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Original article

Protective Effect of *Zingiber Officinale* Against Di-(2-Ethylhexyl) Phthalate (DEHP) Induced Histological Effect in Testes of Adult Male Rabbits

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ABSTRACT

Aims*. DEHP stands for di-(2-ethylhexyl) phthalate, a family of man-made chemical compounds that have been used in the development of plastics, solvents, and personal care products throughout the last century. Ginger (Zingiber officinale) is known to play diverse biological roles including anti-oxidation, anti-inflammation, hypo-lipidemia, anti-carcinogenesis, a protective role against male reproductive dysfunction, anti-nausea, anti-thrombosis, and anti-bacterial process. The purpose of this study was to see if Zingiber officinale ethanolic extract has any anti-oxidant activity against DEHP-induced damage to the male reproductive system of adult rabbits.* ***Methods****. Twenty male New Zealand white rabbits were randomly divided into four groups; first group was a control group were receiving corn oil; second group: rabbits were treated with Zingiber officinale alone; third group: rabbits were treated with DEHP; fourth group: rabbits were giving DEHP and Zingiber officinale.* ***Results****. The histological structure of these treated rabbits showed that the accumulation of spermatogenic and ledig cells was increased and the lumen of the seminiferous tubules was fully packed with sperms in the group 4 compared to group 3.* ***Conclusion****. Zingiber officinale has a protective nutraceutical capacity to help overcome DEHP-induced histological damage in testing.*

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INTRODUCTION

DEHP stands for di-(2-ethylhexyl) phthalate, which are phthalic acid di alkyl or alkyl aryl esters. DEHP is a synthetic plasticizer that improves the flexibility and durability of products. Plasticizers are mostly utilized in construction materials, home furnishings, transportation, fashion, and to a lesser extent in food and medical product packaging [1]. Because DEHP wastes reach the environment in the form of industrial sewage and effluents, they are widely dispersed in nature as a result of their manufacture, use, and transfer. They were identified at rather high levels in the ecosystem, resulting in contamination [1]. Diet, including consumption of contaminated food and water, is the most common human exposure route to DEHP. It can be ingested or breathed in through polluted air. Another possible form of phthalate exposure is dermal contact with phthalate-containing care goods and medical devices contaminated with phthalates [2]. The most prevalent type of endocrine disruptor activity is that of chemicals that imitate or antagonize the activities of natural estrogens [3-5]. Early puberty in females, reduced sperm counts, altered functions of reproductive organs, obesity, altered gender-specific behaviors, and increased rates of some breast, ovarian, testicular, and prostate cancers can all be caused by this compound, which affects the hormone system involved in many biological metabolisms. [6-9]. The thyroid hormone system is thought to be harmed by DEHP [10] and a system vital to normal brain development in the fetus and infant [11]. Contamination of bottled water by endocrine disruptors could happen at the different steps of the bottling process, namely: untreated groundwater from a spring, supply pipes or the filling and cleaning of containers in the bottling process [12-14]. Furthermore, for some authors plastic bottle stress (UV radiation and heat) could also be a source of endocrine disruptors [15]. DEHP is by far the most commonly used plasticizer, annual production being 1-4 million tons. From the late 1960s, leaching of DEHP from PVC formulations, human exposure and, more recently, tissue deposition have been documented

[16-17]. In particular, DEHP induces a wide range of developmental and reproductive toxicities in mammals [18]. While its ability to cause toxicity in people is still unknown, in female manufacturing workers, chronic occupational exposure to high amounts of DEHP is linked to lower rates of pregnancy, greater rates of miscarriage, and an ovulation [19-20].
DEHP is a well-known reproductive system toxin that belongs to the phthalate chemical family of plasticizers with endocrine-disrupting potential. In fetal rat models, DEHP and its metabolites disrupt healthy testicular development [21]. There have been reports of its effects on testosterone, luteinizing hormone, and estrogen-like action [22-23]. DEHP is rapidly converted to monoesters after entering the body and can potentially be oxidized to oxidative metabolites [24]. The presence of phthalates in environmental samples, particularly DEHP, indicates that they can be released during usage and migrate from packaging to contaminate contents such as meals and beverages [25].
Zingiber officinale has been used as a medicine in Asian countries since ancient times. It has been used to cure cold, inflammation, gastrointestinal discomfort, rheumatic disorder, neuralgia and motion sickness [26]. It is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes [27].
Both anti oxidative [28] *Zingiber officinale* has been shown to have anti-androgenic and anti-estrogenic properties in animal models [29], [30] Reported that the main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10-gingerol and these constituents had exhibited strong anti-oxidative activity. The components in ginger include: extractable oleoresins, many fats, carbohydrates, vitamins, minerals and a potent proteolytic enzyme called zingibain. Oleoresins contribute to the sensory perception of ginger. There are 5-8% of oleoresins in crude *Zingiber officinale*, which consist of two distinct groups of chemicals: volatile oils and non-volatile pungent compounds [30].
Zingiber officinale has been demonstrated to have various pharmacological activities, reported that *Zingiber officinale* improve the histological alterations and reduce apoptosis in testis of mice treated with metiram fungicide [31-32]. Injection of ginger extract confirmed a significant improvement in testicular tissues in mice. As a result of infertility is one of the major health problems in life, and approximately 30 % of infertilities are due to a male factor. Thus, this work is a compilation of the more effects of DEHP exposure on the male reproductive systems of rabbits, and described the role of *Zingiber officinale* extract as protective nutraceutical agent towards damage effect on testes due to DEHP- exposure in male rabbits.

