and Saber (2011); Saha *et al*. (2017) and Krim *et al*. (2013) in rats treated with potassium dichromate. Within the current consider, Cr delivered a high critical increment within the serum level of liver chemicals (AST and ALT), typically in understanding with past ponders of Soudani *et al*. (2011); El-Demerdash e*t al.* (2006), who clarified this due to their discharge from the cytoplasm demonstrating harm of liver tissue. In expansion, other ponders credited this due to event of oxidative stretch handle and utilization of the liver glutathione (GSH), that's one of the most antioxidant defense components Gunaratnam and Grant (2008). Another think about clarified that, plasma concentration of ALT is higher than AST within the cytoplasm so it is uniquely raised than AST in cases of inflammations or diseases. But, in infiltrative infections that harm the cytoplasmic and mitochondrial layers, the AST is higher than ALT (Akila *et al*., 1998). The current consider appeared a factual noteworthy increment in plasma level of add up to bilirubin in Cr treated bunch (III) as compared with other bunches. This height of bilirubin level is considered a solid prove of irritated liver capacities, as detailed by El-Demerdash *et al.* (2006) who demonstrated the same results. Cr (VI) could be a poison and carcinogen (Kotyzov´a *et al*., 2015) that influences the dissemination of copper, press, manganese, and zinc in organs and tissues (Tune *et al*., 2012). Cr (VI) can apply its cytotoxicity on neuronal cells by means of enactment of the Akt/ERK/AMPK signaling pathway, which is basically interceded by ROS era (Fu *et al*., 2020). A few ponders have appeared that Cr (VI) causes lipid peroxidation and oxidative harm in hepatocytes (Balakrishnan *et al*., 2013; Patlolla *et al*., 2009). Our discoveries are reliable with past thinks about portraying liver work variations from the norm after presentation to Cr (VI) (Zhao *et al*., 2019; Soudani *et al*., 2013; Xueting *et al*., 2018). ROS and mitochondrion have an critical part in Cr (VI)-induced L02 hepatocyte damage (Zhang *et al*., 2019). Some studies recommended that Cr (VI) might

actuate MT within the pancreas, influencing islets' work and, in turn, driving to anomalous blood glucose levels (Solis-Heredia *et al*., 1999). The component of Cr (VI)-related metabolic clutter is still not well caught on. Within the display ponder, we investigated the impact of Cr (VI) on glucose/lipid digestion system. We found that Cr (VI) might cause glucose metabolic brokenness in rabbits, which was reflected by an increment of fasting blood glucose (FBG), impedance of glucose, and affront resistance. In expansion, past considers have appeared that Cr (VI) can initiate unusual greasy corrosive digestion system in chicken's liver, actuating hepatotoxicity, which appears to be controlled by glucose digestion system and lipid metabolic pathways (Luo *et al*., 2019). Besides, epidemiological considers appeared that Cr (VI) presentation might lead to critical changes in serum Triglyceride (TG), Fasting plasma glucose (FPG), Low-density lipoprotein cholesterol (LDL-C), Apolipoprotein AI (Apo AI), and lipoprotein a LP(a) levels (Feng *et al*., 2018). Cr (VI) has too been detailed to extend add up to lipids, cholesterol glucose levels in rabbits (El-Demerdash *et al*., 2006). Moreover, ponders have found that Cr (VI) can hoist FBG, TG, and LDL-c and diminish HDL-c levels in rats Soudani *et al.* (2013). In this consider, we found that Cr (VI) seem actuate the increment of TG and LDL-C and diminish HDL-C in vivo and in vitro. Generally, combined with past writing, the over discoveries recommended that Cr (VI) might initiate glucose/lipid metabolic clutter.

In conclusion, the comes about of the show ponder convincingly illustrated that potassium dichromate ($K_2Cr_2O_7$) introduction brought about in shifting degree of biochemical parameters in rabbits.

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Accumulation of heavy metals in agricultural soils from the use of pesticides (Study in the Green Mountain)

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Abstract

Since the soil's fertility and minerals are finite and diminish with time, the world's population growth poses a serious threat to food security. In order to meet the expanding population's need for food, agricultural output must be raised. Since oxidative environmental contaminants threaten the ecological balance and human health, a major environmental concern associated with the strong reliance on chemical fertilizers to increase food production is since they are so persistent and poisonous in the environment, heavy metals and pesticides are the top pollutants that endanger the natural world. This essay focuses on the detrimental effects of pesticides (fungicides, herbicides, and insecticides) and heavy metals (cadmium, lead, copper, and zinc) on three locations provided the samples from which the levels of heavy metals were determined. A trustworthy summary of the concentration of heavy metals, also known as metalloids, such as Cd (1.23, 0.09, 0.10 mg/kg), Cu (24.00, 17.33, 20.21 mg/kg), Pb (3.05, 15.84, 15.14 mg/kg), and Zn (52.34, 28.56, 40.26 mg/kg), is made possible by this special compilation of data. In this article, we suggest that human activity, which can be identified at a regional level, may be responsible for some high soil heavy metal concentrations (such as those of mercury and cadmium).

