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Effects of Sodium Benzoate and *Ephedra alata* on Body weight and some organs weight in Male Rats

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Abstract:

One of the most widely used chemical preservatives, sodium benzoate is used to keep a wide range of foods, drinks, and sauces from changing or degrading while being stored due to microorganisms. *Ephedra alata* (*E. alata*) is used in traditional medicine to treat infections caused by fungi and bacteria, cancer, and problems affecting the digestive, respiratory, and circulatory systems. The present study aimed to investigate the effects of sodium benzoate and *Ephedra alata* on body weight and weight Liver, Kidney, Heart and Testis in Male Rats. Twenty male albino rats, weighing between 195 and 300 g, were split up into four equal groups, with five male rats in each group: The first group was retained and given distilled water every day as a control. The second group was given an oral dose of sodium benzoate (100 mg/kg/b. w.) for 2 weeks. The third group was treated with *E. alata* (1 g/kg/b. w.) orally for 2 weeks. The forth group (combination group) was administered with Ephedra and sodium benzoate for 2 weeks. The results of the present study revealed that no significant effects were observed on body weight, liver weight, kidney weight, heart weight, testis weight, relative liver weight, relative kidney weight, relative heart weight and relative testis weight between control and all treated groups, except, there was a significant increase in the body weight and kidney weight in the combination group. In conclusion, the results of this study confirm no effect of sodium benzoate and *E. alata* on body weight and organs weight.

Keywords: Sodium Benzoate, *Ephedra alata*, Body weight, relative organs weight.

Introduction:

Many of food added substances have been progressively recognized as possibly dangerous components for human being such as hepatotoxicity and nephrotoxicity (Radwan *et al.,* 2018; Elghazaly *et al.,* 2020). Sodium benzoate, which is the sodium salt of benzoic acid, is a food preservative that is water soluble and well-stabilized, with fungistatic and bacteriostatic qualities (Pongsavee, 2015). Deuel *et al.* (1954) showed that Rats fed sodium benzoate via diet experienced decreased weight gain and increased liver and kidney relative weights. Rats given a 2.4% concentration of sodium benzoate saw a weight loss, although their relative liver and kidney weight increased. (Fujitani, 1993). Reduced sperm count and epididymal sperm motility, along with weight loss of the gonads, epididymis, and accessory sex organs, are established criteria for characterizing hazardous substances that may cause difficulties with fertility in sodium benzoate-treated patients (Ban *et al.*, 1995; Queiroz-Neto *et al.,* 1997). Saatci *et al.* (2016) showed an insignificant decrease in body weight in rat mothers after 20 days. Redouane *et al.* (2019) found that effects of sodium benzoate on male mice caused significant decrease in body weight gain as well as a significantly increased relative testes weight. Scientists have long been guided by traditional folk remedies derived from plants to find novel pharmaceuticals to sustain and enhance animal and human health (Achterberg, 2002). *Ephedra alata* Decne. (*E. alata*) (The Arabic name is Alanda, family: Ephedraceae) is a perennial genus of non-flowering seed herb belonging to the Gnetales plant, the closest living relative of the angiosperm (Friedman, 1996). Iran, Algeria, Iraq, Chad, Egypt, Palestine, Lebanon, Jordan, Saudi Arabia, Morocco, Syria, Libya, Mauritania, Mali, Somalia, and Tunisia are the native lands of this species. (Abourashed *et al.,* 2003; Al-Qarawi *et al.,* 2014). Moreover, Ghasemi *et al.* (2014) found that the antioxidant activity of *E. pachyclada* extract on mouse significant reduction relative liver weight. Numerous studies have demonstrated the effectiveness of *E. herba*-containing medications in reducing body weight. (Fan *et al.,* 2015; Roh *et al.,* 2017; Lim *et al.,* 2018). Djahra *et al.* (2019) revealed that natural products of *E. alata* caused significant differences in total body weight and relative weight of organs. Lee *et al.* (2019) found that *E. herba* methanol extract had no influence on body weight alterations in mice with hyperlipidemia brought on by a high-fat diet.. A recent study done by El-Shennawy *et al.* (2020) showed that 90 days of exposure to varying dosages of sodium benzoate (0-1000 mg/kg b. w.) in male rats' reproductive systems

altered the weight of the reproductive organs, the properties of the semen, and the plasma levels of sex hormones. Sodium benzoate greatly raised the relative weights of the prostate and epididymis but had no discernible influence on final body weights or the relative weight of the testes.

Materials and Methods:

Experimental animals:

Throughout the current investigation, twenty male albino Rats (*Rattus norvegicus*) weighing between 195 and 300 g were used. They were obtained from the animal house of Zoology Department, Science Faculty, University of Omar Al-Mukhtar. The animals were kept in identical rooms with constant environmental parameters including humidity (50–60%) and temperature (22±3°C) divided into four groups of cages. They were supplied with enough rate feed and drinking water *ad-libitum*. All animals were allowed to acclimatize in the environment for two weeks before the commencement of the study, which lasted for two weeks.

Experimental chemical and medical plant:

Sodium benzoate: Sodium benzoate is a substance that has the chemical formula C_6H_5COONa was used. It was obtained from BDH chemicals Ltd. (England).

***Ephedra alata*:** *E. alata* leaves were gathered in the Al-Jabal Al-Akhdar district on Libya's east coast. The *E. alata* extraction procedure was conducted in accordance with the approach outlined by Dahiru *et al.* (2006).

Preparation of sodium benzoate: According to the group distribution, sodium benzoate was administered orally at a dose of 100 mg/kg/b. w. dissolved in freshly made distilled water every day for two weeks (Tawfek *et al.*, 2015).

Preparation of *Ephedra alata:* The collected leaves weighed and washed with water, dried and cut into small pieces, weighed again. Hit the quantity in the mixer for an hour, filtered with a funnel. Using a rotary evaporator, the samples' solvent was eliminated, and the extract (heavy extract) was collected. Oral administration of *E. alata* at a dose of 1 g/kg/b. w. (Jahromi *et al.*, 2016) was administered every day for a total of two weeks during the experiment.

The two dosages were administered orally using a specialized gastric tube with a smooth tip to prevent damage to the buccal and oral internal linings.

Experimental design:

Twenty male albino rats in all were employed in this experiment. The rats were divided into four equal groups at random, with five male rats in each group, using the following protocol:

1- **Control group (G1):** For two weeks, the animals in this group were orally gavaged with distilled water every day.

2- **Sodium benzoate treated group (G2):** For two weeks, rats were given an oral dosage of 100 mg/kg/b. w. of sodium benzoate every day.

3- ***E. alata* treated group (G3):** Rats in this group received a daily dose of 1g/kg b. w. of *E. alata* for a period of two weeks.

4- **Combination Group (G4):** For two weeks, the animals in this group were given an oral dose of sodium benzoate (100 mg/kg/b. w.) along with an oral dose of ephedra (1 g/kg b. w.).

Determination of the body weight, organs weight and relative organs weight:

Body weight: During the acclimation phase, once before the start of dosing, once every week during the dosage period, and once on the day of sacrifice, the body weight of each animal in the control and treatment groups was measured using a sensitive electronic balance. The percentage of change in body weight during the period of experiment was computed using the differences between the initial and final weights, as follows:

$$Percentage\ of\ \ change\ in\ body\ weight = \frac{Final\ body\ weight - initial\ body\ weight}{Initial\ body\ weight}\ x\ 100$$

Absolute and relative organs weight: Fresh organ weight was computed in relation to total body weight in order to provide an accurate measurement of the change in organ weights. This was done as follows:

$$\text{Relative kidney weight} = \frac{Absolute\ \ organ\ \ weight}{Total\ \ body\ \ weight} \times 100$$

Results:

Body weight: In this study, no significant effects were observed on body weight between normal control, *E. alata* and sodium benzoate groups. While, a significant increase in the mean value of body weight in combination group as compared to control group after the first and second weeks (Table 1 and figure 1).

Liver weight: The data collected, which are displayed graphically in table (1) and figure (2), did not significantly alter the mean liver weight of any of the groups when compared to the control group.

Relative liver weight: Table (1) and Figure (3) display the average liver weight to body weight ratio for each group. After two weeks, no statistically significant variations were observed in the ratio of liver weight to body weight in either the treatment or control groups of rats.

Kidney weight: After two weeks, the data show no differences in the mean kidney weights between the sodium benzoate, *E. alata*, and control groups. However, after two weeks, the mean kidney weight in the combination group increased significantly more than that of the control group (Table 1 and figure 4).

Relative kidney weight: Table (1) and Figure (5) display the average ratio of kidney weight to body weight for each group. The relative weight of kidneys did not differ significantly between the control group and any of the treated groups.

Heart weight:

When comparing the mean heart weight of each group to the control group, there were no discernible changes in any of the data, which are displayed graphically in table (1) and figure (6).

Relative heart weight: No significant differences were found in the relative weight of heart to body weight in control rats and all treated groups after 2 weeks (Table 1 and figure 7).

Testis weight: When comparing the treated groups' mean testis weight to that of the control group, there were no discernible differences, according to the data displayed in table (1) and the visual presentation in figure (8).

Relative testis weight: After two weeks, no statistically significant variations were seen in the ratio of testis weight to body weight in either the control group or any of the treated groups (Table 1 and figure 9).

Table (1): The protective role of *E. alata* aqueous extract against sodium benzoate toxicity on body weight (g), organs weight (g), relative organs weight (%) levels

Parameters	Duration / Group		G1- control Group	G2- Sodium benzoate Group	G3- *E. alata* Group	G4- Comb. Group
Body weight	1st Day	Mean ± S. E. % of change	230.0^{B}±15.76	226.8^{AB}±13.53 -3.2	258.0^{AB}±20.85 28.0	315.2^{A}±11.25 85.2
	1st week	Mean ± S. E. % of change	290.6^{B}±14.82	274.6^{B}±13.30 -16	278.2^{B}±12.50 -12.4	354.6^{A}±15.09 64
	2nd week	Mean ± S. E. % of change	291.2^{B}±10.13	274.8^{B}±11.96 -16.4	299.2^{B}±13.17 8	365.6^{A}±10.89 74.4
Liver weight	2nd week	Mean ± S. E. % of change	8.29^{A}±0.17	8.22^{A}±0.39 -0.07	8.66^{A}±0.29 0.37	9.52^{A}±0.43 1.23
Relative liver weight	2nd week	Mean ± S. E. % of change	2.86^{A}±0.11	3.00^{A}±0.16 0.14	2.91^{A}±0.11 0.05	2.81^{A}±0.14 -0.05
Kidney weight	2nd week	Mean ± S. E. % of change	0.83^{B}±0.28	0.89^{AB}±0.04 0.06	0.80^{B}±0.01 -0.03	1.04^{A}±0.07 0.21
Relative kidney weight	2nd week	Mean ± S. E. % of change	2.86^{A}±0.11	3.00^{A}±0.15 0.14	2.91^{A}±0.11 0.05	2.65^{A}±0.08 -0.21
Hart weight	2ndweek	Mean ± S. E. % of change	0.934^{AB}±0.02	0.926^{B}±0.03 -0. 008	0.980^{AB}±0.01 0.046	1.323^{A}±0.19 0.389
Relative hart weight	2ndweek	Mean ± S. E. % of change	0.32^{A}±0.01	0.34^{A}±0.02 0.018	0.33^{A}±0.02 0.008	0.40^{A}±0.07 0.078
Testis weight	2nd week	Mean ± S. E. % of change	1.490^{AB}±0.038	1.412^{B}±0.074 -0.078	1.402^{B}±0.060 -0.088	1.664^{A}±0.055 0.174
Relative testis weight	2nd week	Mean ± S. E. % of change	0.510^{A}±0.020	0.493^{A}±0.016 -0.017	0.472^{A}±0.028 -0.038	0.469^{A}±0.006 -0.041

A, B: The groups in the same row with different letters are statistically significant ($p < 0.05$).

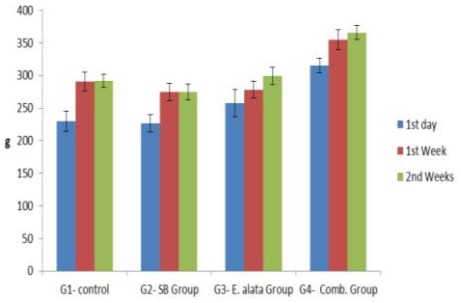

Figure (1): Effects of sodium benzoate and *Ephedra alata* on body weight (g).

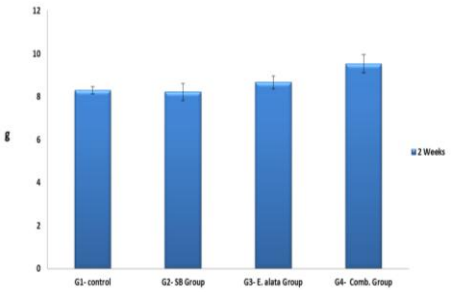

Figure (2): Effects of sodium benzoate and *Ephedra alata* on liver weight (g).

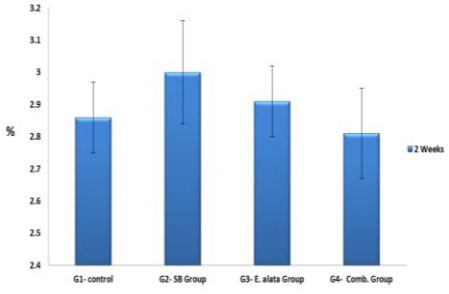

Figure (3): Effects of sodium benzoate and *Ephedra alata* on relative liver weight (%).

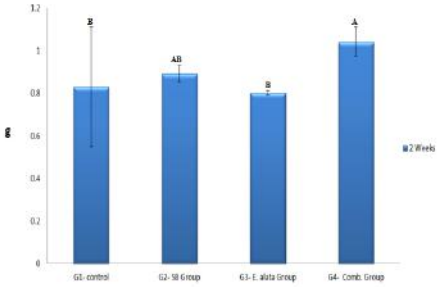

Figure (4): Effects of sodium benzoate and *Ephedra alata* on Kidney weight (g).

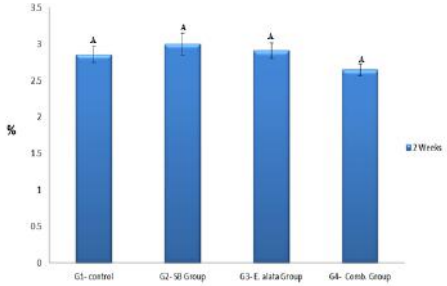

Figure (5): Effects of sodium benzoate and *Ephedra alata* on relative kidney weight (%).

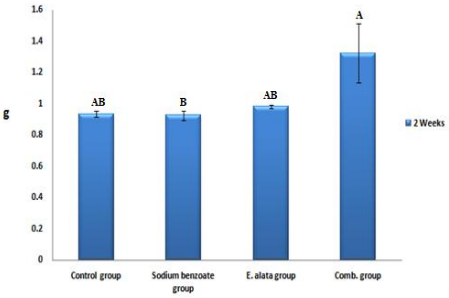

Figure (6): Effects of sodium benzoate and *Ephedra alata* on heart weight (g).

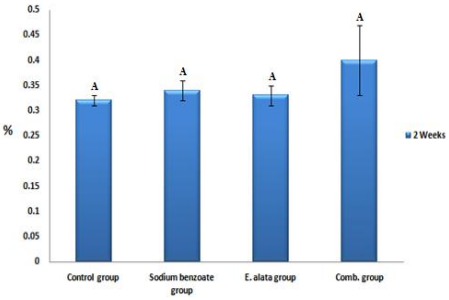

Figure (7): Effects of sodium benzoate and *Ephedra alata* on relative heart weight (%).

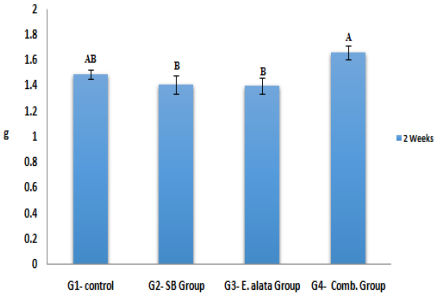

Figure (8): Effects of sodium benzoate and *Ephedra alata* on testis weight (g).

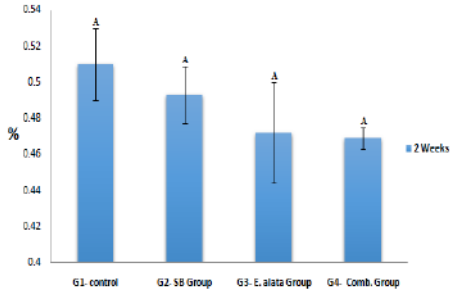

Figure (9): Effects of sodium benzoate and *Ephedra alata* on relative testis weight (%).

