Antioxidant and Protective Effects of Argan Oil and Flaxseed Oil on Diazinon-Induced Thyroid Function Disturbance in Male Rats

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Abstract: Currently, the rate of environmental pollution is constantly increasing globally, which affects the stability of ecosystem, the sustainability and the health and survival of living organism. Pesticides are among the most chemical pollutants affecting the environment and their overuse leads to major health problems. Recently, the use of medicinal plants and natural products has become of great interest due to their safe therapeutic properties, availability and low costs. The current study, for the first time, evaluates the possible protective role of argan oil and flaxseed oil on diazinon (DZN)-induced thyroid function disturbance and oxidative stress in Wistar male rats. Male rats were distributed into six groups. Group 1 was served as normal control. Group 2 was exposed to DZN (100 mg/kg body weight/day). Group 3 was treated with argan oil (500 mg/kg body weight/day) and DZN at the same dose given to group 2. Group 4 was exposed to flaxseed oil (500 mg/kg body weight/day) and DZN at the same dose given to group 2. Groups 5 and 6 were supplemented with argan oil and flaxseed oil individually each at the same dose given to groups 3 and 4 respectively. Blood sera were analyzed after six week of treatments to measure the levels of thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT). From the obtained results, it could be showed that the levels of serum TSH and MDA were significantly increased in rats of group 2. Moreover, serum T3, T4, GSH, SOD and CAT levels were statistically decreased in rats of group 2. Supplementations with argan oil and flaxseed oil attenuated the changes of the measured parameters in rats exposed to DZN (groups 3 and 4). The results suggested that the protective effects of argan oil and flaxseed oil against DZN toxicity attributed to their antioxidant activities.

Key words: Diazinon · Argan Oil · Flaxseed Oil · Thyroid Gland · Hormones · Antioxidants · Blood · Rats

INTRODUCTION

Environmental pollution is reaching worrying proportions worldwide. The global environmental pollution is considered as international public health problems [1]. Pesticide is a group of chemicals which are used in agriculture to control diseases, insects and regulate the growth of plants. Some pesticides are natural compounds and some of them are synthetized by human [2]. Environmental pollution with pesticides is a chronic worldwide problem which plays a vital role in the appearance of numerous diseases affecting animals, plants and humans [3, 4]. Diazinon (DZN) is an organophosphorus insecticide which is widely used around the world [3]. DZN is a moderately persistent organophosphorus pesticide and it has been widely used in industrial agriculture worldwide that would be potentially an exposure risk to workers in this field and the

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public [5-7]. DZN negatively affects several organs of the body including liver, kidneys, pancreas, immune system, urinary and reproductive systems, cardiac and vascular walls and induce hematological and biochemical changes [8].

In recent decades, witnessed a renewed interest in the naturally available botanicals which is as a source of phytochemicals that is used to prevent and treat diseases they are known as the oldest form of human health care [9]. Argan oil is extracted from kernel of the argan tree *Argania spinosa* L. Skeel; *Sapotaceae*, which is one of oldest forest tree in southwestern of Morocco and Algeria [10, 11]. The chemical components of argan oil made it one of the highest quality plant seed oils that have a high nutritional and dietetic value [12]. Moreover, previous experimental studies shown that argan oil was capable of protective against toxic effects and many diseases due to its antioxidant properties [13-16]. Flaxseed (*Linium usitatissimum*) is a member of *Linaceae* family that is also called linseed. Flaxseed oil is one of the famous plant oils which is extracted from the seeds of this plant. Many nutritional, physiological, biochemical and pharmacological researches focused on the effects of flaxseed extracts due to the health properties of flaxseed constituents [17-22]. The present study aims to evaluate the impact of DZN on thyroid function and to explore the possible protective effect of argan oil and flaxseed oil on thyroid gland disturbance and oxidative stress induced by DZN toxicity in male rats.