Keywords: Heavy Metals, pesticides, Environmental Pollutants, Human Health.

Introduction:

Salinization and pesticides have an impact on the AOM breakdown, GPP, and ER ecosystem functions. Furthermore, we conducted a comparative analysis

of several ecosystem management approaches, in which these characteristics yield organic matter originating from the breakdown of allochthonous organic matter (AOM) and photosynthesis, or from the degradation of aquatic biota (autochthonous organic matter). (Schäfer *et al.,* 2012; Knillmann, 2018). Pesticides can affect all kinds of species and are a significant stressor for freshwater ecosystems. (Volety *et al.,* 2008; Schäfer *et al.,* 2012). It has frequently been shown that heavy metal pollution of soil occurs in urban suburbs and industrial areas. (Ferri *et al.,* 2015; Sarwar *et al.,* 2017; Weissmannová and Pavlovský, 2017). The main way that heavy metals are exposed to humans is through their transfer from soil to plants and their subsequent intake. There have been numerous published research on heavy metals in soil-vegetable systems. (Motuzova *et al.,* 2014; Hu *et al.,* 2017; Sarwar *et al.,* 2017). Surface water and groundwater are eventually contaminated by heavy metal stormwater runoff events and heavy metals like Cu or Zn. To minimize transportation risks and evaluate toxicological exposure, it is crucial to forecast the mobilization and export of soil pollutants to aquatic environments. (Schäfer *et al.,* 2012; Tang *et al.,* 2019). Along with aging, the transformation of pollutants, including the speciation of metals and the degradation of organic pollutants, can affect the level of pollutant export from the topsoil. Aging and conversion of pollutants are in turn controlled by non-soil factors such as B. time between an application and a rainfall event (Huang *et al.,* 2015; Voltey *et al.,* 2008). Excessive accumulation of heavy metals in the soil may raise the possibility that dangerous agricultural products will enter the food chain and endanger human health. Because heavy metals are poisonous, have a limited ability to degrade, and can migrate, their accumulations endanger both human health and the natural environment. (Fernández *et al.,* 2018). Using GIS and PCA techniques, identify the real sources of soil heavy metal pollution emissions (roads, mines, and industrial centers), and then use multiple linear regression with suppression to estimate the extent of the pollution source landscapes' effects. (Yuanan *et al.,* 2020).

Materials and Methods:

Study area

Study design and sampling schedule

Three randomly selected agricultural locations within a one-kilometer radius were used for the current study, which was carried out in three of the agricultural fields in 2021. The Green Mountain GPS was used to record the

sampling sites' longitude and latitude. A cylindrical profile sampler was used to gather topsoil (15-30 cm) from each sampling site using the five sub-samples procedures. After being cleaned and kept in breathable paper bags, all of the samples were transported to the lab for the next procedure. All of the soil samples were air-dried in the lab for roughly ten days, then sieved through a 0.3 mm mesh and placed in sealed bags to be examined at a later time. Figure (1) displays the study region as well as the sampling sites' distribution.