Discussion:

The present study showed that, there was no marked changes in all parameters, except, there was a significant increase in the body weight and kidney weight in the combination group. In **sodium benzoate group,** the results showed no changes in the mean body weights and all organs weight when compared with control group after 2 weeks. Supporting the current study, showed insignificant change in body weight with same dose (100

mg/kg) sodium benzoate administration for 2 weeks in rats (Sohrabi *et al.*, 2008; Kehinde *et al.*, 2018). Similar results have been reported by Kaboglu and Aktac (2002), they observed that the total weight of the liver was not changed. Furthermore, Redouane *et al.* (2019) who reported that no significant changes in the absolute testes weight in sodium benzoate treated groups. El-Shennawy *et al.* (2020) they elucidated that the sodium benzoate did not induce a significant effect on the relative weight of the testes. In contrast, disagree with Kaboglu and Aktac, (2002) they reported relative liver weight and absolute liver weights increase in rats administered with 0, 2.08, 2.50 or 3.00% of sodium benzoate in the diet for 10 days. Also, Dewangan (2009) in rat he showed increase in kidney weight when treated with sodium benzoate. However, a study by Al-Ani *et al.* (2019) illustrated a vital reduction in testes masses in sodium benzoate group at dose 400 and 800 mg/kg b. w. throughout the 70 days. Redouane *et al.* (2019) they reported that increase in relative testes weight in 0.5 and 1 % sodium benzoate treated groups after 13 weeks on male reproductive function in mice. These differences of results may be explained by dose and time difference of sodium benzoate. In ***E. atata* group,** the data indicate no changes in the mean body weights and all organs weight between control and *E. alata* after 2 weeks. Similar results have been reported by Fan *et al.* (2015) found that no statistically significant differences in the body weight. In addition, Lee *et al.* (2019), who observed that no significant difference in body weight in mice. In contrast with the present result, Ghasemi *et al.* (2014) found that the antioxidant activity of *E. pachyclada* extract on mouse significant reduction relative liver weight. Also, Shah *et al.* (2009) reported that a significant decrease in heart weight treated with *E. nebrodensis* extract (200 mg/kg) treatment for two weeks. On the other hand, the mean body weight and kidney weight of **combination group** showed increase after 2 weeks. There is no literature on the effect of sodium benzoate with *E. alata* together. So, these result maybe duo to the react between sodium benzoate and *E. alata*.

Conclusion:

The study's findings, taken together, verify that sodium benzoate and *E. alata* extract have no influence on body weights or the weight of any organ.

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A study of Gram positive bacterial profile on computer keyboards and mice in Benghazi University offices, and the efficiency of disinfectants.

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Abstract:

The aim of the study was to evaluate the contamination of computer keyboards and mice surfaces with Gram-positive bacteria in offices of Benghazi University and the efficiency of disinfectant to eliminate contamination. This study included 100 samples 50 samples from keyboards 50 samples from mice. All the bacterial species in this research were specified by morphology characteristics shape (size, odour and color) Gram stains and Biochemical tests. This study showed that the percentage of Gram-positive bacteria isolated reach to 46%. *Staphylococcus epidermis* possess the high rate of contamination reach to 28%, followed by *Staphylococcus aureus* 14% and *Streptococcus mitis* 12%. This study also revealed that the percentage of bacterial contamination on multi users was higher than the personal user. The percent of Gram-positive bacteria among personal from the total of keyboards and mice showed that the highest bacterial contamination *Staphylococcus epidermis* was 39%, followed by *Streptococcus mitis* 13%, *Staphylococcus aureus* 12%. Multi users of both keyboards and mice showed high contamination with *Staphylococcus epidermis* reach to 41%, followed by *Staphylococcus aureus* 27%, *Streptococcus mitis* 20%. The rate of contamination was highest among multi users than personal users. This study demonstrated the rate of contamination among the female was with *Staphylococcus epidermis* reach to 41%, *Staphylococcus aureus* 24%, *Streptococcus mitis* 16%. However, among the male different rate of bacteria was observed such as *Staphylococcus epidermis* 39%, *Streptococcus mitis* 17%, *Staphylococcus aureus* 15%. The effect of disinfectant against isolated bacteria was also studied. The results showed that the Dettol had a great effect against the Gram positive bacteria.

Keywords: Computer key, Mice, Dettol, Gram Positive, Bacterial contamination.

Introduction:

Computers are becoming more and more common in our lives, and keyboards and mice are also a source of bacteria, viruses and potentially fatal infections among users. Contamination of objects and surfaces occurs everywhere in the environment. Computer keyboards and mice are the most open parts of the computer that are 100% contaminated. (Tagoe *et al.*, 2010). Pathogenic bacteria are colonized on animate and inanimate objects; However, most people are not aware that microorganisms are present in many outdoor areas, offices and even homes, the computer keyboards help spread pathogenic microbes, many bacteria have been isolated from computer compounds (Al-Ghamdi *et al.*, 2011). Previous researchers have reported the presence of living pathogenic bacteria on inanimate objects. Several studies of the human environment have found colonization and contamination of items such as doorknobs, telephones, money and computer parts. (Oluduro *et al.*, 2012). In most cases, computers and their accessories such as keyboards and mice are considered pathogens of infection transmission both in society and in science. IT devices can serve as a reservoir for the transmission of potentially dangerous or pathogenic microorganisms. Due to the widespread use of computers, it is necessary to detect and identify whether computer keyboards can serve as attack points and to identify possible routes of contamination or transmission of infectious agents. In the setting of various universities or educational institutions, given the increasing availability of Internet and email in institutions, it is important to recognize that computer equipment can serve as a reservoir for the transmission of potentially dangerous or pathogenic microorganisms. (Hartmann *et al.*, 2004). In the chain of infection, germs can act as a reservoir and pathogens are transmitted from the inanimate to the living environment via the hands. A contaminated PC has been linked to the transmission of a methicillin-resistant strain of *Staphylococcus aureus* to users. Computer keyboards are infected with Staphylococcus spp. contaminated. (Isaacs *et al.*, 1998). Methicillin resistant *Staphylococcus aureus* is of particular concern was also isolated from keyboards reach to 56.4% (Adwan *et al*., 2009). Bacteria present on human body as a member of the commensal flora, it can cause serious opportunistic infections such as urinary tract infections (Anastasiades. 2009). Computer keyboards and mice can serve as vehicles for the transmission of pathogenic microorganisms, directly through superficial mouth contact or indirectly through finger contamination and subsequent hand-to-mouth contact. Cases of

Staphylococcus aureus, *Staphylococcus epidermidis*, Enterococcus, Streptococcus and fungi have also been reported associated with the use of a computer keyboard and mouse in an academic setting. (Enemuor *et al.*, 2012). *Staphylococcus aureus*, including MRSA, survives in dry conditions and can persist for long periods on surfaces touched by human. *S. aureus* and MRSA can be transmitted from person to person, from fomites to humans, and from air to humans. Hartman et al. (2004) reported that *Staphylococcus aureus*, which is normally found on the skin or around the nose and survives only on dry skin on the outside of the body, was found on keyboards. Disinfectants are chemicals that can destroy microorganisms or inhibit their growth. Disinfection removes microorganisms, including potentially pathogenic ones, from the surface of inanimate objects such as (computer keyboards, mice). Dettol and alcohol are commonly used for different purposes including disinfecting skin, objects and equipment, and surfaces in the environment. Dettol is an antiseptic used in houses and various objects.

Materials and Methods:

This was an observational study with a cross-sectional study design. Keyboard and mouse samples were collected from 100 computers using sterile cotton swabs. A total of 100 swabs were randomly collected from different computer keyboards and mice in offices administrative and finance departments of Benghazi university environment during opening hours featuring normal staff traffic at offices administrative and finance departments. A single sterile cotton swab was moistened with sterile saline, passed over the surface (keyboard and mouse), and immediately transported to the laboratory. Identification of Isolates Bacterial isolates were identified based on the culture characteristics of the growth medium, including colony size, color, hemolysis on blood agar, colony pigmentation, swarming, and *odor*. All swabs isolate cultured in Nutrient agar, blood agar, chocolate agar. Bacteria was isolated, and classified to Gram positive or Gram negative based on the reaction of the Gram stain, and the color of the cells under the light microscope. This technique was used to stain a slide such as a fecal smear to observe the presence of bacteria based on their Gram stain reaction and different biochemical procedures used to identify the bacteria isolation included the Catalase, DNase, oxidase, Optochin Novobiocin test. To study disinfectants by Sterile filter paper discs were soaked for 24h with (100%, 75%, 50%) concentration of Dettol and then these discs were placed on incubated nutrient

agar plates which were incubated at 37°C for 24 hours. After incubation, clear areas showed growth inhibition of the bacterial isolate. The area around the disks was measured with a ruler and recorded. The results collected during the research were processed in a computer database. The SPSS software (Statistical Package of Social Science) version 18 was used in analysis. Chai square was used for paired and group comparison. Differences were considered significant whenever p value less than 0.05.

Results:

A simple random technique was used to sample 100 keyboards and mice from offices Benghazi university environment by swabbing their surfaces. These samples were streaked on blood agar and Macconkey agar and chocolate agar for identifying the organisms present in computer keyboards and mice. The results showed 97% of samples had clear bacterial contamination Figure 1. Both the Gram positive and Gram negative organisms were observed from the samples tested. The distribution of Gram negative bacteria was 54%more than the Gram positive bacteria which reach to 46%. All the isolates were classified based on cultural, morphological and Biochemical characteristics of the isolates on different selective, differential, nutrient media. The total percent of bacterial contamination of computer keyboards and mice, showed the highest bacterial contamination in both computer keyboards and mice was the *Staphylococcus epidermis* 28%, followed by *Staphylococcus aureus* 14%, *Streptococcus mitis* 12% Table 1. The percent of Gram positive bacteria among personal from the total of keyboards and mice. The highest bacterial contamination *Staphylococcus epidermis* was 39%, followed by *Streptococcus mitis* 13%, *Staphylococcus aureus* 12%.

Figure 1: Percentage of bacterial growth on culture media.

Multi users of both keyboards and mice showed high contamination with *Staphylococcus epidermis* reach to 41%, followed by *Staphylococcus aureus* 27%, *Streptococcus mitis* 20%. The rate of contamination was highest among multi users than personal users Figure 2.

Table 1: The percent of Gram Positive bacteria from total keyboards and mice.

Bacteria isolate	Keyboards and mice %
Staphylococcus epidermis	28 %
Staphylococcus aureus	14%
Streptococcus mitis	12 %

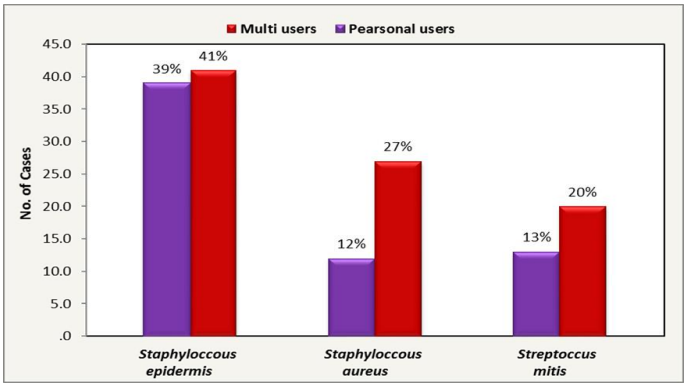

Figure 2: Distribution of Gram positive bacteria among personal and multi users from the total of bacteria isolate from keyboard and mice

In female the highest bacterial contamination was *Staphylococcus epidermis* reach to 41% in both keyboards and mice, followed by *Staphylococcus aureus* 24%, *Streptococcus mitis* 16%, However, In male the highest bacterial contamination was also *Staphylococcus epidermis* was 39%, followed by *Streptococcus mitis* 17%, *Staphylococcus aureus* 15% (Figure 3).

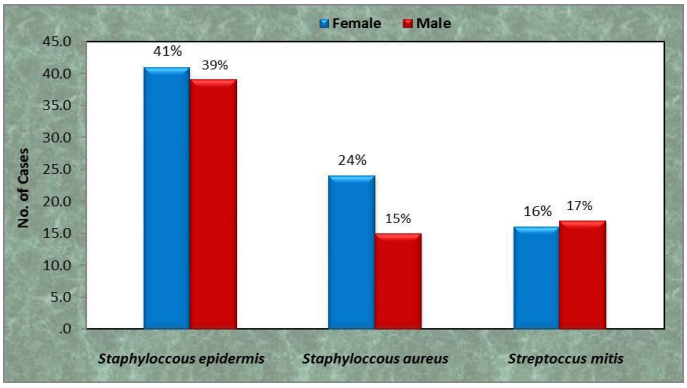

Figure 3: Distribution of bacteria among female and male from the total of bacteria isolate

This study showed the high effect of Dettol at 75% concentration against *Staphylococcus aureus* with inhibition zone reach to (28.3 mm), followed by *Staphylococcus epidermis* with inhibition zone (21.3 mm). However, no effect against *Streptococcus mitis* was observed. Whereas, at 50 % concentration showed high effect against *Staphylococcus aureus* with inhibition zone reach to (18.3 mm), followed by *Staphylococcus epidermis* with inhibition zone (17 mm). However, no effect against *Streptococcus mitis* was observed. While, the 100% concentration showed effect against *Staphylococcus epidermis* with inhibition zone (17.6 mm), followed by *Staphylococcus aureus* with inhibition zone (11.3 mm). While, no effect against *Streptococcus mitis* was observed Table 2.

Table 2: Effects of Dettol at different concentrations on Bacteria isolates

Bacteria isolates	Dettol concentration		
	100% concentration mm	75% concentration mm	50% concentration Mm
Staphylococcus epidermis	17.6	21.3	17
Staphylococcus aureus	11.3	28.3	18.3
Streptococcus mitis	R	R	R

Discussion:

The computer keyboard and mouse can transmit pathogenic microorganisms and thus serve as a gateway for infection (Awe *et al.*, 2013). Previous researchers have reported the presence of living pathogenic bacteria on inanimate objects. While several studies of the human environment have demonstrated colonization and contamination of objects, computers continue to be increasingly present in almost every aspect of our work. (Oluduro *et al.*, 2012). In this study, the various computer keyboards and mice tested were contaminated with considerable number of Gram positive and Gram negative bacteria. The Gram negative bacteria reach to 54% higher than Gram positive bacteria 46%. In contrast, Scott and Bloomfield (2008) found that the percent of Gram positive bacteria is higher than Gram negative bacteria. These results may be due to the fact that survival of Gram positive species on laminate surfaces is greater than that of Gram negative organisms. However, Gram positive bacteria and Gram negative bacteria have been shown to have similar rates of transmission from laminate surfaces to finger tips (Scott and Bloomfield, 2008). Most of these isolate microorganisms were traditional skin flora and probably dust associated organisms especially these from keyboards

and mice. Contamination of these keyboards and mice by bacteria could be traced to the transmission of bacteria that lives on the skin, fingernails, hands to the keyboard. This study revealed that the contamination of *Staphylococcus epidermis* reaches to 28%. This finding are similar with Hartmann *et al*. (2004) who found that high rate of contamination on computer keyboards with *Staphylococcus epidermis*. Similarly, Enemuor *et al.* (2012) found that the contamination of *Staphylococcus epidermis* reaches to 68, 8%. In this study, the percent of *Staphylococcus aureus* isolated reach to 14%. A similar study by Kassem *et al.* (2007) revealed that *Staphylococcus aureus*, *methicillin-resistant Staphylococcus aureus* (MRSA), Staphylococcus epidermidis, was isolated from computer keyboards in science. These bacteria are among the normal microbes of human skin and nasal passages. These bacteria are known to be associated with many disease conditions. *Staphylococcus aureus* can be transmitted by manual transmission from inanimate objects in the environment, including computer keyboards and mice. In this study, the percent of *Streptococcus mitis* reach to 12%. Similar study was observed by Obinna *et al*. (2012) who found that *Streptococcus mitis* were also isolated from the computer keyboards and mice. The present of Streptococcus species may be due to mouth contamination, either directly by surface to mouth contact or indirectly by contamination of fingers and subsequent hand to mouth contact. He also suggested the contamination may be due to mouth contamination. It can cause streptococcal sore throat, scarlet fever, toxic shock syndrome, pneumonia, meningitis, endocarditis, impetigo, urinary tract infection and others (Brooks *et al.*, 2001). This study showed that all Gram negative bacteria were Sensitive to all different concentration of Dettol at (100%, 75%, 50%). Similarly, the Gram positive bacteria isolated were sensitive to all concentration of Dettol at (100%, 75%, 50%) except the *Streptococcus mitis* was resistant. The mechanism of action of disinfectants on bacteria is the production of destructive chemicals that attack membrane lipids, DNA and other essential cellular components of various pathogenic bacteria. (Rutala *et al.*, 2006). Disinfectants effect on bacterial cell by destruction of proteins, lipids or nucleic acids in the cells or its cytoplasmic membrane. This study suggested regular cleaning and disinfection of computers is recommended to reduce the bacterial load.