MATERIALS AND METHODS

Animals: Male albino rats of the Wistar strain (*Rattus norvegicus*), weighing 100-138 g were utilized in the current study. Rats were housed in standard plastic cages. Rats were allocated 10 per cage and maintained under controlled room conditions. Mean daily animal room temperature ranged from 19-21°C and mean daily relative humidity ranged from 60-65% during the study. Light timers were set to provide a 12 h light/12 h dark photoperiod. Experimental animals were fed *ad libitum* on normal commercial chow and had free access to water. The experimental treatments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

Experimental Protocol: A total of sixty male rats were divided randomly into six groups (n=10). The experimental groups were treated as follows:

- Rats of the first group were served as normal controls.
- Rats of the second group were orally given 100 mg/kg body weight of DZN, daily for six weeks.
- Rats of the third group were orally supplemented with argan oil at a dose of 500 mg/ kg body weight/ day for six weeks. Moreover, after 4 h they were treated with DZN at the same dose given to group 2.
- Rats of the fourth group were orally supplemented with flaxseed oil at a dose of 500 mg/ kg body weight/ day for six weeks. Moreover, after 4 h they were treated with DZN at the same dose given to group 2.
- Rats of the fifth group were orally supplemented with argan oil only at a dose of 500 mg/ kg body weight/ day for six weeks.
- Rats of the sixth group were orally supplemented with flaxseed oil only at a dose of 500 mg/ kg body weight/ day for six weeks.

Serum Biochemical Analyses: At the end of experimental duration, all rats were fasted for 8 hours, water was not restricted and then blood samples were taken from orbital venous plexus under total anesthesia using diethyl ether. Obtained blood samples were transferred to test tubes and the sera were separated using the standard centrifugation process. Sera were then collected and stored at -80°C. till the determination time of the levels of thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT). The levels of serum TSH were evaluated using the ELISA TSH kit (Cat. No. KT-29925), Kamiya Biomedical Company, USA. The kit is a competitive inhibition enzyme immunoassay technique for the quantitative measurement of TSH. Serum T3 levels were measured using the ELISA T3 kit (Cat. No. KA0925), Abnova Company, Taipei City, Taiwan. The T3 ELISA Kit is intended for the quantitative measurement of T3 in serum or plasma. The levels of serum T4 were determined using the ELISA T4 kit (Cat. No. KT-60863), Kamiya Biomedical Company, USA. T4 ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of T4. The method of Beutler *et al*. [23] was used to determine the value of GSH. Nishikimi *et al*. [24] method was used to determine the level of serum SOD. The method of Ohkawa *et al*. [25] was used to determine the value of MDA. The value of CAT was measured according to the method of Aebi [26].

Statistical Analysis: Quantitative data were presented as mean ± standard deviation (S.D.) and were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison among all groups. Statistical Package for Social Sciences (SPSS) for windows version 22.0 software was used for statistical analysis. The $P \leq 0.05$ level of probability was used as the criteria of significance.

RESULTS

Figure 1 represented the level of serum TSH in all experimental groups. In comparison with control rats of group 1, the levels of serum TSH were significantly increased in rats exposed to DZN (+ 91.5%, $P \leq 0.000$), argan oil plus DZN (+ 46.7%, $P \leq 0.01$) and flaxseed oil plus DZN (+ 35.4%, $P \leq 0.05$). Significant decreases in the level of serum T3 were observed in rats of group 2 (- 25.7%, $P \leq 0.002$), group 3 (- 11.4%, $P \leq 0.03$) and group 4 (- 11.8%, $P \leq 0.01$) compared with control rats (Fig. 2). In comparison with control rats of group 1, the levels of serum T4 were significantly decreased in rats of group 2 (- 20.4%, $P \leq 0.000$), 3 (- 13.0%, $P \leq 0.03$) and 4 (- 7.4%, $P \leq 0.04$) (Fig. 3). Moreover, insignificant changes were observed in the levels of serum TSH, T3 and T4 in rats treated with argan oil (group 5) and flaxseed oil (group 6) compared with control levels.
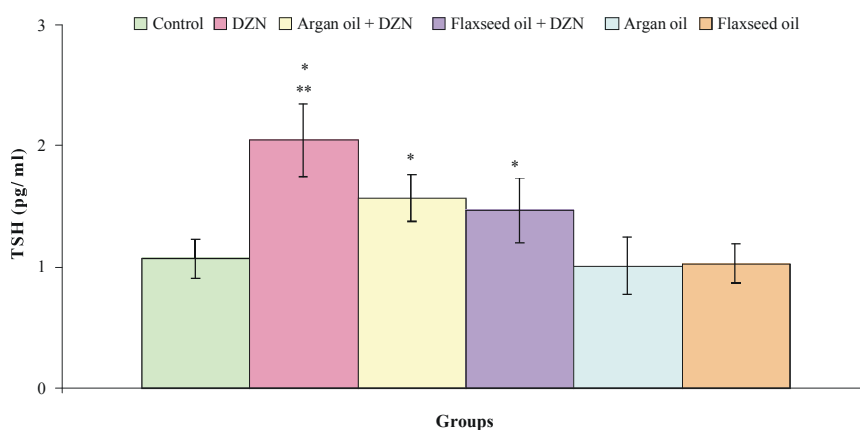