METHODS

Tested compound

In this study DEHP (purity 99.0%) was purchased from Sigma–Aldrich (USA). All other chemicals used in the experiment were of analytical grade. Mature male New Zealand White rabbits (age of 7 months and initial weight of (2.917 ± 28.9 Kg) were used.

Study design and setting

Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits): Group I: Rabbits were used as control and received an equivalent volume of the vehicle (corn oil) alone by oral gavage daily for 12 successive weeks. Group II: Rabbits were treated *Zingiber officinale*. It was given daily by gavage at a dose of 100 mg/kg B.W, [33], [34] which dissolved in corn oil for 12 successive weeks. Group III: Rabbits were treated daily with DEHP by gavage at a dose of 500 mg/kg B.W/day 1/50 of DEHP lethal dose [35] , [36] for 12 successive weeks. Group IV: Rabbits were given DEHP daily at a dose of 500 mg/kg B.W by gavage like group III and given the *Zingiber officinale* concurrently daily at a dose of 100 mg/kg B.W by gavage like group II for 12 successive weeks. The tested doses of DEHP and *Zingiber officinale* were given daily for 12 weeks. The serum samples obtained were analyzed to determine the concentration of testosterone. The analysis was carried via the tube-based enzyme immunoassay (EIA) method. The protocol used for the hormone was according to the method described for the kit (Immunometric Limited UK) and meet the WHO standards in research programme for human reproduction.

Histological examination

Specimens of testes were put in 10% buffered formalin for histopathological examinations. Histological preparation of testes was carried out according to previous study [37].

Statistical analysis

Statistical analysis was carried out in Minitab software (version17; statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test and appropriate $P < 0.05$ consider significant.

RESULTS

Table 1 was shown the overall means of the data of different parameters. Administration of DEHP caused significantly decreased in most parameters including the body weight, testicular weight, and levels of testosterone compared to control. While treatment with *Zingiber officinale* was caused significant increases in previous parameters compared to control. In combination group levels of theses parameters were found close to control values.

Table 1: The overall means (±SEM) of body weight, relative testes weight and level of testosterone in male rabbits which treatment with Zingiber officinale, DEHP and their combination compared to control.

Parameters	*Groups*			
	Control	***Zingiber officinale***	***DEHP***	***Zingiber officinale+ DEHP***
Body weight (gm)	3402 ± 28.2^{a}	3581 ± 78.2^{a}	3019 ± 29.9^{b}	3218 ± 60.2^{ab}
Testes weight (g/100 g body weight)	3.1 ± 0.003^{b}	4.1 ± 0.002^{a}	2.8 ± 0.007^{c}	3.3 ± 0.004^{b}
Testosterone (ng/mL)	1.59 ± 0.034^{b}	2.53 ± 0.130^{a}	1.05 ± 0.069^{c}	2.02 ± 0.063^{b}

Values are means ± SEM of 5 rabbits in each group. Mean with different letters (a- d) are significantly difference ($p \leq 0.05$). Mean with the same letters (a-d) are non-significantly difference ($p \geq 0.05$)

Histopathological observations

Histopathological examination of rabbit testicular tissue of the different studied groups showed the following changes:

Group I (Control)

The light microscopic examination of the testes showed that complete active spermatogenic cycle was regular in all male rabbits of the control group (Fig1 A). The structure components of the testes are the seminiferous tubules and interstitial tissues (Leydig cells), each testicle is surrounded by a capsule of dense, irregular fibrous connective tissue containing some elastic fibers called the tunica albuginea, followed by a layer rich in blood vessel branches called the tunica vasculosa.