Conclusion:

The main goal of this study was to isolate and identify the bacteria present on computer keyboards and mouse surfaces in the offices of Benghazi University and the effectiveness of the disinfectant (Dettol). This study showed that the total percentage of Gram Positive bacteria reach to 46%. The effect of disinfectant against bacteria was also studies. The results showed that the Dettol had a great effect against the Gram Positive bacteria with maximum inhibition zone the high effect of Dettol at 75% concentration against *Staphylococcus aureus* with inhibition zone reach to (28.3 mm), followed by *Staphylococcus epidermis* with inhibition zone (21.3 mm), However, no effect against *Streptococcus mitis* was observed. Whereas, at 50 % concentration showed high effect against *Staphylococcus aureus* with inhibition zone reach to (18.3 mm), followed by *Staphylococcus epidermis* with inhibition zone (17 mm). However, no effect against *Streptococcus mitis* was observed. While, the 100% concentration showed effect against *Staphylococcus epidermis* with inhibition zone (17.6 mm), followed by *Staphylococcus aureus* with inhibition zone (11.3 mm). While, no effect against *Streptococcus mitis.*

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Determination of Some Heavy Metals Content in Chicken Shawarma Samples from a Restaurant in El-Beida City, Libya

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Abstract:

The purpose of this study was to identify certain heavy minerals in samples of chicken shawarma from some fast-food restaurants in El-Beida city Libya. Using UV-Visible spectrophotometry. The concentrations of the minerals (pb, Cu, and Fe) were measured, and the content was represented as ppm. The highest concentration of lead (0.2640-0.7249 ppm) has been identified in chicken shawarma. Conversely, the largest concentrations of copper (5.98-10.91 ppm) and iron (0.6973-1.6066 ppm) have been detected in this type of food. Every sample that was analyzed had a content of heavy metals that above the limits of safety for human consumption established by various international public health organizations, such as WHO.

Keywords: Heavy Metals, UV-Visible spectrophotometry, Chicken Shawarma.

Introduction:

Protein, fats, minerals, vitamins, and other bioactive ingredients make up meat, along with trace amounts of carbohydrates. Meat's significance from a nutritional perspective stem from its premium protein, which includes all necessary amino acids, as well as its highly accessible minerals and vitamins (FAO, 2013). The street fast food is widely recognized to offer low-cost, easily accessible. Where in both urban and rural areas there are enticing and varied food options for tourists and higher income groups; and affordable in poor areas (HWO, 2020). Furthermore, because it is readily available, reasonably priced, and requires little preparation, fast food has become the lunchtime staple for most metropolitan school-age children. These quick meals can therefore expose consumers, particularly children, to harmful concentrations of heavy metals (Byuro, 2011). Among all the snacks that are most popular in the Arab world is shawarma. Usually made with meat from cattle. It is sliced thinly and arranged on a skewer that is vertical so that each serving can be shaved off. Shawarma is often served as a sandwich, where it is

rolled into a flat circle of bread and topped with tahini (sesame) sauce, finely chopped onions, lettuce, and tomatoes (Essa *et al.*, 2006). Metallic elements with densities higher than waters are known as heavy metals. However, some micronutrients (such as Cu, Cr, and Ni) may be dangerous at high concentrations. Not many are necessary for plant growth or human nutrition. Furthermore, a number of metals, including As, Pb, and Cd, are unsafe to human health even at low concentrations. (Wang and Shi, 2001). Eating these heavy metal-contaminated foods can severely deplete the body of certain essential nutrients, which can lead to immunological deficiencies, intrauterine growth retardation (IVGR) in fetuses (due to Pb, Mn, and Cd), psychological problems, and malnutrition-related disabilities. (FSANZ, 2003). Moreover, there is research linking As and Pb-containing foods to cancer. For instance, there is a link between high levels of Cu, Cd, and Pb in fruits, vegetables, and other foods and a higher risk of upper gastrointestinal cancer. (Tchounwou *et al.*, 2003). Heavy metals can affect the neurological, cardiovascular, renal, and reproductive systems. They may also result in sulfaturia by impairing the sulfate/bicarbonate transporter in the liver and kidney of mammals through abnormalities in cellular uptake processes. (Javed *et al.*, 2009). Because lead is difficult to eliminate from the body, it is be toxic and to accumulate there. The nervous system, kidneys, stomach, and reproductive organs are all impacted by lead poisoning. Furthermore, in laboratory animals, it has been connected to mutagenesis, teratogenesis, and carcinogenesis. (Pitot and Dragan, 1996). Also, levels of two essential elements, copper and zinc, can poison both humans and animals. (Pond, 1975) It is commonly known that copper is required at low concentrations, it can be dangerous at higher ones. On the other hand, excessive copper consumption can cause bloody diarrhea, severe nausea, jaundice, and hypotension. Wilson's disease, characterized by nerve cell death, liver cirrhosis, ascites, edema, and hepatic failure, may result from persistent copper poisoning. (Pond, 1975; Thomas *et al.*, 2001). An accumulation of heavy metals in bodily organs, particularly the spleen, liver, and kidney, is gradual and irreversible, making the contamination of meat and poultry tissues a serious health and food safety risk. Furthermore, heavy metals cannot be removed from food by prolonged heating. Therefore, there is a considerable risk associated with consuming ready-to-eat meat products that are made from previously contaminated raw meat. (Chitmanat *et al.*, 2010). Determining the number of heavy metals in fast food is essential to raising public awareness about its pollution because the negative effects of consuming heavy metals do not manifest themselves for a long time. Therefore, the purpose of this study is to quantify the number of heavy metals, such as lead, copper, and iron, present in frequently consumed fast foods, especially chicken shawarma.

Materials and Methods:

Collection of samples:

Nine arbitrary samples of chicken Shawarma were gathered from various fast-food restaurants in Elbeida, Libya. The collected samples were sent directly to the lab to evaluation the residues of heavy metals and trace elements determined.

Table 1: The sources of samples under study

NO	Name of source of samples	Type of samples	Place
S1	Bouhweh Restaurant	chicken Shawerma	Outside restaurant
S2	Al Nawaer Restaurant	chicken Shawerma	Outside restaurant
S3	Crispy Restaurant	chicken Shawerma	Outside restaurant
S4	Babai Restaurant	chicken Shawerma	Outside restaurant
S5	Al Madina Restaurant	chicken Shawerma	inside restaurant
S6	Istanbul Restaurant	chicken Shawerma	inside restaurant
S7	Cuba Cabana Restaurant	chicken Shawerma	inside restaurant
S8	Turkish Restaurant	chicken Shawerma	inside restaurant
S9	Al Anwari Restaurant	chicken Shawerma	Outside restaurant

Chemical and standards:

Stock solutions Cu^{+2}: Stock standards solution was prepared at (1000 µg/ml), added 10 ml conc. H_2SO_4 to stock solution to make the solution acidic. the diluted solution was made (100 ,150.200,250 and 300 ppm), 25 ml of NH_3 (1 M), was added to diluted solution to form blue color.

Stock solutions Fe^{+3}: Stock standards solution was prepared at (1000 µg/ml), added 10 ml conc. HCl to stock solution. Diluted solution was made (2, 4, 6, 8 and 10 ppm), 5 ml KSCN (0.2 M), was added to diluted solution to form red color.

Determination of Maximum Wavelength of pb^{+2}:

Pipette up to 5 mL of a 100 ppm. Pb $(NO_3)_2$ solution and transfer it into a 50 mL volumetric flask. Add aqua demineralization until the desired concentration of lead is reached (10 ppm),–5 mL of 10% KCN and added NH_4OH (1N) solution until reach pH 10, the extraction process was performed in a separating funnel using a 0.001% dithizone solution in chloroform (until it was not red). Next, using a UV-Vis spectrophotometer, the maximum wavelength of the Pb-dithizonate complex was determined to be between 400 and 800 nm. (Ari Wardani *et al.,* 2020).

Sample Digestion:

The collected samples were acid digested to identify the heavy metals as described by (Hadiani *et al.*, 2014). Each sample was taken and put on a hot plate in Becker at a weight of 0.5 g. The sample was treated with concentrated 10 ml HNO_3 (MERCK 70 % v/v) and heated until it took on a black color. To get rid of the derbies, the digested sample was filtered. The sample was then filled to a final volume of 50 ml in a volumetric flask and examined with a spectrophotometer.

Determination of some heavy metals:

After digestion and to preparing samples to measure all the reagent and chemical which we add to standard must add to the samples under study, the measuring done by using UV- VIS spectrophotometer.

Results and discussion

This study proved a good linearity regression for the study standard (lead, iron, and copper). (R^2=0.99) and lower detection of limit (LOD). The method's linearity was tested in a variety of (100 – 300) ppm of Cu^{+2}, and the method showed good linearity regression (R^2 = 0.99), with Y = 0.01884X + 0.00243, and (0.8 – 4) ppm of Pb^{+2}, with Y = 0.37207X - 0.04763, and (R^2 = 0.99), and (2-10) ppm of Fe^{+3} with Y= 0. 09678X-0.055491, and (R^2 = 0.99) and which is showing good linearity, precision, accuracy and sensitivity, which could be used for determination heavy metals (figs., from 1- 3) and table 2. It is well predictable that an increase in the body's accumulation of heavy metals, which can enter through ingestion and inhalation and include Pb, Cd, As, and Cr, can cause major systemic issues. (Wang and Shi, 2001). It's interesting to note that eating habits affect the number of heavy metals ingested. Nowadays, fast food is the main food preference of most people, especially school-age children. Due to the high concentration of heavy metals in these fast foods, children may be seriously at risk for health problems. Children are especially vulnerable to biological hazards and are known to absorb metals more quickly than adults, so there is a particular concern for them. (Cui *et al.*, 2005; Hussain *et al.*, 2013).

Table 2: Limit of detection (LOD) and limit of Quantification (LOQ) of metals determined in chicken Shawarma, and their wavelength and calibration range

Elements	Wavelength (nm)	Calibration range ppm	Correlation Coefficient	Limit of detection LOD	Limit of Quantification LOQ
Fe	467 nm	0-10	0.996	0.14	0.45
Cu	602 nm	0-300	0.999	0.06	0.36
Pb	516 nm	0-4	0.995	0.18	0.18

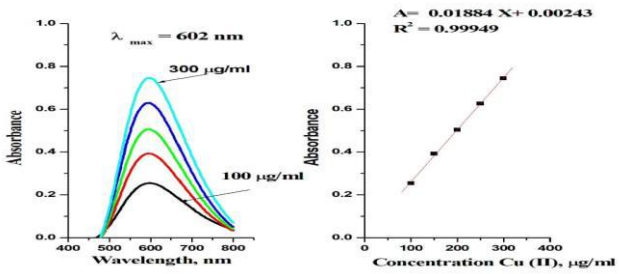

Fig. 1: Show the absorption spectrum and standard calibration curve of Cu (II)

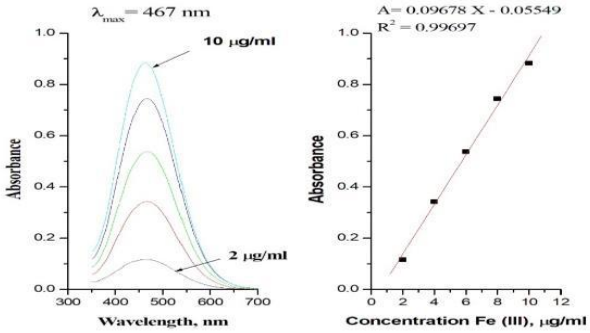

Fig. 2: Show the absorption spectrum and standard calibration curve of Fe (III)

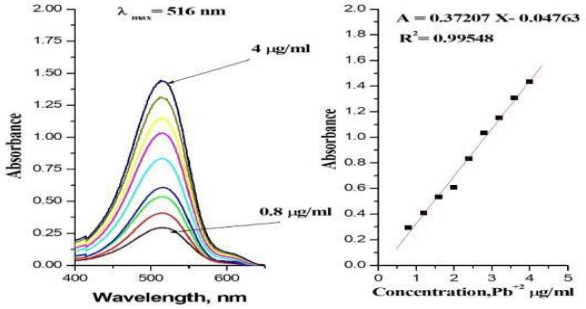

Fig 3: Show the absorption spectrum and standard calibration curve of pb (II)

The safe permissible limit for copper and zinc residues, as outlined by the Australian Nuclear Safety Authority (ANZFA, 2001) was found to be exceeded in 100% of the chicken shawarma samples that were analyzed. Table 3. Copper plays a crucial role in bone formation, skeletal mineralization, and the preservation of connective tissue integrity. It is an

essential component of many enzymes. Copper is essential for good health, but very high intake can cause health problems such as liver and kidney damage (Services, 2002).

In high concentrations, copper can potentially pose a threat to public health (Brito et al., 1990). According to Macrae et al. (1993), excessive copper accumulation in the liver can cause cirrhosis or hepatitis as well as a hemolytic crisis that resembles acute copper poisoning. In humans, 10–30 mg of oral copper ingested from foods stored in copper vessels may cause intestinal discomfort, headaches, and dizziness.

Table 3: The concentration of different studied elements

Sample	Concentration of Cu^{+2}, ppm	Concentration of Fe^{+3}, ppm	Concentration of pb^{+2}, ppm
1	6.79	0.8626	0.6539
2	10.13	0.8730	0.6021
3	9.84	0.8316	0.5435
4	9.60	0.7800	0.7249
5	8.03	0.6973	0.4516
6	10.91	0.8006	0.5612
7	7.98	0.8110	0.4247
8	8.18	0.7593	0.2640
9	5.98	1.6066	0.3808

However, it was not possible to rule out other potential sources of the metal during baking, such as the baking flour or additional water. Food vendors at terminals typically shop at a variety of bakeries, making it difficult to pinpoint the origins of the samples. The most likely and significant source of lead in the food samples under analysis may have been atmospheric deposition of lead compounds from vehicle exhaust. (Awofolu, 2004).

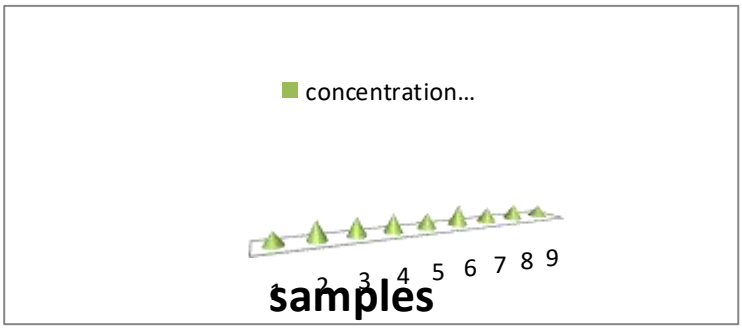

Fig. 4. Show a comparing between copper content in different samples

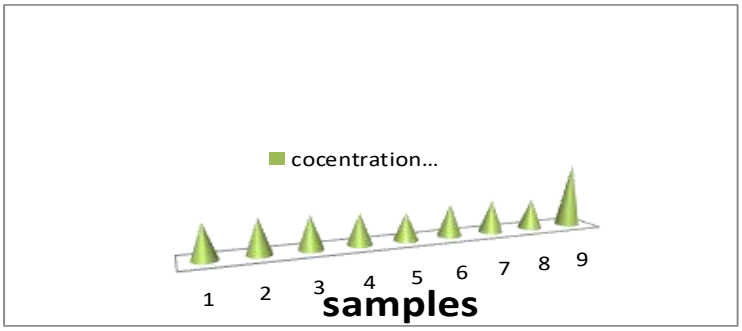

Fig 5. Show a comparing between iron content in different samples

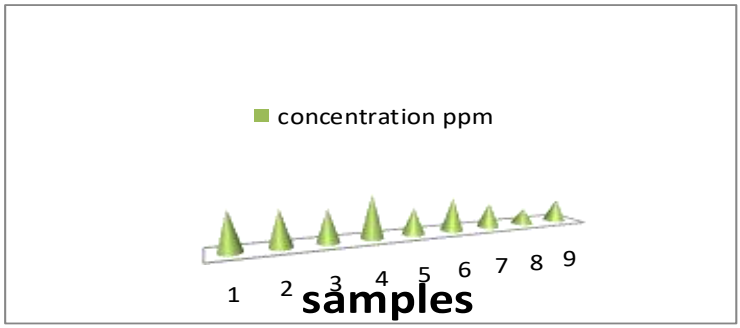

Fig. 6: Show a comparing between lead content in different samples

Table 4: Statistical analytical results of heavy metals and trace elements residues (ppm) in examined chicken Shawarma (n=9).