Fig. 1: Level of serum TSH in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. ** Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil
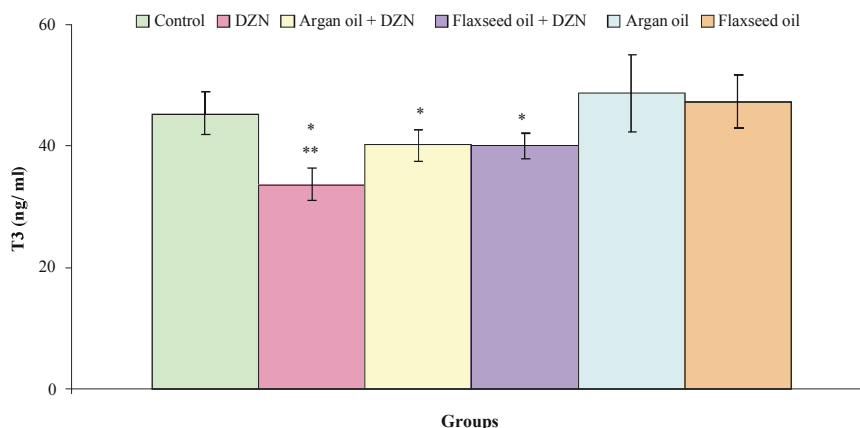

Fig. 2: Level of serum T3 in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. **Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil
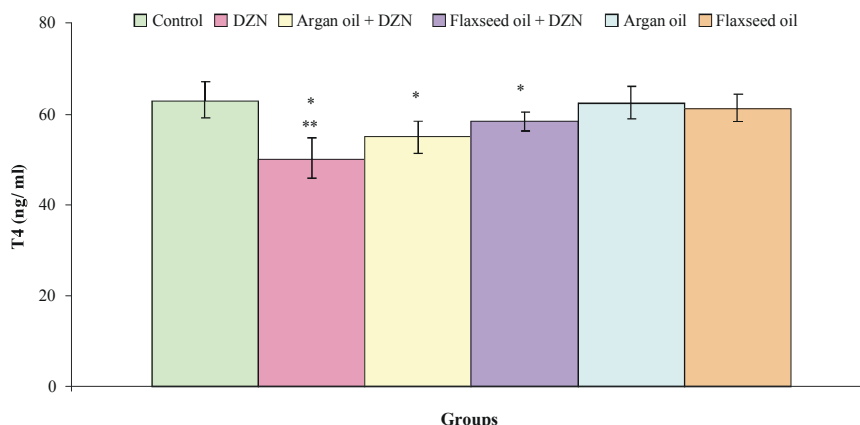
Fig. 3: Level of serum T4 in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. **Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil.
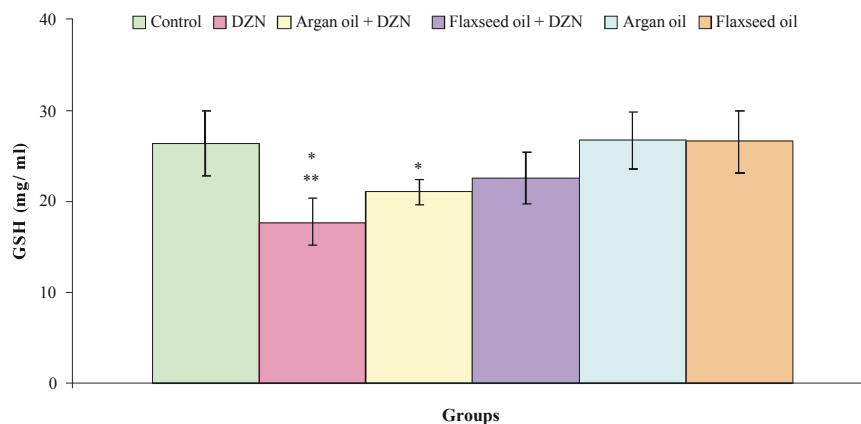