The tunica albuginea tissue extends into the testicle, forming septulae testis. The testicle is divided into lobules testis, The lobules of the testicle contain a number of seminiferous tubules surrounded by interstitial connective tissue containing fibroblasts, blood and lymph vessels, nerves, and myoid cells. The seminiferous tubules are lined with stratified epithelium. Two types of cells were identified in rabbit seminiferous tubules, the Sertoli cells and the spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms). The Sertoli cells, rest on the thin basal lamina, while the spermatogenic cells are arranged in many layers, namely, the spermatogonia, primary and secondary spermatocytes, spermatids and finally mature spermatozoa, (Fig1 B & C).

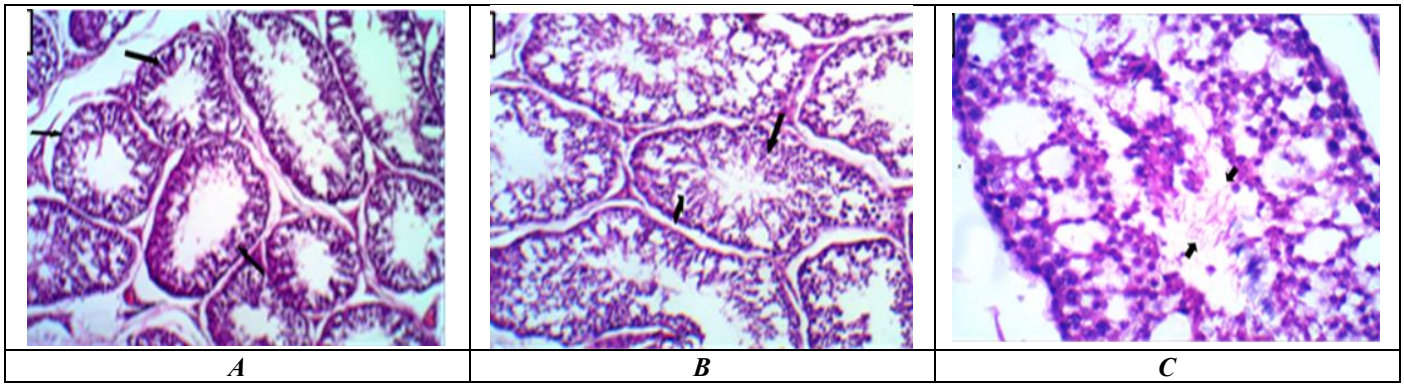

Figure 1. Histological slices of rabbit testicles in the group treated with phthalate and ginger together. *A: section in the testes of the control cases showing seminiferous tubules lined by germinal epithelium at various stages of maturation till the mature sperm stage (H&E stain, X 200). B: Control case showing the lining germinal epithelium where the spermatogenic maturation is full and complete (H&E stain, X 400). C: section view in the seminiferous tubules in a control case demonstrating the presence of mature sperms within the tubular lumen (H&E stain, X 400).*

Group II (Ginger)

The light microscopic examination of the testes of ginger treated rabbits showed normal testicular morphology similar to that of control group (Fig 2 A, B&C).

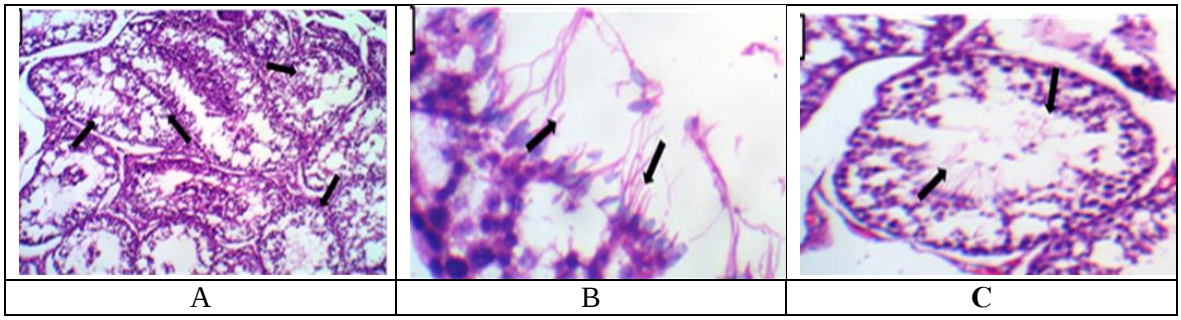

Figure 2. Light microscopic examination of the testes of ginger treated rabbits. *A: section in the testes of the cases receiving ginger alone. Note the crowding of the seminiferous tubules and the full range of germinal epithelium till the mature sperm stage (H&E stain, X 200). B: high power view of the tubules receiving ginger alone showing high number of well-formed sperms (H&E stain, X 400). C: high power view of the tubules receiving ginger alone showing high number of well-formed sperms (H&E stain, X 400).*