Elements	No. of examined samples	Minimum µg/ml	Maximum µg/ml
Fe	9	0.6973	1.6066
Cu	9	5.98	10.91
Pb	9	0.2640	0.7249

Table 5: Acceptability of examined meat and chicken Shawarma for toxic heavy metals and trace elements residues (ppm) in examined chicken Shawarma (n=9).

Metals	Maximum permissible limits (ppm)	Unaccepted chicken shawarma	
		No	%
Cu	200		.
Pb	0.1	9	100

Conclusion:

The results obtained from analyzing chicken shawarma sold along highways, main roads and traffic intersections in the study area (Al-Bayda) indicated that the concentrations of heavy metals such as lead and copper were higher than those inside the restaurants. Lead contamination scores were the highest.

Other metals such as copper also showed a high degree of pollution in chicken shawarma purchased from highways and traffic intersections, and for this reason, it can be expected that the sources of pollution of these metals such as lead and copper. The lead is likely from anthropogenic sources. Hence it can be said that these minerals found in chicken shawarma purchased along highways and traffic junctions originate from waste streams from atmospheric deposition emissions from vehicular traffic. Hence the overall levels of lead contamination are those that require closer monitoring in the area under study.

Recommendations

- There are numerous ways for owners of cars and trucks to mitigate the impact of automotive pollutants on the environment. Electric, hybrid, and other clean, fuel-efficient vehicles have the least impact, but old, poorly maintained cars are the main culprits
- Rationalizing human consumption of fast foods and dispensing with healthy foods prepared at home
- Follow-up of the relevant government agencies for restaurants and prevent them from preparing food outside restaurants

We recommend conducting chemical analyzes on the rest of the fast foods and using other techniques

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Histopathological Changes Induced by Heavy Metals (Lead and Cadmium) in *Siganus rivulatus* fish Collected from the Benghazi Sea Port

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Abstract:

The marine pollution is problematic and its impacts are having devastating effects on marine resources and the ecosystems. *Siganus rivulatus* (*S. rivulatus*) one of five species of Siganidae. The objective of this study was to determine how the heavy metals lead (Pb) and cadmium (Cd) affected the histological structure of the fish *S. rivulatus's* liver and gills. In September 2020, fish was taken from Benghazi Sea Port. Gills and liver tissues were removed for histopathological investigation. The results of this study illustrated in gills section of *S. rivulatus* showed extensive hypertrophy leading to severe lamellar fusion of secondary lamellae and congestion in gill lamellae. Also, secondary lamellae are irregular and curved with vasodilation and epithelial lifting. Hyperplasia of chloride cells revealed cleavage and congestion. Moreover, substantial lifting of the lamellar secondary, leukocyte infiltration, respiratory epithelium sloughing, congestion and ballooning dilatation of primary lamellae were seen. Chloride cell hypertrophies with filament chondrocytes, hypertrophic fusion lamellar of gills were seen. Examination of liver sections from *S. rivulatus* revealed interstitial hemorrhage, congestion, and some hepatocytes with larger, atypical nuclei. The findings also revealed certain hepatocytes to have vacuoled and the hepatic sinuses to have narrowed. Also, areas of fatty degeneration were noticed. Moreover, odema in some cells were indicated, beside necrosis and congestion of central vein surrounded by areas of enlarged hepatocytes containing hydropic degeneration. Dilatation with congestion of blood vessels and blood sinusoids, some of hepatocytes lost their boundaries. The results of this study confirm that minimal effects of Pb and Cd on histological structure of gills and liver tissues in fish organs.

Keywords: Histopathology, Gills, Liver, Muscle, *Siganus rivulatus*.

Introduction:

The Mediterranean Sea is a semi-enclosed and roughly isolated marine ecosystem, placed at amid-latitude (Robinson *et al.*, 2001). Five species of Siganidae are known in the northwestern area of Red Sea (Hashem, 1983). Nonetheless, the nation's economy remains heavily depends on the fishing industry, which also sustains a sizable export sector (Food and Agriculture Organization (FAO), 2005). *Siganus rivulatus* (*S. rivulatus*) (Figure 1) known with other names for instance Rivulated rabbitfish, Surf parrotfish or the Marbled spinefoot. *S. rivulatus* is a herbivorous, gregarious, demersal fish that typically ranges in length from 5 to 25 cm. Body of fish is oval and laterally compressed and its length is 2.7-3.4 times its depth (Stefani *et al.*, 2012).

Figure (1): Morphological of *Siganus rivulatus*

According to several studies by Au (2004), Van der Maaten and Hinto (2008), and Schlenk *et al.* (2008), histopathological biomarkers are frequently employed in fish environmental observation studies. In teleost fish, the liver serves as the main organ for the biotransformation of organic xenobiotics (Hinton *et al.*, 2001). Also, several studies by Myers *et al.* (1998); Stehr *et al.* (2004); Johnson *et al.* (2008) demonstrated that exposure to xenobiotics caused hepatic lesions in different fish species. In addition, Thophon and Pokethitiyook (2004) demonstrated that in certain organs, Cd caused pathological alterations of differing degrees of severity. Furthermore, greater dosages of Cd were reported to produce apparent exterior lesions on the livers of *Cyprinus carpio*, *Carassius auratus*, and *Corydoras paleatus*, including coloring and necrosis, by Çavaş and Ergene-Gözükara (2005). Additionally, Giari *et al.* (2007) demonstrated that Cd induced endoplasmic reticulum swelling, also known as cloudy swelling, in the kidney of *Dicentrarchus labrax* and epithelial swelling of the renal tubules. The histopathological changes caused by heavy metals were observed in gills, liver, kidneys, gonads and other organs of fish (Monteiro *et al.*, 2005; Saxena and Saxena, 2008;

Deshpande *et al.*, 2011; Deore and Wagh, 2012). According to El-Ghazaly *et al.* (2006), pollutants damaged the gill structure of *S. rivulatus*, which was taken from the Saudi Arabian coast of the Red Sea. These damages included pillar cell destruction, epithelia lifting, and chloride cell growth in the basilamellar regions of the gill. Abdel-Aziz *et al.* (2006) showed hepatocyte vacuolization, ballooning degeneration cellular, coagulative necrosis, cellular infiltration, granuloma inflammation and bile duct proliferation. Gills of *S. rivulatus* taken from Suakin site carry out epithelial lifting, hyperplasia and hypertrophy of the respiratory epithelium, lamellar fusion and aneurysms in the gills. Furthermore, the liver showed hepatocytes with hypertrophy, cytoplasmic and nuclear hypertrophy, melanomacrophage aggregates, bile stagnation and one case of focal necrosis. In addition, the lesions were comparatively more severe (Mohammed *et al.*, 2016). Eldurssi *et al.* (2023) showed normal condition factor, also, positive relation between body weight, head length, standard length and total body length were presented. Conversely, the mean values of Pb and Cd were dispersed in all tissues. In addition, Eldurssi *et al.* (2023) reported that highly significant relationships between standard body length and Pb concentration in gills. Thus, this study was designed to evaluate the marine pollution in Benghazi Sea Port including histopathological changes in gills and liver of the fish *S. rivulatus*.

Materials and Methods:

Description of the study area (Benghazi Sea port):

The Benghazi Sea Port (Figure 2) is a fantastic natural commercial and fishing port with an oil shipping terminal. In the surroundings of the city, municipal and untreated sewage water is released directly into the sea. Rainwater that falls on the city's numerous rubbish disposal sites eventually finds its way into the sea. These factors contribute to a slew of environmental issues in the seas surrounding the city.

Figure (2): Benghazi Sea port (Abmdas, 2018)

Collection of fish samples:

Fish samples of *S. rivulatus* mean body weight (158.9 g) were collected from Benghazi Sea port by fisherman using fishing net at same tame. The samples were kept directly in ice in plastic tanks and samples were transported to the marine biology research lab. of Zoology Department, Faculty of Science, University of Benghazi.

Preparation of tissue samples

Upon opening the abdominal cavity during the sacrifice, the livers and gills were quickly removed, cleaned in saline to get rid of any blood or other debris, and then dried on filter paper. For histological analysis, tissue samples from the liver and gills were preserved in 10% neutral buffered formalin solution. Liver and gill specimens were dehydrated using increasing concentrations of ethyl alcohol (70%, 90%, and 100%), cleaned in xylene, then impregnated and embedded in paraffin wax after being fixed in 10% buffered neutral formalin solution (Lillie, 1954). Using a rotary microtome, serial sections five microns thick were created, and for general histological analysis, Harris's hematoxylin and eosin stain (Harris, 1900) was applied.

Results:

Histopathological examination of gills section of *S. rivulatus* showed extensive hypertrophy leading to severe lamellar fusion of secondary lamellae and congestion in gill lamellae (Figure 3). Also, secondary lamellae are irregular and curved, with vasodilation, epithelial lifting. Hyperplasia of chloride cells revealed cleavage and congestion, beside, epithelial lifting and hyperplasia of chloride cell (Figure 4). Moreover, substantial lifting of the lamellar secondary, leukocyte infiltration, respiratory epithelium sloughing and congestion, ballooning dilatation of primary lamellae (Figure 5). Chloride cell hypertrophies with filament chondrocytes, hypertrophic fusion lamellar of gills were seen in figure (6). Inspection of liver sections in *S. rivulatus* displaying interstitial hemorrhage and congestion, as well as some hepatocytes has enlarged nuclei with irregular shape (Figure 7). Hepatocyte vacuolation and hepatic sinus narrowing. Also, areas of fatty degeneration were noticed (Figure 8). Moreover, odema in some cells were indicated, beside necrosis and congestion of central vein surrounded by areas of enlarged hepatocytes containing hydropic degeneration (Figure 9). Dilatation with congestion of blood vessels and blood sinusoids, some of hepatocytes lost

their boundaries (Figure 10). The cytoplasm of some cells are pale become vacuolated with degenerating hepatocytes and hydropic swelling, As well as, the blood vessel's endothelial linings had somewhat thickened (Figure 11).

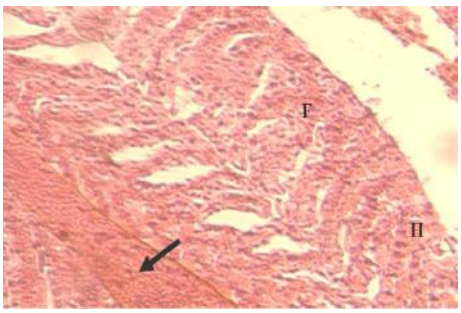

Figure (3): Photomicrograph of a section of gill in fish (*Siganus rivulatus*) showing extensive hypertrophy (H) leading to severe lamellar fusion of secondary lamellae (F) and congestion in gill lamellae (Arrow) **(H & E, X400)**.

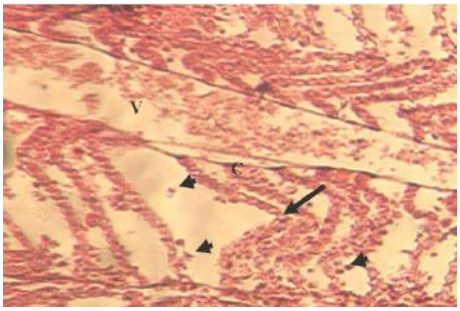

Figure (4): Photomicrograph of a section of gill in fish (*Siganus rivulatus*) showing irregular and curled secondary lamellae (Arrow), with vasodilatation (v), epithelial lifting (Head arrows) and hyperplasia of chloride cell (C) **(H & E, X400)**.

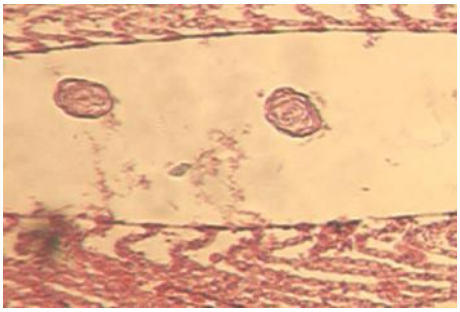

Figure (5): Photomicrograph of a section of gill in fish (*Siganus rivulatus*) showing loss of lamellar architecture organization and ballooning dilatation of primary lamellae **(H & E, X400)**.

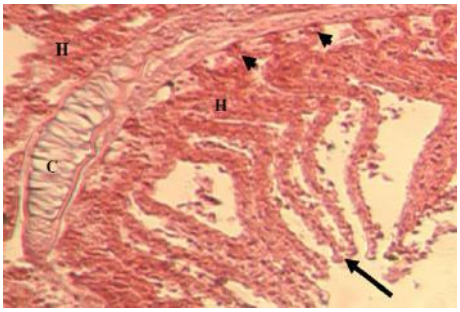

Figure (6): Photomicrographs of sections of gill in fish (*Siganus rivulatus*) showing damage and congestion was prominent in gill lamellae. Notice lamellar fusion and hyperplasia (H) of the twisted irregular secondary lamellae with blunt ends (Arrow). Hypertrophy of chloride cell (Head arrows) with filament chondricytes (C) **(H & E, X400)**.

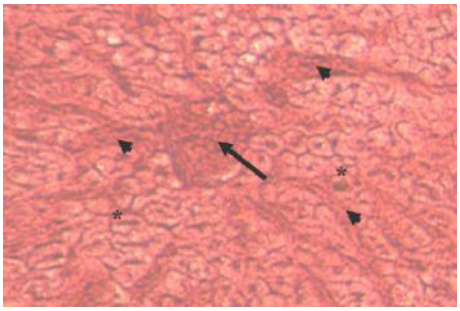

Figure (7): Photomicrograph of a section of liver in fish (*Siganus rivulatus*) showing interstitial hemorrhage (Head arrows) and congestion (Arrow) some hepatocytes have enlarged nuclei with irregular shape (*) **(H & E, X400)**.

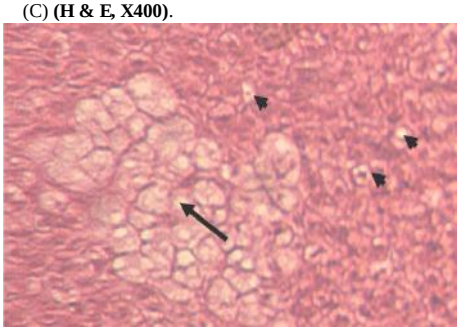

Figure (8): Photomicrograph of a section of liver in fish (*Siganus rivulatus*) showing narrowing of hepatic sinuses and vacuolation of hepatocytes (Head arrows). Note area of fatty degeneration (Arrow) **(H & E, X400)**.

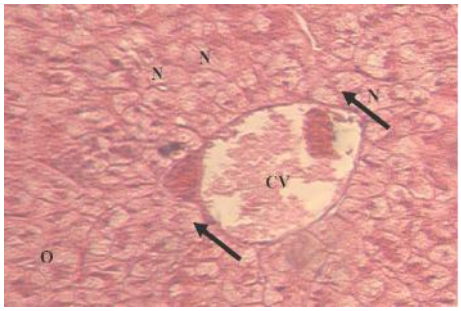

Figure (9): Photomicrograph of a section of liver in fish (*Siganus rivulatus*) showing odema (O), necrosis (N) and congestion of central vein (CV) surrounded by areas of enlarged hepatocytes containing hydropic degeneration (arrows) **(H & E, X400)**.

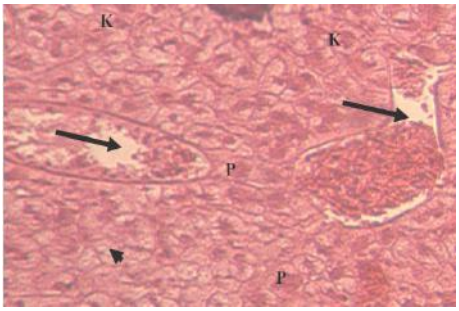

Figure (10): Photomicrograph of a section of liver in fish (*Siganus rivulatus*) showing dilatation with congestion of blood vessels (arrows). Note odema (O) and series of necrosis [pyknotic (P), karyorrhexis (K) and karyolysis (Head arrow)] **(H & E, X400)**.

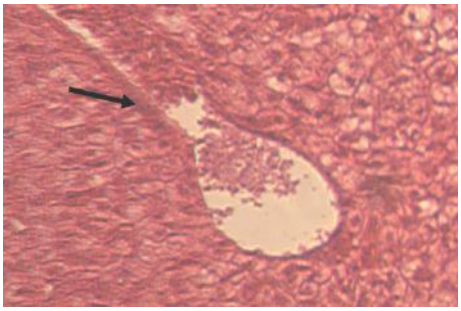

Figure (11): Photomicrographs of sections of liver in fish (*Siganus rivulatus*) showing Loss of hepatic architecture with degenerating hepatocytes and hydropic swelling, pale cytoplasm become vacuolated, dilated and congested central vein and pyknotic nuclei. The endothelial linings of blood vessels were slightly thickened (Arrow) **(H & E, X400)**.