Fig. 4: Level of serum GSH in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. ** Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil

Fig. 5: Level of serum SOD in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. ** Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil
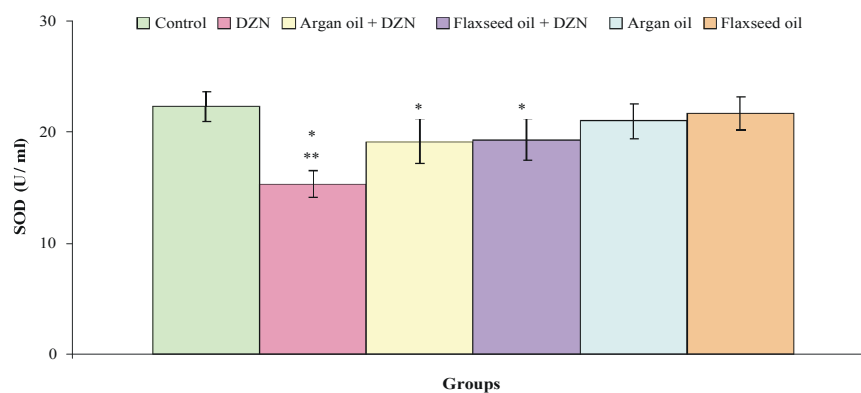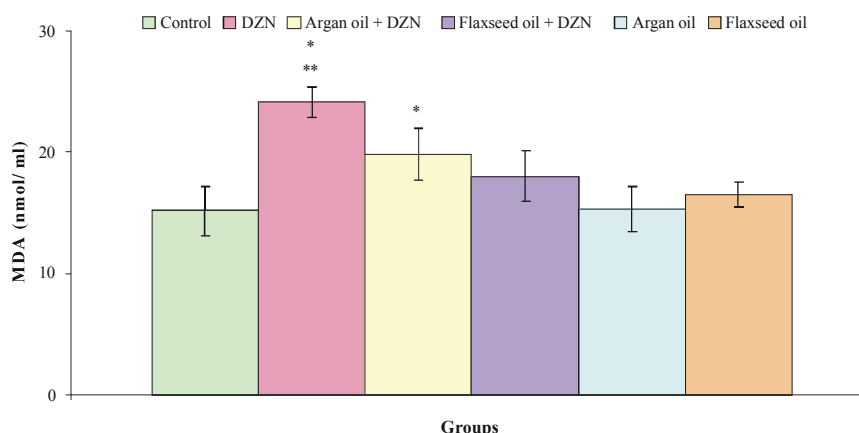
Fig. 6: Level of serum MDA in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. ˚˚Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil

Fig. 7: Level of serum CAT in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats after six weeks. *Indicates a significant difference between control and treated groups. ** Indicates a significant difference between rats treated with DZN and argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil
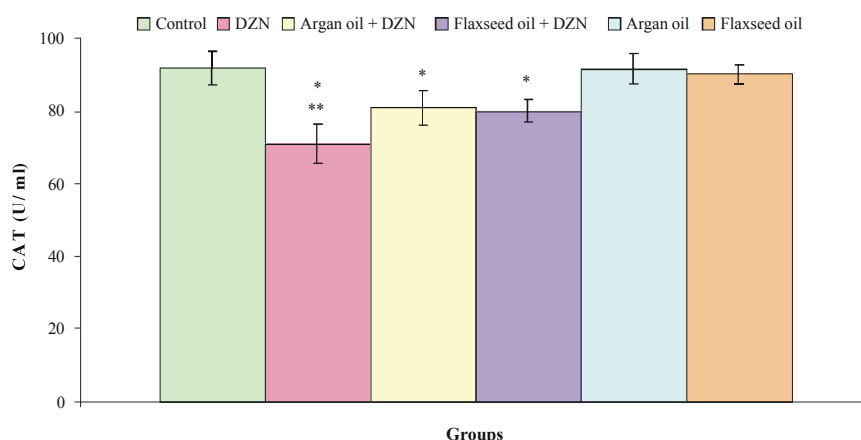
Figure 4 showed the level of serum GSH in all experimental groups. There is significant decrease in the level of serum GSH in rats treated with DZN (- 32.9%, $P \leq 0.000$) and argan oil plus DZN (- 20.2%, $P \leq 0.03$) compared with control rats, while the level of serum GSH was statistically unchanged in flaxseed oil plus DZN, argan oil and flaxseed oil treated rats. The level of serum SOD was significantly declined in rats of groups 2 (- 31.4%, $P \leq 0.000$), 3 (- 14.2%, $P \leq 0.001$) and 4 (- 13.4%, $P \leq 0.003$) compared with control rats of group 1. The level of serum SOD was insignificantly changed in rats of groups 5 and 6 (Fig. 5). Noticeably increases of serum MDA were noted in rats treated DZN (+ 59.4%,