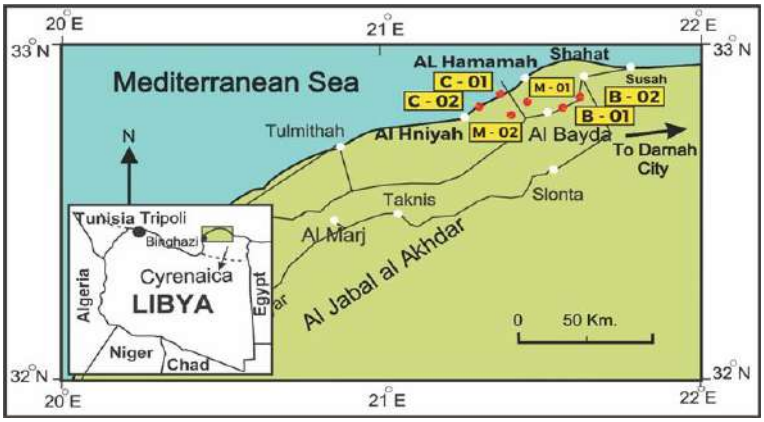

Figure 1: The distribution of the sampling sites are Shown

Sample Analysis

In accordance with the Chapman and Pratt method (Volety, 2008), 0.1 g of soil was combined with 1 mL HNO3, 3 mL HCl, 1 mL HF, and 0.5 mL HClO4 for every soil sample prior to digestion. Coupled Plasma-Optical Emission Spectrometry (ICPOES) was used to measure the total Cd, Cu, Pb, and Zn concentrations in the soil samples. Standard reference materials (GB Testing & Certification Co., Ltd.) were employed in the determination processes (Comero *et al.,* 2015). For the study of the SOM of the 100 samples, the potassium dichromate oxidation technique was utilized to measure the amounts was measured. Three duplicates of each sample were examined during analysis. Assessing the pH of the soil samples with a pH meter.

Evaluation of Pollution from Heavy Metals

Index of Single-Factor Pollution

The single-factor pollution index was commonly used to measure the degree of single heavy metal pollution in soils (Atiemo *et al.,* 2011).

The following is the formula:

$$Pi = Ci/Si \quad (1)$$

where Ci is the actual measured value of the metal under examination, and Pi is the single contamination index of the heavy metal element in soil. The assessment standard is known as the soil environmental quality risk control standard for soil contamination of agricultural areas, and Pi >1.0 indicates soil contamination, while Pi ≤ 1.0 suggests that the soil is not contaminated. I and Si are the relevant background standard concentration of metal in research.

In the meantime, the accumulation of heavy metals is more problematic the higher the value of Pi.

The risk screening levels for soil contamination of national agricultural land and the soil.

Index of geoaccumulation (Igeo)

By comparing the levels of heavy metals acquired to the background levels initially used with bottom sediments, the index of geoaccumulation (Igeo) is extensively employed in the assessment of pollution (Atiemo *et al.,* 2011). Equation is used to calculate it.

$$Igeo = \log2(Cn/1.5Bn)$$

Where Cn, indicates the measured concentration of the elements investigated and Bn is the geochemical background value of the element in fossil argillaceous sediment (average shale) (Taylor & McLennan, 1985).

For the geoaccumulation index, the following classification is provided: 0–1 denotes no pollution to moderate pollution, 1-2 denotes moderate pollution, 2-4 denotes moderate to severe pollution, 4-5 denotes strong to extremely pollution, and > 5 denotes extremely pollution. (Lu *et al.,* 2009).

Data Processing and Statistical Analysis

The heavy metal concentration in soil was spatially interpolated using inverse distance weighted methods. used to complete the data statistics and analysis. used to refine the chart. With ArcGIS 10.3, spatial analysis and mapping were completed.

Results and Discussion

Soil physicochemical properties

Table 1. Descriptive statistics for (B) Heavy metal concentrations and PH in agriculture soils (Mg kg−1; SOM: %)

Heavy metals	Mean	Median	SD	Min	Max	(%) CV
pb	13.80	3.05	2.34	11.44	17.18	16.96%
Cd	1.80	1.23	0.56	0.13	1.54	31.1%
Cu	19.97	20.21	2.50	13.38	20.16	12.52%
Zn	52.18	52.34	3.86	47.24	57.08	7.39%
PH	7.84	7.85	0.012	7.69	7.97	0.15%

Table 2. Descriptive statistics for (C) heavy metal concentrations and PH in agriculture soils (Mg kg−1; SOM: %**)**

Heavy metals	Mean	Median	SD	Min	Max	(%) CV
Pb	15.51	15.84	1.81	12.44	16.85	11.67%
Cd	0.36	0.09	0.66	0.01	1.54	183.3%
Cu	16.92	17.33	2.60	13.38	20.16	15.4%
Zn	29.05	28.56	2.63	25.77	32.44	9.05%
PH	8.16	8.12	0.11	8.06	8.33	1.34%

Table 3. Descriptive statistics for (M) heavy metal concentrations and PH in agriculture soils (Mg kg−1; SOM: %)

Heavy metals	Mean	Median	SD	Min	Max	(%) CV
Pb	14.31	15.14	2.42	11.12	17.18	16.9%
Cd	0.07	0.10	0.60	0.01	0.14	857.1%
Cu	22.35	24.00	4.25	16.00	26.16	19.02%
Zn	37.82	40.26	8.60	27.83	47.77	22.7%
PH	8.52	8.53	0.061	8.44	8.60	0.72%