Discussion:

The present study showed that extensive hypertrophy leading to severe lamellar fusion of secondary lamellae and congestion in gill lamellae. Besides, the twisted irregular secondary lamellae with blunt ends have lamellar fusion and hyperplasia with vasodilation. Similar results have been reported by (Mallatt, 1985; Mazon *et al.*, 2002) they showed hypertrophy fusion lamellar, as well as partial separation of some secondary lamellae. According to the results of the current investigation, Triebskorn et al. (2008), who described epithelial lifting, proliferation of primary and secondary lamellae epithelial cells, and hyperplasia of mucous cells in the gills of heavy metal-polluted *Coilia nasus* and *Mugil cephalus* from River Mures, Western Romania Likewise, our results illustrated gills of *S. rivulatus* were showed hyperplasia of in some chloride cells revealed cleavage and congestion. Also, other

chloride cell suffered hypertrophy. This finding agrees with Fivelstad *et al.* (2003) they showed hyperplasia of chloride cells. Mucus cell hyperplasia that is somewhat widespread, along with an increase in mononuclear cellularity. In addition, Fivelstad *et al.* (2003) reported that lesions on the gills included gill filaments with hypertrophy of chloride cells and adhesion of lamellae at the base and distal ends. Chloride cell proliferation is thought to be a compensatory reaction to ion loss; consequently chloride cell hyperplasia could be a helpful biomarker of adaptation (Hinton *et al.,* 1992). In addition, results of this study showed inspection of liver sections in *S. rivulatus* displaying interstitial haemorrhage and congestion, as well as some hepatocytes have enlarged nuclei with irregular shape and vacuolation of hepatocytes. Also, areas of fatty degeneration and odema in some cells were noticed. Moreover, hydropic degeneration, pyknotic nuclei and most of hepatocytes lost their boundaries were detected. Marked necrotic of large areas in hepatocytes were discovered. The cytoplasm of some cells are pale become vacuolated with degenerating hepatocytes and hydropic swelling. This finding similar to results that observed in *S. canaliculatus* by Agamy (2012), who noted that lipid accumulation, pyknotic nuclei, vacuolation of hepatocytes, larger, irregularly shaped nuclei, and areas of hepatocyte necrosis all manifest as large, spherical, clearly defined lipid droplets inside the hepatocytes. Also, agree with Mohammed *et al.* (2016) in *S. rivulatus*; Mahmoud and Abd El-Rahman (2017) in *Mugil capito* and Kaur *et al.* (2018) in *Labeo rohita*, they showed liver lesions included degeneration in hepatocytes, fatty degeneration, haemorrhage, oedema and congestion of blood sinusoids. Furthermore, our results similar to results that indicated by Mohammed *et al.* (2016) They demonstrated that the hepatocytes in *S. rivulatus* grew pale and balloon-like. This is most likely the result of water and electrolyte buildup as a result of decreased membrane permeability after autolysis (Burkitt *et al.,* 1996). Hepatocyte vacuolization may be a sign of an imbalance between the pace at which chemicals are released into the systemic circulation and the rate at which they are synthesized in parenchymal cells (Gingerich, 1982). Also, our results demonstrating narrowing of hepatic sinuses in some tissues and dilated in other blood sinusoids with congestion in some hepatic sinuses. There was dilatation along with congestion and blood vessel bleeding, and the central vein was congested around regions of larger hepatocytes. As well as, blood vessel endothelium linings have somewhat thickened. The similar results were investigated in work by Agamy (2012)

who displayed regions of increased hepatocytes encircling a clogged central vein. Additionally, there was a modest thickening of the blood vessel endothelial linings. Dilation of blood vessels and degeneration were observed in *Tilapia mossambicus*, *Clarias gariepinus* and *Mugil cephalus* from Nile River (Ibrahim and Mahmoud 2005). Similar results showed by Agamy (2012) in *S. canaliculatus*, they observed hepatocyte vacuolation, necrosis, and constriction of the hepatic sinuses. Additionally, Gaber (2007) found that fish treated with Pb and Cd had severe sinusoidal congestion and varied degrees of liver deterioration. After the stressor is removed, the alteration's ability to be reversed is reflected in the significance factor. Degenerative modifications, such as general necrosis, are assigned the highest priority factor three because they are thought to be a direct result of toxicants, are typically irreversible, and may eventually result in the loss of organ function entirely or partially (Agamy, 2012).

Conclusion:

The study's findings, taken together, demonstrate that Pb and Cd have no effect on fish organs. Additional marine contaminants may be the source of histopathological alterations. The findings of this study suggest that additional research be done to concentrate on other metals found in fish tissues.

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Antibacterial Activity of *Ulva lactuca* Extracts from the Coast of Sousa City (Libya)

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Abstract:

During the past several years, the indiscriminate use of antibiotics has resulted in the emergence of microbial resistance to common antibiotics, which ultimately threatens the effectiveness of treatment for infections caused by bacteria. This forced researchers to search for novel antimicrobial substances from various sources, e.g., seaweeds. In the present study, the green alga *Ulva lactuca* has been taken and evaluated for its antimicrobial activity against pathogenic bacteria, including two species of Gram-negative bacteria (Escherichia coli and Salmonella ssp.) and Gram-positive bacteria (*Streptococcus pneumoniae* and *Staphylococcus aureus*). The green algae *Ulva lactuca* was extracted separately with ethanol, acetone, and water as solvents by using a rotary evaporator. All extracts revealed antimicrobial activity by using the disc diffusion method. The obtained results of this study showed that the average diameter of the inhibition zones resulting from the effect of algae extracts against four types of bacteria ranged between 2 and 15 mm. Ethanol extract showed the strongest activity against bacteria compared to the other solvents used. Also, the rate of the lowest inhibitory concentration of the tested bacteria was in water. These results give an indication of the presence of compounds in marine algae showing antibacterial activities and their most promising applications.

Keywords: Anti-bacterial, Pathogenic bacterial, *Ulva lactuca*, *Staphylococcus aureus*, *Escherichia coli*.

Introduction:

In the marine environment, marine algae have the potential to be renewable resources. Some 6,000 species of seaweed have been identified and categorized into three types depending on color: chlorophytes, pheophytes, and rhodophytes (Khaled *et al.*, 2012). Because of seaweed's nutritional and medicinal benefits, awareness of it has grown dramatically on a global scale.

Marine herbs of nutritional value have been used as fresh or dried vegetables or components in a variety of ready foods, and in nutritional terms, they are low-calorie foods with a high concentration of metals (Mg, Ca, P, K, and I), vitamins, proteins, carbohydrates, and a low concentration of fat. (Ambreen *et al.*, 2012). Infectious disorders are among the many natural anti-microbial chemicals that have been found in the maritime environment compared to Earth (Ireland, 1988), and infectious diseases are one of the leading causes of high human morbidity and mortality worldwide, particularly in underdeveloped nations (Waldvogel, 2004). The indiscriminate use of antibiotics has led to a common occurrence of drug-resistant illness-causing bacteria, and severe infections have caused a major increase in disease severity in recent years. Antibiotic resistance of bacteria and fungi is one of the biggest rising health care problems around the globe, and it is becoming an increasingly greater challenge in the delivery of treatment against resistant pathogenic bacteria (Sieradzki *et al.*, 1999). The development of novel alternatives has become necessary due to the limited efficacy and resistance of antibiotic infections. (Smith *et al.*, 1994; Ireland, 1988). Among the many sources of novel, structural, and biological active compounds (Ely *et al.*, 2004). There are numerous reports of several pathogens inhibiting large sea algae from causing viral, microbial, and innate diseases. Various sea algae extracts have been demonstrated to show antibacterial activity against positive and gram-positive bacteria (Lima-Filho *et al.*, 2002). Marine herbs have also attracted considerable interest in the pharmaceutical industry, given the wide variety of species available and the ability to produce secondary receptors with various pharmaceutical activities such as toxic cell activity, anti-reproductions, anti-microbials, antivirals, anti-sensitives, anti-coagulants, and antioxidants. (Bouhlal *et al.*, 2011; Zubia *et al.*, 2009). As an aid to protecting themselves from other living organisms in their environment, large algae produce a variety of chemically active metabolites, including alkalis, polyketides, peptides, scaroids, fluorotanes, dyterboids, steroids, quinone, fat, and glycerol, which contain a wide range of biological substances. (Al-Saif *et al.*, 2014). However, the effectiveness of seaweed antimicrobials lies in the efficiency of the extraction method (TÜney *et al.*, 2006), the types of algae (Vlachos *et al.*, 1997), and the solvents used (Cox *et al.*, 2010). Some of the materials extracted from algae were used in the pharmaceutical industry, such as iodine, carotine, glycerine, genes, and caragnan (Kharkwal *et al.*, 2012; Mahadhebi *et al.*, 2011). The main objective of the study was to assess the

anti-microbial activity of extracts from green algae and, in particular, local Ulva algae against five types of pathogenic bacteria.

Materials and Methods:

Study Area

The algae samples were collected from the port area of Sousa in March 2022. Figure 1 illustrates the area of the port of Sousa. The port is located at (32 N° and 21 E°).

Figure 1: The port area of the city of Sousa

Sample collection methods

The manual harvesting of seaweed has been practiced for centuries and is still common for naturally growing species in coastal areas (van den Burg and others, 2013). Plants, impurities, and salts are carefully removed in the laboratory using tap and distilled water. Marine grasses have been examined with the naked eye or two-eye microscope and identified according to the following (Aleem, 1993; Demirbas, 2010). The sample collected, *Ulva lactuca*, shown in figure 2, was defined in Botany Department, Faculty of Sciences, University of Omar Al-Mukhtar.

Figure 2: *Ulva lactuca* algae

Preparation of samples

The samples were repeatedly washed with seawater to dispose of plankton and dust, then with distilled water (Caccamese, 1980). It was dried in the air for seven days, as shown in Figure 3, and then placed in the oven at 40°C for 20-30 minutes to remove the remaining moisture. The samples were then well ground with an electric mill and stored well for use (Rao and Parekh, 1981).

Figure 3: Methods for drying algae samples used in the study

Antibacterial activity test

Preparation of algae extracts

Twenty grams of dried algae powder were recovered at 100 ml with three different solvents: methanol (water, 80:20 volume/volume), ethanol (water, 80:20 volume/volume), and acetone (water, 80:20 volume/volume). For two days at room temperature, the solution was filtered through the sterile filter paper (Whatman No. 1). The resulting solvent extracts were vaporized by the rotor evaporation، and then stored at -20°C until testing (Alghazeer *et al*., 2013).

Bacterial strains

The Microbiology Department of El-Beida Hospital provided two strains of Gram-positive bacteria (*Staphylococcus aureus* and *Stryptococcus pyogenes*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Salmonella spp*.).

Agar well diffusion method

The agar-well diffusion method was followed to determine the antimicrobial activity of Mueller-Hinton agar (MHA). Plates were swabbed with sterile cotton swabs, and respective bacteria wells with a diameter of 4mm were made in each of these plates using a sterile cork borer with about 100 µm of

different organic solvents. Added sterile syringes into wells. The plates were incubated at 37°C for 18-24 hours for bacteria. Zones of inhibition were measured using a meter ruler as described by Drago *et al*. (1999).

Results and Discussion

Evaluation of antibacterial activity

The antibacterial efficacy of the marine algae extract was studied using different solvents (water, ethanol, and acetone) by agar-well diffusion against four bacterial strains. The results in Table 1 show that all marine algae extracts from different solvents have demonstrated their effectiveness against bacterial strains.

Table 1: The effect of organic solvent extracts from the green algae *Ulva lactuca*

Pathogenic bacteria	Inhibition zone (in mm) of solvent extracts of *ulva lactuca* algae		
	Acetone	Ethanol	Water
Salmonella spp.	2	6	NA
Escherichia coli	10	10	NA
Streptococcus pyogenes	12	15	3
Staphylococcus aureus	7	13	NA

NA= NO Activity

Table 1 shows that the effect of the organic solvent extracts from *Ulva* Lactic algae on the growth of bacteria was recorded with the highest ethanol inhibition on *Streptococcus pyogenes* at 15 mm, as shown in figure 6. Less inhibition from the acetone extract on *Salmonella spp*. at 2 mm, as illustrated in figure 7, and no inhibition of *Salmonella spp*., *E. coli*, and *Staphylococcus aureus* by the water extract, as shown in figures (7,8 and 9). In the marine environment, marine algae have the potential to be renewable resources. Some 6,000 species of seaweed have been identified and categorized into three types depending on color: chlorophytes, pheophytes, and rhodophytes (Khaled *et al*., 2012). Because of seaweed's nutritional and medicinal benefits, awareness of it has grown dramatically on a global scale. Marine herbs of nutritional value have been used as fresh or dried vegetables or components in a variety of ready foods, and in nutritional terms, they are low-calorie foods with a high concentration of metals (Mg, Ca, P, K, and I), vitamins, proteins, carbohydrates, and a low concentration of fat. (Ambreen *et al*., 2012). Infectious disorders are among the many natural anti-microbial chemicals that have been found in the maritime environment compared to Earth (Ireland, 1988), and infectious diseases are one of the leading causes of high human

morbidity and mortality worldwide, particularly in underdeveloped nations (Waldvogel, 2004). The indiscriminate use of antibiotics has led to a common occurrence of drug-resistant illness-causing bacteria, and severe infections have caused a major increase in disease severity in recent years. Antibiotic resistance of bacteria and fungi is one of the biggest rising health care problems around the globe, and it is becoming an increasingly greater challenge in the delivery of treatment against resistant pathogenic bacteria (Sieradzki *et al.*, 1999). The development of novel alternatives has become necessary due to the limited efficacy and resistance of antibiotic infections. (Smith *et al.*, 1994; Ireland, 1988). Among the many sources of novel, structural, and biologically active compounds (Ely *et al.*, 2004), there are numerous reports of several pathogens inhibiting large sea algae from causing viral, microbial, and innate diseases. Various sea algae extracts have been demonstrated to show antibacterial activity against positive and gram-positive bacteria (Lima-Filho *et al.*, 2002). Marine herbs have also attracted considerable interest in the pharmaceutical industry, given the wide variety of species available and the ability to produce secondary receptors with various pharmaceutical activities such as toxic cell activity, anti-reproductions, anti-microbials, antivirals, anti-sensitives, anti-coagulants, and antioxidants. (Bouhlal *et al.*, 2011; Zubia *et al.*, 2009). As an aid to protecting themselves from other living organisms in their environment, large algae produce a variety of chemically active metabolites, including alkalis, polyketides, peptides, scaroids, fluorotanes, dyterboids, steroids, quinone, fat, and glycerol, which contain a wide range of biological substances. (Al-Saif *et al.*, 2014). However, the effectiveness of seaweed antimicrobials lies in the efficiency of the extraction method (TÜney *et al.*, 2006), the types of algae (Vlachos *et al.*, 1997), and the solvents used (Cox *et al.*, 2010). Some of the materials extracted from algae were used in the pharmaceutical industry, such as iodine, carotine, glycerine, genes, and caragnan (Kharkwal *et al.*, 2012; Mahadhebi *et al.*, 2011). The main objective of the study was to assess the anti-microbial activity of extracts from green algae and, in particular, local Ulva algae against five types of pathogenic bacteria.

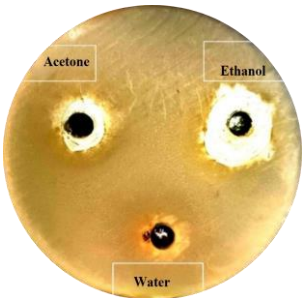

Figure 6: Effects of *Ulva lactuca* extracts against *Stryptococcus* pyogenes

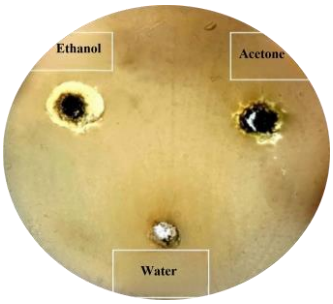

Figure 7: Effects of *Ulva lactuca* against extracts *Salmonella spp*.