$P \leq 0.001$) and argan oil plus DZN (+ 30.1%, $P \leq 0.02$). The level of serum MDA was insignificantly altered in flaxseed oil plus DZN, argan oil and flaxseed oil treated rats as compared with control group (Fig. 6). The measured levels of serum CAT in control, DZN, argan oil plus DZN, flaxseed oil plus DZN, argan oil and flaxseed oil treated rats are given in Figure 7. Serum CAT levels were markedly decreased in rats exposed to DZN (- 22.7%, $P \leq 0.001$), argan oil plus DZN (- 11.8%, $P \leq 0.02$) and flaxseed oil plus DZN (- 12.7%, $P \leq 0.01$). Insignificant changes in the level of CAT were observed in rats treated with argan oil and flaxseed oil compared with control rats of group 1.

DISCUSSION

The present study is the first experimental investigation aimed to evaluate whether pretreatment with argan oil and flaxseed oil would have a protective effect on DZN induced thyroid function disturbance and oxidative stress in Wistar male rats. The present results showed that the exposure to DZN increased the level of serum TSH, while the level of serum T3 and T4 was decreased. Endocrine disruptor (ED) is an exogenous substance or mixture capable of altering the function of the endocrine system. Endocrine disruptors (EDs) are exogenous environmental molecules affecting the synthesis, secretion, transport, metabolism, binding, action and catabolism of the natural hormones [27]. Thyroid hormones, which consist of T3 and T4, are mostly found in the form of T4 [28]. Thyroid hormones (T3 and T4) exert critical regulatory roles in cellular homeostasis as they control a number of physiological processes, including organ development, cell differentiation, cell growth and maintenance and metabolism (i.e. anabolism and catabolism of carbohydrates, proteins, lipids and damaged organelles) [29]. In the thyroid gland, the activation of TSH receptor (TSHR) by TSH stimulates iodine uptake in thyrocytes through a sodium/iodide symporter (Nis) [30]. Afterwards, iodine is carried out through the apical membrane to colloid by pendrin (Pds), an anionic channel [31]. In the colloid, the thyroperoxidase (Tpo) enzyme catalyzes iodide oxidation, iodination of the thyroglobulin tyrosine residue and iodothyronine coupling [32]; which are essential steps for T4 and T3 biosynthesis under TSH regulation. Then, thyroid hormones are hydrolyzed from thyroglobulin protein and transported from the cytosol through the basolateral membrane to the bloodstream by monocarboxylate transporter 8 (Mct8) [33]. Moreover, the synthesis and release of thyroid hormones in the body are mainly regulated by the hypothalamic–pituitary–thyroid (HPT) axis [34-36].