The heavy metal contents of the agricultural soil in the three areas

The table shows that if this element was (1.80, 0.36, 0.07 mg/ kg) respectively, the presence of cadmium in the cultivated soil samples in the above seasons is attributed to the addition of chemical fertilizers that contain some cadmium as impurities during 40 years of cultivation (Lehoczky *et al.,* 1998). The current results also indicate that the addition of phosphate fertilizers for more than 40 years did not significantly affect the available content of cadmium in the soil. The current findings corroborate earlier findings, which showed that high concentrations of cadmium had been found in soil that had been irrigated with sewage water, in areas with heavy traffic, in areas that used an excessive amount of agricultural chemicals, or in close

proximity to industrial areas (Ebong and Ekong, 2015; Bhatti *et al.*, 2016). The average concentration of zinc in the soil samples was (52.18, 29.05, and 37.82mg/kg), respectively. The results indicate that the concentrations recorded are very low in the study area (c) compared to the rest of the regions as shown in the tables (1, 2 and 3). Sandy soil and the accumulation of zinc ion in the cultivated soil was probably due to the addition of chemical fertilizers. Previous reports also indicated a low concentration of zinc in soil samples in Accra, Ghana (Fosu-Mensah *et al.,* 2017), while high concentrations of zinc were recorded in soil samples collected from electronic and other landfill sites (Li *et al.,* 2011). As for the copper element, in the three study areas (Table 1, 2 and 3) high concentrations of the element were recorded, it was an average Its concentration (19.97, 16.92 and 22.35 mg/kg) respectively Copper pollution is attributed to various human activities such as excessive addition of chemical fertilizers and low-quality irrigation water (Hang and Jin, 2008; Bhatti *et al.,* 2016). Among the heavy metals that were studied in the soil samples is lead, where the average concentration in the three study areas ranges between (13.80, 15.51 and 14.31 mg/kg) due to the low concentration in the study area (A), and it is believed that the possible reason for this is the use of water Underground irrigation and lead-free chemical fertilizer High concentrations of lead have been recorded in the study areas (B and C), the reason for this is likely due to the presence of one of the sources of these elements, such as Traffic of cars, industrial processes, or the use of any type of waste water for irrigation, which is from Possibly contaminated with lead High concentrations of lead were also reported in the agricultural areas of Amritsar district of Punjab province in India, which amounted to 350 mg/kg, as a result of the use of compounds containing lead (Anwar and Vanita, 2014) and in Olkusz district in Poland, the concentration of lead in some arable soils reached 404 mg/kg near the areas industrial (Miskowiec *et al.,* 2015). The results of the analysis of the physicochemical properties of the studied soil samples indicate the three regions, tables (1, 2 and 3). The average pH of the soil samples was, (8.52, 8.16 and7.84) respectively, and the low pH may be due to it acidity in the soil due to the addition of urea fertilizer. As for The pH is considered to be an essential factor affecting cation movement and regulates 8.14 and 8.22. Solubility of heavy metals in soil, most of which tend to pH in acidic media (Rodriguez *et al.,* 2008). The results also indicate that soil samples tend to be slightly alkaline, which does not harm soil fertility because these values still tend to be

neutral. These results are in agreement with what was indicated by (Sharma *et al.*, 2017). The addition of containing fertilizers is working On nitrogen such as diammonium phosphate and urea in the long term, the soil pH decreases as a result of acidification, as well as the release of H^+ ions by the roots (Belay *et al.*, 2002).

Table 4. Average geochemical accumulation and pollution level in soil sample

Station	Igeo-Cd	Igeo-Cu	Igeo-Zn	Igeo-Pb
B	1.70	-0.53	-0.33	-0.34
C	-2.70	-0.60	-0.59	-0.28
M	-0.81	-0.48	-0.47	-0.32

The Muller-proposed Igeo index of categorization was used to gauge the quality of the soil (Muller, 1981) (Table 4). According to the findings of the Igeo examination, C and B were essentially free of Pb, Zn, Cu, and Cd contamination. Since M was heavily and severely polluted by Cd, there are no sources of pollution there, such as human activity, excessive traffic, or irrigation with contaminated water. This pollution can be attributed to the long-term use of chemical fertilizers containing impurities of these elements. correspond the present results with previous findings reported in (FosuMensah *et al.*, 2017; Rahman *et al.*, 2012).

Conclusion:

In this study, it can be concluded that regular use of heavy metals and pesticides are associated with acute and chronic toxicity occasioned by bioaccumulation of toxic trace metals and pesticides in the soil , where could increase the potential risk to human health from the entry of hazardous agricultural products into the food chain due to their migratory capacity, their low degradability and their toxicity.

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