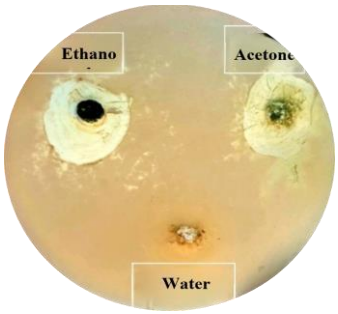

Figure 8: Effects of *Ulva lactuca* extracts against *Staphylococcus aureus*

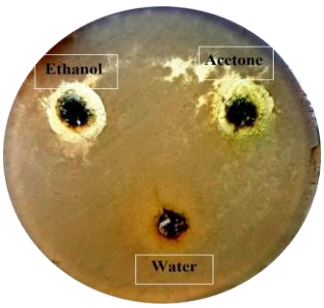

Figure 9: Effects of *ulva lactuca* extracts against *Escherichia coli*

Conclusion

1. From the present study, we can conclude that the *ulva lactuca* selected for this study has a potential source of bioactive compounds. This proves that sea algae contains biologically active compounds that are effective in resisting the growth of the nurse bacteria, whether the bacteria are positive or negative.
2. Marine algae is becoming increasingly important in pharmaceutical manufacturing industries around the world.

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Effect of Flame Retardants and 1% Stabilizer on melting and dripping behaviour of thermoplastic polymers due to the furnace test
Part 1: Furnace modulated and calibrated

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Abstract:

The goal of this study was to comprehend the mechanism underlying the combination of various flame retardant (FR) actions on melting, dripping behavior, and their moderation. Polypropylene polymer was chosen to be blend in a twin-screw extruder with the flame retardants and an additive, which is a 1% Stabilizer, to investigate Polypropylene's melting, dripping moderation to reduce it by studying its melting behaviour and dripping. Melting and dripping behaviour tests which are known as Furnace test melting and dripping tests conditions were applied in this study after furnace setup developed, for furnace test. The development is set up by following 3 stages, which are applied modulated whilst adjusting the furnace set up, ready for the mean furnace melting and dripping test in this experimental work. PP polymer samples at various furnace temperatures was examined. The relationship between melting and dripping behaviour could be obtain and proven by experimental results. A degree of degradation may be predicted by analyzing the polymers and their molten drops thermo gravimetrically (TGA) and differential thermal analysis (DTA). To determine the relationship between the melting and dripping behaviors of thermoplastic polymers, the values obtained from the two methods have been compared in order to comprehend the melt dripping and degradation behavior of polymers. The majority of earlier research on melt and dripping behaviors focused on modeling the thermal process and studying fire operating conditions..

Keywords: Materials treatment, melting of polymer, dripping of polymer, polypropylene polymer, flame retardants.

Introduction:

This series of studies is a part of a larger project exploring the production of fire-retardant synthetic nano/micro composite fibres. This present work is a part this series of studies which are concentrated on burning/ melting / dripping behaviors of thermoplastic polymer such as Polypropylene (PP) which is a useful commodity polymer mainly used in clothing, furniture, floor coverings, medical, geotextiles and automotive applications, due to its low cost, light weight, good mechanical properties and low reactivity towards other chemicals (Quincy *et al.,* 2007). Polypropylene (PP) had higher values for tensile strength at break, the Polypropylene polymer degradation occurs at high temperatures, the Polypropylene polymer (PP) Melting temperature is 174°C if PP is 100 % isotactic and the temperature of glass transition of PP polymer is -17°C. The primary benefit of PP is its exceptional resistance to numerous chemical solvents, acids, and bases due to its manufacturing as an addition polymer from the monomer propylene. But because of its entirely aliphatic hydrocarbon structure, it burns extremely quickly, producing a flame that is largely smoke-free and leaves no char behind (Quincy *et al.,* 2007). Reactive flame retardants react more difficultly with the structure's lack of polar groups. If added, the amounts of additive flame retardants needed to give products the necessary fire protection must be high (> 20% w/w). (Wang *et al.,* 2010; Mastura, 2018), this effects flame retardancy, which increases with increasing irradiation and vanishes with decreasing irradiation (Qin *et al.,*. 2005). Nevertheless, excessive addition levels complicate the processing of polymers, especially when it comes to extrusion into thin films or fibers. flame retardancy is sensitive to modification of the flame retardant (Wang *et al.,* 2010; Mastura, 2018) and flammability tests still require some amount of conventional flame retardants (Schartel *et al.,* 2006. Mastura, 2019). It is important to acknowledge that the utilization of flame retardants as additives results in a lower polymer content in the formulation when compared to the original polymer. In past works (Zhu *et al,* 2001; Qin *et al.,* 2005, Morgan *et al.,* 2007; Wang *et al.,* 2010; Mastura, 2018), they have demonstrated that nanoclays can be nanodispersed in polypropylene with a proper choice of compatibilizer, and the compounded polymer can be extruded into fibers (Xie *et al.,* 2001; Qin *et al.,* 2005). Nanoclays, although increase the thermal stability of polypropylene and helping in char formation (Zhu *et al.,* 2001; Mastura, 2018), they do not reduce the flammability of PP fibers to a large extent (Xie *et al.,* 2001). Clay, nanoclay, and a small amount of flame

retardant (5%) (Zhu *et al.,* 2001; Qin *et al.,* 2005; Schartel *et al.,* 2006; Morgan *et al.,* 2007; Quincy *et al.,* 2007;. Wang *et al.,* 2010; Mastura, 2019) when added together to PP containing certain compatibilizers, the extruded fibers could be self-extinguished. In the previous work, we have only used ammonium polyphosphate (Qin *et al.,* 2005; Schartel *et al.,* 2006, Mastura, 2018) However, we use a different type of phosphorus in this study. The primary goal of this research is to comprehend how various flame retardant types combine to affect the thermal stability of polypropylene. Using a twin-screw extruder, a variety of polypropylene samples with compatibilizer, clay, stabilizer, and various flame retardants (Table 1) have been compounded.

Materials and Methods:

The following materials were obtained from commercial sources and tested after blended with additives and FR

1- Polymer Preparation:

The 7 polypropylene samples composition (wt %) and additives were blended with 1% Stabilizer (Nor 116), and 5% FR as shown in (Table 1). However, the nanoclays which could be used (Cloisite 20A, and Southern Clay Products, USA) are montmorillonite clay modified with dimethyl, and dihydrogenated tallow quaternary ammonium chloride. This modified clay was chosen because of its nonpolar alkyl substituents. A Thermoelectron Prism Eurolab 16 twin screw extruder with a temperature profile over six heating zones between 179-1900C was used for compounding. The polymer samples (diameter 1.8 ± 0.2 mm) were collected before pelletising.

Table 1: Mass percentages of various components in the formulations where Stabilizer 1% is NOR 116.

Sample	*pp %*	*Clay 3%*	*Graft 1%*	*FR 5(%)*
1- PP-Nor 116	100	---	---	---
2- PP-APP 107	96	107	Polybond (pb)	---
3- *PP-NOR-NH*	91	107	Polybond	APP
4- PP FR 107	91	107	Polybond	[1]FR245
5- PP Amgard 107 NH	91	107	Polybond	Amgrad
6- PP 3OB	96	3OB	Polybond	---
7- PP APP 3OB	92	3OB	---	APP

Note: [1]*FR = APP, NH, FR245, also PP-NOR-NH is* PP 107, FR372 or *FR245.*

2- Melting and dripping behaviour experimental technique setup:

I have developed and industrialised the experimental technique setup for inspection and study of melt dripping behaviour of thermoplastic compounded polymers using an electric furnace as shown in (fig.1), then assisting in temperature measurement tests in this furnace test set up in 3 stages experimental work.

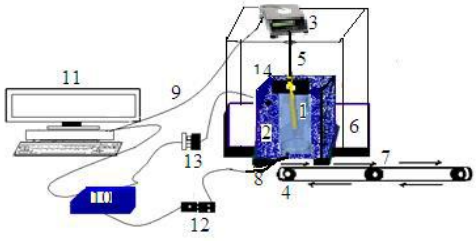

Fig.1: The outline of the planned experimental methodology, where 1- the polymer sample, 2- is the electric furnace, 3- the scale, 4- wells, 5- sample hooker wire, 6- the furnace stand, 7- the conveyor belt, 8- the electric wire set at 200°C, 9- wire to circle connections, 10- adjustable temperature controller, 11- personal computer, 12- two transistors, 13- electric point sours connection, 14- furnace cover.

A tube furnace, capable of moving upwards and downwards will be used in vertical position. This in fact contains of an 800-Watt home-grown, transportable electric furnace with an exhaust of 120mm length and 25mm diameter. The furnace is achieved by a temperature controller with adjustable temperature limit up to 600 - 610°C. Dynamic recording of the mass of the polymer sample (100 x 6 x 4 mm) is made by a digital mass balance connected to a computer. The sample is fixed, and a pre-heated furnace is raised on rails via a pulley arrangement until the bottom of sample is in the centre of the furnace. The melting drops are collected on an aluminium large strips foil placed on a conveyer belt placed beneath the furnace and moving at a pre-determined uniform speed, by known speed of the belt; the rate of melt dripping and the distance between one drop to the next will be measured. A constant rate of this speed is 11.2 cm/s. The melting drips they hit the foil so that the drops are kept separated from each other. By weighing the foil, before and after the test, also the total mass of drops produced can be evaluated. In addition, the number, thickness and diameter, also the distance and time between the drips, and additional, the pictures taking of the aluminium foil during and after the test to record the environmental effects on the dripping size and more (Hu, et. 2007). I calibrated the furnace using the three stages

experimental work which were before applying the furnace test which I will discuss in part 2 of this work. This paper (part 1) records and explains this work.

Results:

Furnace tube modified and adjusted:

The 3 stages of regulating and adjusting the furnace, to achieve the melting and dripping test correctly as follows:

1- The first stage

To find out the maximum overshoot for a range of temperature likely to be used for the furnace melt dripping test part 2, and this stage of the experiment running between 500°C and 600°C with 11 degree increments, and noting the minimum and maximum temperature, to find that the furnace overshoot by a certain level, this is to know what the temperature value to set the furnace at during the furnace test later, as recorded in fig.2 and table 2 (a, b) which I chose to find the furnace appropriate setting temperature,

Table 2 (a, b) shows the furnace minimum and maximum temperatures selected

Max. temperature	Min. temperature	Max. temperature	Min. temperature
512	496	513	496
529	506	522	506
531	516	532	516
541	526	542	526
550	537	551	536
565	545	562	546
570	557	571	556
582	566	580	566
589	576	590	576
598	586	599	587
610	596	610	596

(a) (b)

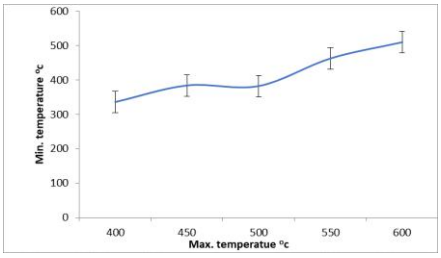

Fig.2: Shows the average of furnace minimum and maximum temperatures selected.

2- Second stage

By using thermocouple (T/C) type K, measured the temperature inside the furnace at 3 different positions of 4cm, 6cm and 8cm, (fig. 3), each of these positions tested at temperature degrees of 400,450, 500, 550 and 600°C, as a following results shown in table 3(a, b) and fig.4

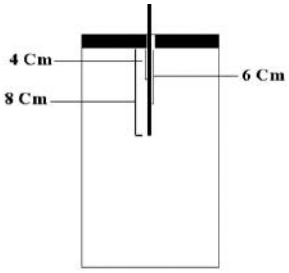

Fig.3: Shows the sketch of the 3 different positions of thermocouple place inside the furnace.

Table 3 (a, b): Recorded the melt dripping temperature of the Polymer plaques (size 100 x 6 x 4 mm) at 3different places inside the furnace (4, 6 and 8cm); to determine the electric furnace temperature settings for melt dripping experiment.

0	4cm	6cm	8cm
400°c	360.873	311.2699	334.5238
450°c	463.1746	264.7619	424.127
500°c	424.0476	343.5714	379.2064
550°c	502.0635	460	427
600°c	535	495.4762	500.6349

(a)

0	4cm	6cm	8cm
400°c	339	346	357
450°c	366	325	338
500°c	531	464	465
550°c	539	554	521
600°c	591	612	580

(b)

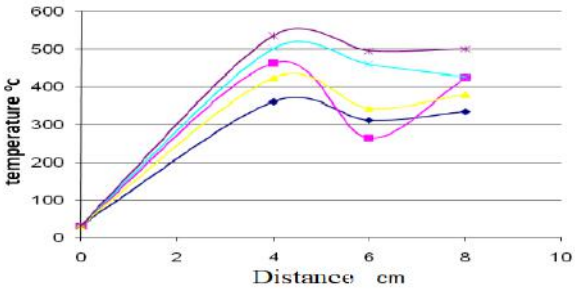

Fig. 4: Temperatures of molten drops and degradation behaviour at the 3 different places inside the furnace

3. Stage 3

3.1 Third stage, which was completed to find the auto-ignition temperatures of the standard PP polymer samples with the current samples standard sized 100 x 6 x 4 mm and the temperature values around 600 **°**C as stated in table 5.

Table 5: Stage 3.1; to find out the auto-ignition temperatures of the standard PP polymer samples with the current samples' stander and the temperature values around 600 °C

Sample / Temperature ^{0}c	Thickness mm	Width mm	Length mm
1 / 600	3	6	40
2 / 600	3	3	40

3.2 The data that is produced by these steps are as mentioned in the following table 6 and fig. 6, to collect advanced information to complete the part 2 of this work.

Table 6: shows the stage 3 (1.3 and 3.2) data to find out the auto-ignition temperatures of the standard PP polymer

Temp. °C / Position cm	400	450	500	550	600
At 4 cm	360.873	463.1746	424.0476	502.0635	535
At 6 cm	311.2699	264.7619	343.5714	460	495.4762
At 8 cm	334.5238	424.127	379.2064	427	500.6349

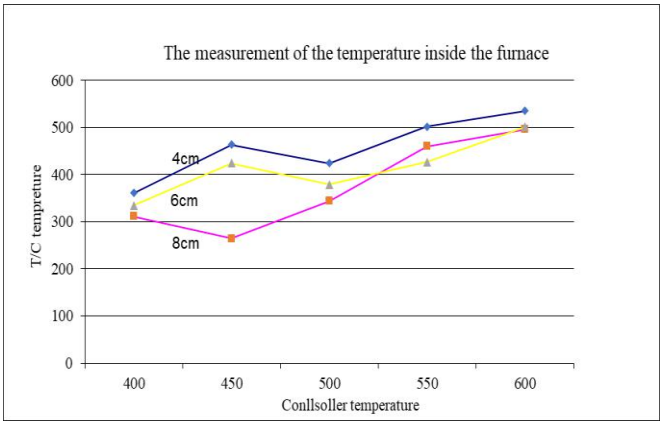

Fig. 6: shows the temperature recorded inside the furnace at 3 different positions

Resultant these stages, furnace appropriate setting temperature for the melt drop temperature, to avoid any ignites or burn sample, and I discovered that the temperature should be less than 600°C.

Discussion:

Recorded the melt dripping temperature of the Polymer plaques (size 100 x 6 x 4 mm) at the 3 different places inside the furnace (4, 6 and 8cm) as mentioned in table 3 (a, b); to determine the electric furnace temperature settings for melt dripping experiments in part 2, and I discovered that the temperature is less than 600°C as seen as in fig. 2. In addition, the collection data of molten drops temperatures and degradation behaviour at 3 different places inside the furnace as shown in fig. 4, to help understand the degradation to discuss and find out the physical properties such as the viscosity and more which are affected on melting and the drops behaviour to draw the relationship between melting and dripping behaviour of thermoplastic polymers and correlation pattern between the temperature and the distance, which that the melting temperature increases with the increasing of the distance (D) inside the Furnace. Also, the dripping temperature decreases with the decreasing of the distance within the furnace, the air effects the test results, which means if the belt situated at the bottom of the furnace is at a different distance from the furnace, which is the effect of the air (environment) between, which consequently will affect the results. The curves and data help to figure out the temperature inside the furnace to complete part 2, the next step of experiment, to find out the following:

The first drip time and size; using a digital camera. And secondly, count the total drips. The data and curve that are produced in this part 1 as mentioned in table 6 and fig.6, to collect advanced information to draw the relationship between melting and dripping behaviour of PP polymers. In furnace test the drops are collected on a known speed of conveyor belt for any belt length, the rate of melt dripping will be measured. The constant rate of this speed is 11.2 cm/s. According to part 2, this belt speed is calibrated for the distance measured between one drop to the next. The melting drips hit the foil so that the drops are kept separated from each other. By weighing the foil, before and after the test, also the total mass of drops produced can be evaluated. In addition, the number, size (diameter and thickness), shape, distance, and time between individual drops were evaluated by taking pictures of the aluminium foil during and after the test to record the environmental effects (air) on the

dripping size and more (Kempel *et al.,* 2010). In part 2 the melting and dripping behaviour were recorded using high speed video to measure the drop size (diameter), weight (balance), the distance and the time of one drop to the next were measured for collection of date about the physical properties such as viscosity and density. The furnace is heated rapidly to the required temperature (stage 1). When maximum temperature (less than 600°C) is reached, the sample is weighed, using a personal computer connected to a Oahus balance is recorded at a particular temperature, the mass loss % versus time can be plotted to draw the curve of this mass loss with time. The empirical results in part 2, will be finally compared with the experimental ones in order to observe the influence of heat on the sample itself as well as its physical properties.