Group III (DEHP)

Examination of the histological slides of rabbit testis in the group treated with Phthalate only and stained with hematoxylin and eosin dye showed that there was bleeding in the interstitial connective tissue that covers the seminiferous tubules, and also between the seminiferous tubules within the testicular lobules. With some degeneration of the stratified epithelium lining the seminiferous tubules, where histological results showed that giving rabbits a dose of phthalates at a dose 500 mg/kg B.W/day for a period of for 12 successive weeks a sufficient dose to cause damage to the seminiferous tubules and their irregularity within the testicular lobule, and thus be ineffective. (Fig3 A & B). Vascular congestion was also observed (Fig3 C).

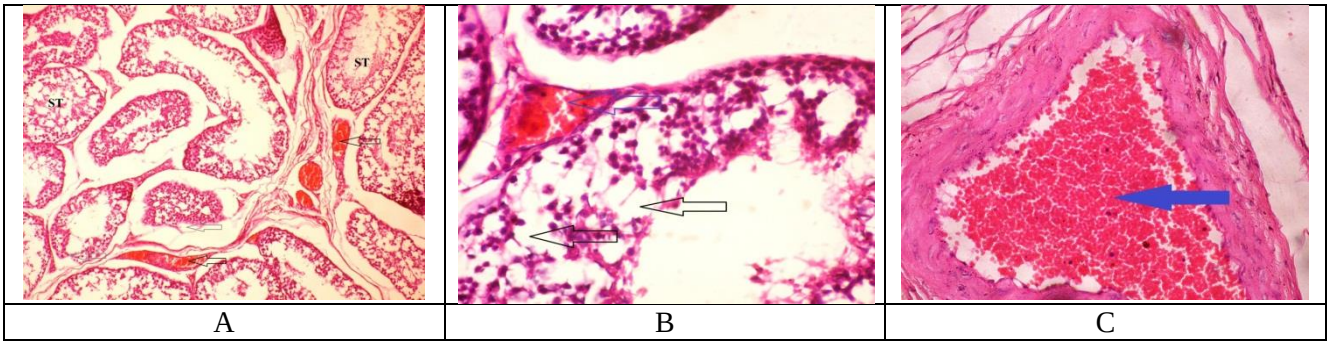

Figure 3. Histological slides of rabbit testis in the group treated with Phthalate only and stained with hematoxylin and eosin dye. *A: section of testis of rabbit from the treated with Phthalate only showing bleeding between the seminiferous tubules (Arrows), the seminiferous tubules (ST), degeneration of the stratified epithelium lining the seminiferous tubules (Grey Arrows) (H&E stain, 200X). B: section of testis of rabbit from the treated with Phthalate only showing bleeding in the interstitial connective tissue that covers the seminiferous tubules (Blue Arrow), Damage to the seminiferous tubules (Arrows), (H&E stain, 400X). C: section of testis of rabbit from the treated with Phthalate only showing Vascular congestion was also observed (Blue Arrow), (H&E stain, 400X).*

Group IV (the treated group)

While examining histological slices of rabbit testicles in the group treated with phthalate and ginger together (the treated group) and stained with hematoxylin and eosin dye showed a significant improvement in the organization and arrangement of seminiferous tubules within the testicular lobe, Also, a decrease in vascular congestion was observed compared to the group treated with phthalate only. There was decreased bleeding between the seminiferous tubules, as well as decreased bleeding in the interstitial connective tissue covering the seminiferous tubules (Fig.4 A). The process of spermatogenesis was seen in the seminiferous tubules (Fig.4 B).