EDCs can interfere with thyroid function at different levels including the central regulatory system in the hypothalamus and pituitary, thyroid hormone production at the thyroid gland, thyroid hormones transfer, as well as hormone bioavailability, function and metabolism [37, 38]. Regulatory, experimental and epidemiological studies point to a positive association between pesticide exposure and impaired thyroid homeostasis [39-41]. The thyroid endocrine system is highly susceptible to the effects of EDCs, which, in turn, are capable of altering the physiological homeostasis of the hypothalamic-pituitary-thyroid axis at different levels, including the synthesis, metabolism and biological effects of thyroid hormones [42]. In agreement with the present results, various experimental investigations on rats and mice showed that the DZN and other pesticides induced similar observations in the levels of serum TSH, T3 and T4; and some of these experimental investigations revealed that the exposure to pesticides induced obvious histopathological changes of thyroid gland structure [43-48]. Toft *et al*. [49] investigated the thyroid function in Danish greenhouse workers, to evaluate if greenhouse workers classified as highly exposed to pesticides experiences altered thyroid levels compared to greenhouse workers with lower exposure. Significant increase of TSH and decrease of T3 and T4 were observed in high exposure compared to the low exposure groups. The authors concluded that the pesticide exposure among Danish greenhouse workers results in only minor disturbances of thyroid hormone levels. Farokhi and Taravati [50] investigated the association between exposure to pesticide and serum levels of thyroid hormones in pesticide sprayers. TSH level in sprayers was significantly increased compared to control subjects. Significant decreases in T3 and T4 levels of sprayers were observed. The results suggested that the exposure to pesticides may be responsible for increasing TSH and decreasing T3 and T4 levels, therefore supporting the hypothesis that pesticides cause hypothyroidism in pesticide sprayers. Moreover, Quraishi *et al*. [51] studied the effects of pesticides on blood circulating thyroid hormones (TSH and T3) in agriculture workers. The analysis showed a decrease in the level of TSH and an increase in the level of T3 hormone in group of people who have been exposed to pesticides in comparison to the other group of people who have not been exposed to pesticides (control). Quraishi *et al*. [52] attributed the variation between their obtained results and the results of Toft *et al*. [50] to the change of environmental factor, differences in immunity of the selected population and differences in the use of pesticides.

The current study showed that DZN induced oxidative stress which confirmed by the decreases of serum GSH, SOD and CAT levels and an increase of MDA level. These findings clearly showed that DZN induced oxidative stress in male rats. Free radicals are naturally present in the living organism and they include reactive oxygen species (ROS) and reactive nitrogen species (RNS) [52]. Pollutions of air and water, toxins, drugs, heavy metals, pesticides and cigarette smoke play an important role in the production of ROS [53]. Various

environmental stresses lead to excessive production of ROS causing progressive oxidative damage. When the ROS present in physiological concentration, they play an important role in the maintenance and the functioning of the body, but when their production exceeds the capacity of the cells to trap them, they start a state of oxidation called oxidative stress [54]. Oxidative stress has been shown to be involved in the pathophysiology of many diseases. Recently, ROS have gained more and more attention, because of their central role to the progression of many diseases [55-57].

The present study revealed that the treatment with argan oil and flaxseed oil attenuated the alteration of TSH, T3, T4, GSH, SOD, MDA and CAT induced by DZN toxicity in male rats. This indicated the effectiveness of argan oil and flaxseed oil in prevention of DZN toxicity. From the present new results, the possible mechanism of argan oil and flaxseed oil attributed to its antioxidant activities which evaluated by GSH, SOD, MDA and CAT levels. The protective effect of argan oil is probably due to its high contents of powerful antioxidants, particularly polyphenols, tocopherols and sterols, which are known as powerful antioxidants [58]. Antioxidants present in argan oil are believed to prevent or delay the onset of ROS after lipid peroxidation observed in rats or human plasma [59-61]. Flaxseed has regained its popularity from its traditional usage as raw material in oil production due to its high content of alpha-linolenic acid (ALA), which belongs to the family of omega-3 fatty acids. Flaxseed has a higher level of omega-3 and omega-6 and showed higher antioxidant capacity [62]. Naqshbandi *et al*. [63] attributed the ameliorative effect of dietary flaxseed oil supplementation on cisplatin induced specific metabolic alterations and oxidative damage due to its intrinsic biochemical antioxidant properties. Additionally, previous studies showed that the flaxseed has strong antioxidant activities against different toxicants induced physiological and biochemical alterations and oxidative stress [64-67].

In summary, the present study revealed that the exposure to DZN induced severe alterations of thyroid function which confirm by the evaluation of TSH, T3 and T4 levels and oxidative stress markers including the levels of GSH, SOD, MDA and CAT. The obtained results indicated that the argan oil and flaxseed oil are promising chemotherapeutic agents against DZN toxicity due to their antioxidant activities. Accordingly, it can be suggested that the supplementation of argan oil and flaxseed oil has a beneficial effect on thyroid function disturbance induced by DZN and may be also by other toxicants and pathogenic factors. Finally, the present

results justify the development of additional physiological, pharmacological and biochemical studies in order to clarify the nature of the substances responsible for the effect and the exact mechanisms action of active constituents of argan oil and flaxseed oil.

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