Conclusion:

This part 1 of the present work for furnace modulated and calibrated to complete the furnace test within Part 2 to obtain and draw the relationship between melting and dripping behaviour of PP thermoplastic polymers due to the furnace test. In this part I found the data and results that are produced by these experimental steps are as mentioned in table 6 and fig.6, to collect advanced information to complete the part 2 of this work. And all these results are the leader for using the outline of the planned experimental methodology of furnace test analysing the melting and dripping of the thermoplastic compounded polymers strands was studied to draw the relationship between melting and dripping behaviour of PP thermoplastic polymers due to the furnace test in part 2. As a result of part 1 of the present study, the furnace was set to the appropriate setting temperature for the melt drop temperature, to avoid any ignites or burn sample in part 2 study, and I discovered that the temperature is less than 600 °C. Because of all these standing results of this current study (part 1), there is a greater chance to build a new scientific comparison with conclusion for the next studies. These results of furnace tube modified and adjusted (part 1), are the suitable test results to use for numerical method , in part 2, and for theoretical understanding of the scientific formulations. The numerical, applied in part 2, are the Particle Finite Element Method (PFEM), to the modeling of the melt flow behavior of thermoplastics method (Butler *et al.,* 2007).

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Phytochemical screening, total phenolic content and antibacterial activity of fruits, peels and seeds of some watermelon and muskmelon cultivars from Al-Marj region

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Abstract

This study investigated the content of phytochemical screening extracted by aqueous and ethanol solvents, total phenol compounds and antibacterial effects by agar well diffusion method of muskmelon and watermelon fruits, peels and seeds from four locations around Al-Marj town (Al-Jabal Al-Akhder) namely Farzogha, Botraba, Sidi Arhoma and Al-Ewilia locations. The results revealed that the phytochemical screening of aqueous extracts, that flavonoids, carbohydrates glycosides, cardiac glycosides and saponins were present in all parts of muskmelon and watermelon. While the phytochemical screening for ethanol extracts of watermelon and muskmelon parts showed that flavonoids, tannins, cardiac glycosides and alkaloids were present in all parts for ethanol extracts of watermelon and muskmelon, carbohydrates glycosides and saponins were, absent in all parts of muskmelon and watermelon. High percent of total phenol were found in the seeds followed by the fruits and finally peels from different locations. The results indicate that, all the aqueous extracts of different parts of muskmelon and watermelon samples did not exhibited any inhibition zone against all tested microorganisms. On the other side, few ethanol extracts exhibited different values of inhibition zones.

Key words: frying, muskmelon, watermelon, Farzogha, Botraba, Sidi Arhoma, Al-Ewilia

Introduction:

The Cucurbitaceae family, which includes multiple genera and species, includes watermelon. Usually, the seeds, rind, and peels are thrown away or added to animal feed. It is said that these frequently overlooked areas have health advantages. It is well known that the rind has vasorelaxant properties (Rimando *et al.*, 2005); the islet of Langerhans' β-cell releases insulin in response to the seed, which controls blood sugar levels (Omigi and Agoreyo,

2014), but it is known that the peels contain analgesic properties (Kumari *et al.,* 2013). Watermelon species are collectively known as ground or *cucurbits*, which include watermelon, melon, cucumbers, and pumpkins and so on. The Cucurbitaceae family, watermelon (*Citrullus lanatus*) accounts for around 40% of global crop production. *Cucumis sativus* (about 27%), melon (*Cucumis melo*) (about 20%), and pumpkin (Cucurbita) (about 13%) follow in order of importance (Bomfim *et al.,* 2013). There are Latin and Greek roots to the watermelon's scientific name. The term "citrus" in Greek refers to the fruit, hence the name *Citrullus*. The Latin word "*lanatus*," which refers to the tiny hairs on the fruit's stems and leaves, means "wooly." (Erhirhie *et al.,* 2013). About 68% of the weight is made up of the flesh, 30% is the rind, and 2% is made up of the seeds. There are significant differences in the flesh, seed, and rind compositions. Edible and non-edible parts of fruits such as peels pulps and seeds resources are known to have abundantly bioactive secondary metabolism. Peels, seeds, husks, and other materials are created annually as wastes and improperly collected. Terpenes and terpenoides are the three primary categories into which these bioactive secondary metabolites can be divided (approximately 25000 types), alkaloids (approximately 12000 types) and phenolic compounds (8000 types) and can extracted using conventional or non-conventional extraction techniques. Non-edible plants provide cheap renewable feedstock and are thought to be rich in bioactive secondary metabolites. There is a shifted attention to the search for antimicrobial and antioxidant compound from natural sources (Switaj *et al.,* 2015). This is partially caused by the adverse effects of some synthetic medications combined with pathogens' resistance to their actions (Byford *et al.,* 2000). Phenolic compounds are secondary plant metabolites commonly found in plants, which have multiple biological effects, including antioxidant activity. Despite the fact that the phenolic content of popular melon fruit does not rank particularly highly—values range from 109.6 mg/100 g in cantaloupe to 59.3 mg/100 g in honeydew (INRA, 2009), one of the most significant groups of phytochemicals found in plants that are biologically active is phenolics. Because of their redox characteristics, which enable them to function as reducing agents, hydrogen donators, and singlet oxygen quenchers, phenolics have antioxidant action. Since antibiotic resistance is becoming a global public health concern, particularly with regard to food-borne illnesses and nosocomial infections, the antimicrobial chemicals found in plants are of interest (Anderson *et al.,* 2001; Vattem *et al.,* 2004; Hsueh *et al.,* 2005; Lin *et al.,* 2005; Mora *et al.,* 2005; Navon-Venezia *et al.,* 2005). Numerous studies indicate that endogenous antioxidants, also known as exogenous antioxidants obtained through diet, have the ability to act as scavengers of free radicals and enhance human health (Parr and Bolwell, 2000; Mojšilovê and Kuchta, 2001; Connor *et al.,* 2002; Oktay *et al.,* 2003). Therefore, eating a range of plant foods, such as muskmelon and watermelon,

may offer further health advantages. Antioxidants that retard the oxidation process may additionally exhibit antimicrobial activity (Hao *et al.*, 1998; Cutter, 2000).

Material and methods:

Area of samples collection

Fresh watermelon and muskmelon fruits samples were collected from four locations around Al-Marj town, northeastern Libya. The Al-Marj plain is located close to the Mediterranean coast on the western side of Al-Jabal Al-Akhdar. With 16 inches (400 mm) of annual precipitation, the surrounding plain produces fruits, vegetables, cereals (barley and wheat), and other goods. The commercial hub of the plain is Al-Marj. Three muskmelon fruit samples were collected from Farzogha, Botraba and Sidi Arhoma locations, while one watermelon fruit was collected from Al-Ewilia location. During the month of April 2022, fruits of several local types of muskmelon and watermelon were gathered from the previously mentioned regions. The samples were delivered to the Herbarium Unit of the Department of Plant Biology for verification. Every component of various places was assigned a symbol and categorized, as indicated in Table (1).

Table (1): Locations, part of fruits and symbol of each

Location	Part of fruit	Symbol
Farzogha	Fruits of muskmelon	F1
	Peels of muskmelon	P1
	Seeds of muskmelon	S1
Botraba	Fruits of muskmelon	F2
	peels of muskmelon	P2
	Seeds of muskmelon	S2
SidiArhoma	Fruits of muskmelon	F3
	peels of muskmelon	P3
	Seeds of muskmelon	S3
Al-Ewilia	Fruits of watermelon	F4
	peels of muskmelon	P4
	Seeds of watermelon	S4

Sample preparation

Before being analyzed, the gathered fruits were rinsed multiple times with distilled water after being cleaned with distillated water. Fresh samples of fruits were sliced with cleaned knife to separate the peels from the pulp. From the pulp, the seeds were carefully extracted. The fruit was shred, and the peels were cut into tiny cubes. Prior to nutritional analysis, each sample was moved into a foil-lined tray, properly labeled, and stored in a laboratory refrigerator at 4°C.

Preparation of extracts

Solvent extraction: The powdered materials of Watermelon and Muskmelon were extracted with different solvents of (water and ethanol), where 10 grams of each plant powders were added to 100 ml of aqueous and non-aqueous solvents (Ethanol). Crude extract was evaporated at 60ºC, with the rotary evaporator the extracts were collected and stored at 4ºC until further use (Akinpelu *et al.*, 2008; Al-Shammary and Ibrahim, 2014).

Phytochemical screening: The extracts underwent a qualitative phytochemical examination utilizing recognized laboratory methods, as described by Nagalingam *et al.* (2012). Tests on the presence of sterol and/or triterpens, flavonoids, anthraquinones, tannins, carbohydrates glycosides, cardiac glycosides, alkaloids and saponins were conducted accordingly.

Determination of phenolic compound content: The phenolic compound were estimated by using the standard methods. By using folin-Ciocalteau reagent.

Antibacterial activity: The extracts were individually tested against pathogenic bacteria, the following bacteria were tested:

Bacterial strains

Gram positive bacteria: One species of Gram positive (*Staphylococcus aureus*) was selected in this study.

Gram negative bacteria: Two species of Gram negative (*Escherichia coli* and *Salmonella enteric*) were selected in this study

Agar well diffusion method: Agar well diffusion method was followed to determine the antibacterial activity, mueller-Hintion ager (MH), Plates were swabbed (sterile cotton swabs) with pathogenic bacteria, well 4 mm diameter were made in each of these plates using sterile cork borer, about 100 µl of different organic solvents. Were added by sterile syring into wells. For 18 to 24 hours, the plates were incubated at 37°C. zone of inhibition were measured using a meter rule as described by Mukherjee *et al.* (1997).

Results and Discussion:

From the table (2) of the phytochemical screening of aqueous extracts, they showed that flavonoids, carbohydrates glycosides, cardiac glycosides and saponins were present in all parts of muskmelon and watermelon, while sterols and/or triterpines and anthraquinine were absent in all part of watermelon except peels of watermelon (P4) and fruits of watermelon (F4) from Al-Ewilia location. Tannin were found in all parts of muskmelon and watermelon except the fruits of watermelon from Al-Ewilia location (F4) only.

Table (2): Phytochemical screening of aqueous extracts:

Phytochemical screening test	S1	S2	S3	S4	P1	P2	P3	P4	F1	F2	F3	F4
Sterol and/or triterpines	-	-	-	-	-	-	-	+	-	-	-	+
Flavonoids	+	++	++	++	+	+	+	++	+	+	+	++
Anthraquanine	-	-	-	-	-	-	-	+++	-	-	-	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	-
Carbohydrates glycosides	+	++	+	+	+	+	+	++	+	+	+	+++
Cardiac glycosides	+	+	+	++	+	+	++	+	+	+	++	+
Alkaloids	+	+	+	+	+	++	+	-	++	++	+	+
Saponins	+	+	+	+	++	+	+	+	+	+	+	+

Table (3) indicated the phytochemical Screening of ethanol extracts of watermelon and muskmelon parts. They showed that flavonoids, tannins, cardiac glycosides and alkaloids were present in all parts of watermelon, while carbohydrates glycosides and saponins were absent in all parts of muskmelon and watermelon, the highest amount was in flavonoids for peels of watermelon (P4) from Al-Ewilia location only.

Table (3): Phytochemical Screening of ethanol extracts:

Phytochemical screening test	S1	S2	S3	S4	P1	P2	P3	P4	F1	F2	F3	F4
Sterol and/or triterpines	+	+	+	-	+	+	+	++	+	+	+	+
Flavonoids	+	+	+	++	+	+	+	+++	+	+	+	++
Anthraquanine	-	-	-	-	-	-	-	++	-	-	-	+
Tannin	+	+	+	+	+	+	+	++	++	++	++	+
Carbohydrates glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	+	+	++	+	+	+	++
Alkaloids	+	++	+	+	+	++	+	++	++	++	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-	-

Parts of watermelon and muskmelon could not be the only source of fat, protein, and carbohydrates, They also have notable antioxidant activity and are incredibly rich in phytochemicals. Condensed tannins and flavonoids are examples of phenolic substances that are known to inhibit certain molecular targets of pro-inflammatory mediators during inflammatory responses. Moreover, the phytochemicals scavenge free radicals to function as antioxidants, which reduces inflammation (Charoensiri *et al.*, 2009; Abdelwahab *et al.*, 2011). Furthermore, alkaloids are known to have an effect on the central nervous system and some act as a pain killer (such as morphine). Alkaloid has been found in the majority of plants that have historically been used to treat malaria by phytochemical screens (Eleazu *et al.*, 2010). Similarly, plants that contain alkaloids have also long been used to cure degenerative diseases including rheumatism and gout. In addition, Phenolic substances have been utilized as purgatives, including anthraquinones (Sodipo *et al.*, 2000). Watermelon fruit extract contains a moderate amount of anthraquinones, which are traditionally used to relieve constipation and stomachaches. Dietary anti-nutrients called tannins are what give foods and

beverages their harsh flavor (Charoensiri *et al.*, 2009). Red blood cells can precipitate and coagulate due to the characteristic of saponin. Strong anticancer activity and the prevention of oxidative cell damage are two characteristics of flavonoids, which are water-soluble antioxidants and free radical scavengers. The fruit of the watermelon and muskmelon contains flavonoids, which may indicate that the plant can provide protection against free radicals, inflammation, bacteria, and tumor development (Okwu, 2004). Total phenolic content (ppm) of the studied watermelon and muskmelon samples were shown in table (4). It is a clear that high percent of total phenol were found in the seeds followed by the fruits and finally peels from different locations. The highest value (2.20 ppm) was recorded in seeds of muskmelon from Farzogha location (S1) followed by the seeds of muskmelon (2.18 ppm) from Botraba location, while the lowest value was (0.65 ppm) for peels of watermelon from Al-Ewilia location.

Table (4). The total phenolic content (ppm) of the studied samples

Parts	Code	Total phenol (ppm)
Seeds	S1	2.20
	S2	2.18
	S3	1.90
	S4	2.10
Fruits	F1	1.05
	F2	1.20
	F3	1.70
	F4	1.13
Peels	P1	0.78
	P2	0.92
	P3	0.83
	P4	0.65

According to Ramazan *et al.* (2012), watermelon seeds have total phenol levels ranging from 0.13 mg GAE/g to 0.30 mg GAE/g. The highest total phenol was established in Forage watermelon kernel. The polyphenolic content of the peels and pulps of four cucurbit fruits—pumpkin, ash gourd, watermelon, and muskmelon—was examined by Singh *et al.* (2016). They discovered that the pulp from muskmelon had the highest overall polyphenolic content, followed by pulp from watermelon, pumpkin and ash gourd in all solvents while in case of peel, Muskmelon had the largest percentage, followed by watermelon, pumpkin, and ash gourd. These findings demonstrated that the polyphenolic content of muskmelon fruit is higher than that of other cucurbits. According to Neglo *et al.* (2021), the peels had the highest total phenolic content (0.087 mg GAE/g), which was followed by the seed (0.042 mg GAE/g) and the rind (0.026 mg GAE/g). Table (5) displays the antibacterial properties of the aqueous and ethanol extracts of the watermelon and muskmelon in all of its parts. The results indicate that all the aqueous extracts of different parts of muskmelon and watermelon samples did not exhibited any inhibition zone against all tested

microorganisms. On the other side, few ethanol extracts exhibited different values of inhibition zones. The highest antimicrobial activity was against for Salmonella bacteria. The diameter of each of their separate inhibition zones for watermelon seeds from the Al-Ewilia area was 17 mm (S4). The lowest inhibition zone (6 nm) recorded for ethanol extract of fruits of muskmelon from Botraba location (F2). Escherichia coli was the most microorganism affected by four parts of muskmelon and watermelon ethanolic extracts.