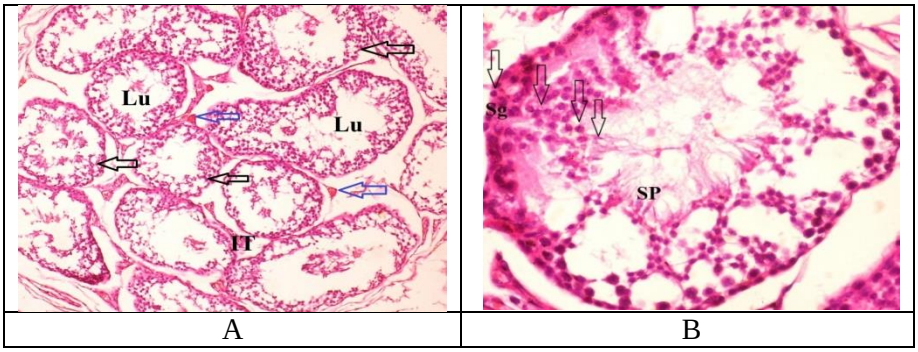

Figure 4. Histological slices of rabbit testicles in the group treated with phthalate and ginger together (the treated group) and stained with hematoxylin and eosin dye. *A: section of testis of rabbit from the treated with phthalate and ginger together (the treated group) showing significant improvement in the organization and arrangement of seminiferous tubules within the testicular lobe (Arrows), Decreased bleeding between the seminiferous tubules (Blue Arrows), Lumen (**Lu**), interstitial tissue (**IT**). (H&E stain, 100X). B: section of testis of rabbit from the treated with phthalate and ginger together (the treated group) showing the process of spermatogenesis was seen in the seminiferous tubules (Arrows), Spermatogonia (**Sg**), Spermatocyte (**SP**). (H&E stain, 400X).*

DISCUSSION

DEHP have been detected in the atmosphere [38] in aquatic environments [39],[40] Reduced fertility, atrophic changes in male gonads, degenerative changes in the epididymis, as well as a reduction in sperm count and motility, cryptorchidism, hypospadias, poor sperm quality, and other genital defects were among the most common effects of DEHP exposure (decreased testicular weight, delayed spermatogenesis, Leydig cell aggregation, impaired Sertoli cell maturation, and significant inhibitions of testicular enzymes) [41]. The current study investigated the histological effects of DEHP

exposure on rabbit testes, as well as the role of *Zingiber officinale* extract as a preventative nutraceutical agent against this impact.

The present results indicated that treatment with DEHP caused significant reductions in body weight (BW) and relative testes weight (RTW) (Table 1). The reduction in BW and RTW of the DEHP treated rabbits is in agreement with those reported in previous studies [42],[43],[44]. Also, [45] DEHP (1000 mg/kg/d, 28-day treatment) damaged seminiferous tubules and spermatogenic cells in mice, resulting in worse semen quality. The testes' weight was reduced due to a decrease in the number of germ cells and lengthened spermatids in the testes [46]. DEHP's histological effect in certain mammals, including humans, has previously been studied [47].

According to report from Generally Recognized as Safe" (GRAS) document of the US FDA that has been listed ginger as safe to use. As a result of, a dose of 0.5 – 1.0 g of ginger powder ingested 2-3 times for periods ranging from 3 months to 2.5 years did not cause any adverse effects [48]. From our results, *Zingiber officinale* treated rabbits has shown normal testicular morphology similar to results of control group. These histopathological results agree with the work of [49].

The results of this study have shown that the DEPH treated rabbits for 12 weeks cause numerous histopathological changes in testes. This agrees with a number of studies using DEPH the most common effects of exposure included reduced fertility [41].

The present study was indicated that *Zingiber officinale* improved the histological alterations in testes of rabbits treated with DEHP. These alterations may refer to the antioxidant property of *Zingiber officinale*. Similarly, [32].

Confirmed that injecting ginger extract into the testes of mice significantly improved tissue [50]. demonstrated that *Zingiber officinale* extract reduced the extent of cisplatin-induce sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants. *Zingiber officinale* rhizome was found to overcome reproductive toxicity of gentamicin and induced spermatogenesis through the elevation of testosterone levels [51], [52] reported that co-administration of aqueous *Zingiber officinale* extract with arsenite was found to protect against adverse change in the reproductive organ weight, attenuate the decrease in sperm functions. Study, [53]. reported that antioxidant activity of ginger evaluates testicular toxicity induced by formalin in rat.

CONCLUSION

The findings given in this study add to the growing body of evidence supporting DEHP's extensive effects in experimental animal research and the importance of *Zingiber officinale* as a safe herbal treatment with few and minor side effects. This study also discovered and concluded that *Zingiber officinale* has a protective nutraceutical capacity to help overcome DEHP-induced histological damage in testing.

To assess how much human exposure to phthalates affects reproductive function and thus human health, more epidemiological and toxicological research is needed. To decrease the risk, toxicological data and chemical analysis must be combined, especially when the reactions are positive, and feasible entry channels and concentrations of substances must be determined.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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