Table (5) - Microorganisms and their zone of inhibition using various parts of Watermelon and muskmelon. (Diameter of zone of inhibition (mm)

Samples		Staphylococcus aurous		Escherichia coli		Salmonella	
		Aq extract	Eth extract	Aq extract	Ethext ract	Aq extract	Ethextr act
Seeds	S1	-	-	-	-	-	-
	S2	-	-	-	10	-	-
	S3	-	9	-	-	-	9
	S4	-	-	-	7	-	17
Peels	P1	-	-	-	-	-	-
	P2	-	-	-	7	-	-
	P3	-	-	-	-	-	-
	P4	-	-	-	-	-	-
Fruits	F1	-	-	-	10	-	-
	F2	-	6	-	8	-	-
	F3	-	-	-	-	-	-
	F4	-	-	-	-	-	-

A number of investigators studied the effects of fruit extracts and their active components as antimicrobial agents to inhibit the growth of pathogenic microorganisms. According to some research, antimicrobial components found in fruit extracts, such as terpenoid, alkaloid, and phenolic compounds, interact with proteins and enzymes in the cell membrane, rupturing it and causing a flow of protons outside the cell, which either causes cell death or inhibits enzymes necessary for the biosynthesis of amino acids (Burt 2004; Gill and Holley, 2006). Various researchers have presented conflicting findings in this area. According to Braid et al. (2012), Saxena et al. (2013), and Rahman (2013), water extract has a superior response to antibacterial activities in comparison to methanol. In contrast, Braid *et al.* (2012) found the opposite. Variability in strain and variations in methodology could be the cause of this disagreement.

Conclusion:

Thus, based on the current study's findings, it can be said that the majority of muskmelon and watermelon components' aqueous or ethanol extracts contain trace levels of phytochemicals, The seeds had the highest percentage of total

phenol content, followed by fruits and, lastly, peels from different locations. All the aqueous extracts of different parts of muskmelon and watermelon samples did not exhibited any inhibition zone against all tested microorganisms. On the other side, few ethanol extracts exhibited different values of inhibition zones. Because of this, many muskmelon and watermelon samples include a variety of chemicals that have a wide range of therapeutic uses.

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Effect of hibernation on testis genomic DNA fragmentation and comet assay in *Uromastyx acanthinra* (BELL, 1825)

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Abstract:

This study aim to investigate the effect of hibernation on testis genomic DNA fragmentation and comet assay in *Uromastyx acanthinra* during two seasons (hibernation season and summer season). The results revealed that, serum testosterone level was markedly increased during summer while declined during hibernation season, and during the hibernating season, the degree of laddering (total DNA fragmentation) was more expressed and had a higher genomic expression. There was no detected genomic DNA damage during summer.

Keywords: hibernation, testis, DNA damage.

Introduction:

Numerous authors have researched the reproductive cycle of reptiles and the seasonal variations in their assays (Droge*et al.*, 1982; Elliott, 1985; Said and Hussein, 1992; Licht, 2005; Nora and Bertona, 2006). One gets the idea from reading a lot of the research on hibernation that ectotherms become inactive in the winter because they can't sustain their regular metabolic rates at the lower temperatures that occur during that season. (El-Masry and Hussein, 2001). Although many scientists have studied lizard reproductive cycles, not much is known about reproductive activity during periods of activity and hibernation (El-Ghazaly*et al.*, 1987; Bhagyshri *et al.*, 2000). When compared to nearly normal structure and less fatty liver during hibernation with nearly normal pattern, *Uromastyx acanthinra* experiences increased fatty liver and pigment cells with abundant damage during hibernation. A separation of genomic DNA was seen during hibernation. In addition, compared to activity season, caspase3 and caspase7 activity in the liver tissue increased during hibernation (Abdelhafid, 2013), this study aim to investigate the effect of hibernation on testis genomic DNA fragmentation and comet assay during

two seasons (hibernation season and summer season) in *Uromastyx acanthinra*.

Materials and Methods:

Ten mature male *Uromastyx acanthinra* individuals were captured in south Libya and transported straight from their natural habitats to the laboratory. The specimens were split into two groups according to the annual cycle: the activity season (summer: late June to mid-July) and the hibernation season (winter: late November to mid-January).

Determination of hormone testosterone:

The IBL-AMERICA Testosterone ELISA Kit (Immuno-Biological Laboratories, Inc. (IBL-America) is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an antibody directed towards an unique antigenic site on the testosterone molecule. When it comes to binding to the coated antibody, endogenous testosterone in a sample faces competition from a testosterone horseradish peroxidase conjugate. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate and the sample's testosterone concentration are inversely correlated. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of testosterone in the sample (Tietz, 1986).

DNA Fragmentation Assay:

An adaptation of the methodology used by Arends et al. (1990) and Bortner et al. (1995) was used to measure DNA fragmentation. Freshly isolated specimens were washed twice with ice-cold and suspended in 100 ml of lyses buffer (10 m MTris HCl/10 mM EDTA/0.5% Triton X-100, pH 8.0), vortex-mixed, sonicated, and incubated on ice for 20 min. After centrifugation for 20 min at 40°C, 14.000 rpm the supernatant containing fragmented (soluble) DNA was transferred to another tube. Lyses buffer (100 ml) was added to the pellet containing insoluble DNA. Both samples were treated with RNase A (0.5 mg/ml) for 1 hr at 37°C and then with proteinase K (Sigma, 0.4 mg/ml) for 1 hr at 37°C. After adding 20 ml of 5 M NaCl and 120 ml of isopropanol, the samples were incubated overnight at 220°C, and the DNA concentrations were determined. Fragmented DNA was calculated as 100% X soluble DNA/ (soluble+insoluble DNA). The soluble fraction of DNA was determined by electrophoresis on 1.5% agarose gel and has a ladder-like appearance.

Single cell gel electrophoresis (Comet assay):

Fresh samples of whole testis of specimen aptured during the mentioned seasons were separated and immediately stored at -80°C for Comet assay. The specimens were homogenized in chilled homogenizer buffer, pH 7.5 containing 75mM NaCl and 24mM Na_2EDTA pH 13 to obtain a 10% tissue solution. Apotter-type homogenizer was used and samples were kept on ice during and after homogenization. Six microliters of the homogenate were suspended on 0.5% low melting agarose and sandwiched between a layer of 0.6 % normal-melting agarose and a top layer of 0.5 % low melting agarose on fully frosted slides. Throughout each gel layer's polymerization, the slides were maintained on ice. After the solidification of the 0.6% agarose layer, the slides were immersed in a lyses solution (1% sodium surcosinate, 2.5m NaCl, 100mM Na_2 EDTA, 10mm Tris-HCl, 1% tritonX-100 and 10% DMSO) at 4°C. After 1 h, the slides were placed in electrophoresis buffer (0.3M NaOH, 1 mM Na_2EDTA, Ph 13) for 10 minutes at 0°C to allow DNA to unwined. Electrophoresis was performed for 10 min. at 300mA and 1V/cm. The slides were neutralized with tris–Hcl buffer, pH 7.5, and stained with 20µg/ml ethidium-bromide. Each slide was analyzed using the Leitz Orthoplan (Wetzlar, Germany) epifluorescence microscope. The Comet Assay II automatic digital analysis device was utilized to analyze one hundred cells on each slide. The distance of DNA migration from the nuclear core's center, measured in µm, is known as the perspective tail length and is used to assess DNA damage. The product of the tail length and the percentage of total DNA in the tail is known as the tail moment (Tail moment =tail length X % of DNA in the tail). Image analysis software automatically measures the tail's length and intensity (Sasaki *et al.*, 1997; Robbiano *et al.*, 2004).

Results:

Effect of hibernation on serum testosterone levels:

Figure (1) illustrates serum testosterone levels during hibernation season and summer season. The hormonal level was markedly increased duringsummer and declined during hibernation season.

Effect of hibernation on testis genomic DNA fragmentation and comet assay (single-cell gel electrophoresis):

Figure (2) illustrates effect of hibernation on testis DNA fragmentation of *Uromastyx acanthinra*. The genomic expression of the degree of laddering

(total DNA fragmented) increased during hibernation season. There was no detected genomic DNA damage during summer season. Also, following applying comet assay (single-cell gel electrophoresis) during hibernation, the single strand nucleotide testis was detached with increased tail length and DNA concentration, comparing with normal pattern structure during summer (figure 3).

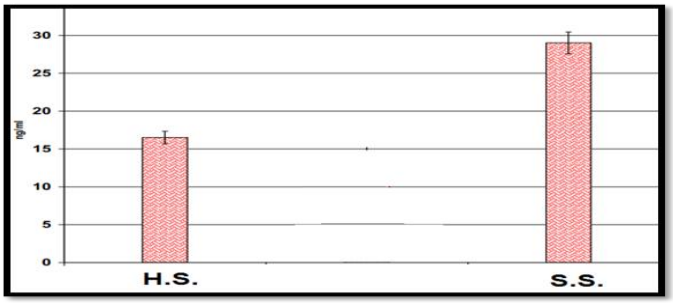

Fig. 1: Effect of hibernation on serum testosterone content in of *Uromastyx acanthinra* (H. S.: Hibernation Season, S. S.: Summer Season).

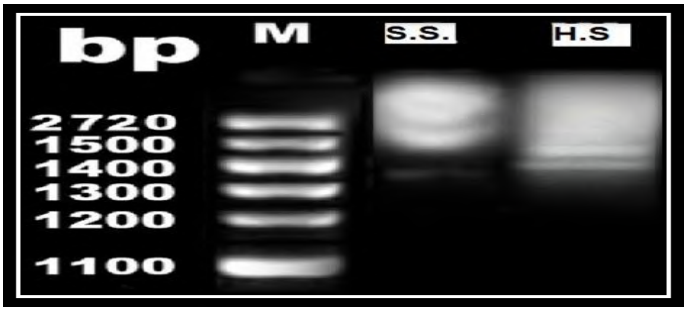

Fig. 2: DNA fragmentation detected with agarose gel electrophoresis of DNA extracted from testis of *Uromastyx acanthinra* (H. S.: Hibernation Season, S. S.: Summer Season).

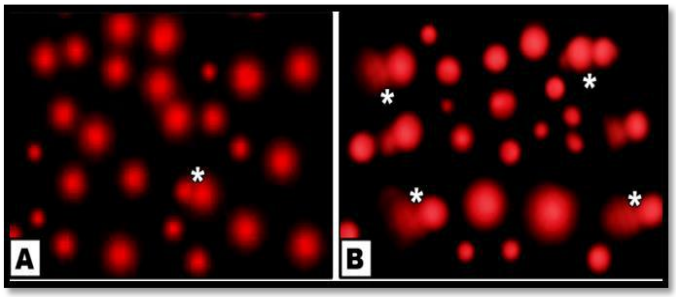

Fig. 3: Photomicrographs of comet assay of testis neuronal cell of *Uromastyxacanthinra*(A) Hibernation Season, (B) Summer Season). (*) means stretched damage of cells.

Discussion:

Despite significant progress in the last decade, there is still a dearth of knowledge regarding the ecology and physiology of *Uromastyx acanthinra* in south Libya. The understanding of how ecological and physiological variations may occur from changes in the environment with respect to seasonal variation is hampered by this lack of knowledge. For reptile species, hibernation is a highly complex and delicate biological phenomenon that involves several physiological processes. Moreover, certain brain areas continue to function during deep torpor (Heller, 1979), when the brain of a hibernator can drop below the freezing point of water and exhibit electrical quiescence when subjected to surface electroencephalography (Heller and Ruby, 2004). As a hibernator transitions into profound torpor, the brain actually goes "asleep." This could be due to the activation of the reticular thalamic nucleus, which inhibits arousal activity, as indicated by the expression of c-fos. The "biological clock" is in operation during hibernation; the choroid plexus and the ependymal cells of the lateral and third ventricles exhibit significant activity, indicating that the production of cerebrospinal fluid or an as-yet-unidentified function of these cells may be crucial for maintaining torpor (Bratincsak *et al.* 2007). Active control stops protein synthesis during hibernation, a state characterized by barely noticeable blood flow and drastically decreased nutritional supply. While in non-hibernators, persistent disruptions in protein synthesis during ischemia accurately predict postischemic cell necrosis (Hossmann, 1993). Reptiles respond to varying environmental conditions by displaying a diversity of reproductive techniques. The gonadal cycle phases (recrudescence, climax, and gonadal quiescence) in animals that reproduce seasonally appear to be temporarily arranged based on their thermic and energy requirements, as well as potentially their duration. (Saint Girons, 1985). Consequently, an extensively documented process in reptile reproduction, gonadal cycle separation between sexes is caused by differential gamete maturation needs (Moore and Lindzey, 1992; Whittier and Tokarz, 1992). Sex steroid hormones, which are necessary for all vertebrate reproduction, are secreted by the gonads. Sex steroid hormones have a direct impact on gametogenesis, but they also have an impact on sexual behavior, brain structure, and sexual development (Young *et al.*, 1995). In this study the results revealed that, effect of hibernation on testis DNA fragmentation of *Uromastyx acanthinra*. During the hibernating season, there was an increase in the genomic expression of the degree of laddering (total DNA fragmented).

There was no detected genomic DNA damage during summer season. Additionally, the single strand nucleotide testis was detached with increased tail length and DNA concentration after using the comet assay (single-cell gel electrophoresis) during hibernation, contrasting with typical pattern structure during summer. All results similar finding to Hossmann (1993) who revealed that active control stops protein synthesis during hibernation, a condition marked by a substantial reduction in nutritional supply and only a trickle of blood flow. Prolonged disruptions of protein synthesis following ischemia in non-hibernators accurately predict post-ischemic cell necrosis; however, in an in vitro model of cerebral ischemia, suppressing protein synthesis during hibernation not only survives without adverse effects but is also linked to tolerance to an additional insult.

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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.

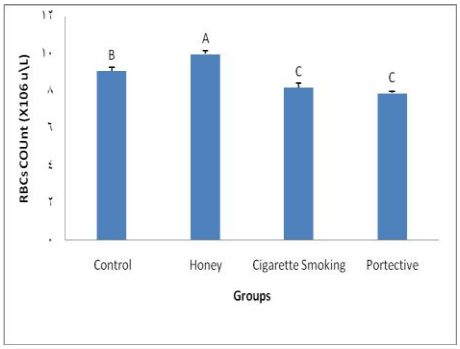

Figure 2: Averages of the mean value of the RBCs count ($X10^6$ µl/L).

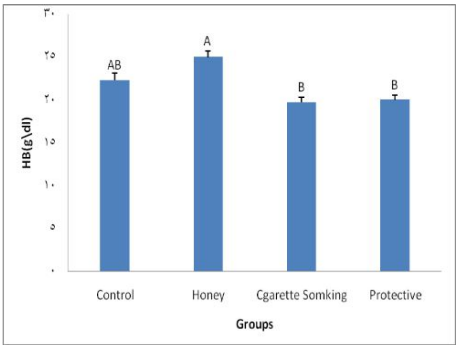

Figure 3: Averages of the mean value of the HG (g/d) level.

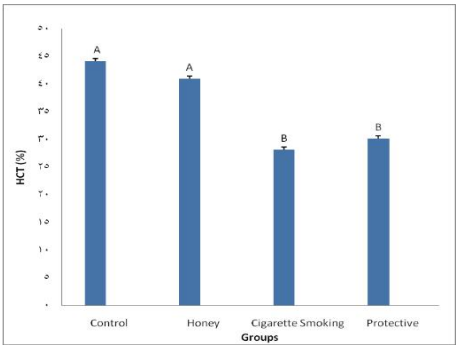

Figure 4: Averages of the mean value of HCT level (%).

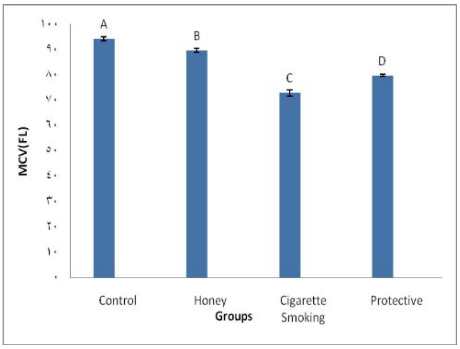

Figure 5: Averages of the mean value of MCV (FL) level.

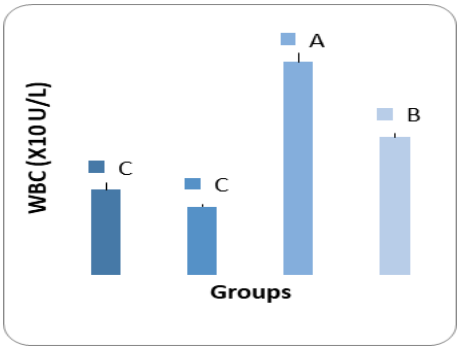

Figure 6: Averages of the mean value of WBCs count ($X10^3$ µl/L).

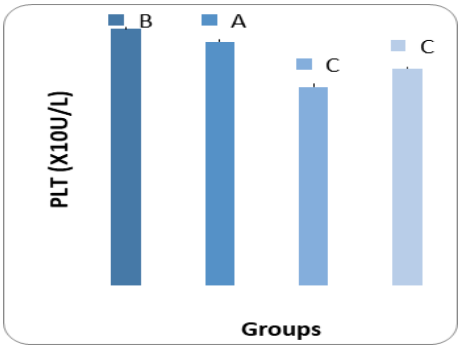

Figure 7: Averages of the mean value of PLT count ($X10^3$ µl/L).

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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