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ORIGINAL ARTICLE

The possible ameliorative effects of ginger extract against toxic effects of atrazine on the thyroid gland of albino rats model.

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ABSTRACT

Background: atrazine is widely used organophosphate herbicide that adversely affects the structure of the thyroid gland. So this work was performed to study the possible ameliorative effects of ginger extract against toxic effects of atrazine on the thyroid gland of albino rats.
Methods: Forty male adult albino rats were subdivided into four equal subgroups each contain 10 rats. Group I: each rat was given 5ml of water once daily orally for thirty days. Group II: each rat received (750 mg/kg body weight) of ginger extract by oral gavage, once daily for thirty days. Group III: each rat received (100 mg/kg body weight) of atrazine by oral gavage for thirty days. Group IV: each rat received (100 mg/kg body weight) atrazine and 750mg/kg body weight of ginger aqueous extract by oral gavage once per day for thirty days.
Results: Atrazine caused structural damage of thyroid gland in the form of disruption of the shape of the follicles. The colloid was vacuolated, increased collagen deposition, decreased amount of colloid and increased caspase-3 intensity immunoreaction with decreased serum levels of T3&T4 and increased serum level of TSH also there was increased level of MDA in thyroid tissue. On the otherwise the concurrent administration of ginger extract aqueous solution with atrazine cause improvement in the previous changes that caused by atrazine only.
Conclusions: Ginger extract administration with atrazine could ameliorate the toxic effects of atrazine on thyroid gland.
Keywords: Atrazine; Ginger; Caspase-3; Thyroid hormones

INTRODUCTION

The thyroid gland is the most affected endocrine gland by organophosphate herbicides, also it is one of the most sensitive organs to it, causing disruption of thyroid function [1]. Thyroid hormones are necessary to maintain normal growth development and metabolism, where any disruption in the thyroid hormones levels may cause many clinical conditions [2]. Organophosphorus compounds as herbicides and pesticides which are commonly used in agriculture are harmful chemical compounds that have toxic effects on human and vertebrate animals [3]. Agricultural pesticides are extensively used especially triazine chemicals and atrazine (ATZ). They possess disrupting effects on endocrine system especially mammals reproductive systems [4,5,6]. Atrazine a famous environmental pollutant is reported to cause hazardous effects in many organs of mammalian experimental models. It is reported to cause rat liver damage, it also induces erythrocytes damage, genotoxicity and DNA damage in rat stomach and kidney [7]. The toxic effects of atrazine were due to oxidative stress which caused reactive oxygen species generation (ROS) [8], peroxidation of lipid and depletion of antioxidant (6). Todays, many people utilize herbal drugs as one of complementary and alternative medicine, as they possess low cost and few side effects. A great importance was given to medicinal plants because they have profound antioxidant effect of the phytochemicals that reduce free-radical oxidative damage. Overwhelming the green parts of the plants play a key role in protection against the oxidative damage because it possess huge quantity of antioxidant and phenolic compounds [9].

One of the most important plants which have been utilized for many hundreds of years as an anti-vomiting, treatment of diabetes mellitus, and cancer therapy is ginger. Ginger, belongs to the Zingiberaceae family, having polyphenolic and flavonoids contents that possess many properties as analgesic, anti-inflammatory, antidiabetic and

antioxidant properties [10]. So, the current study was done to study the possible ameliorative effect of ginger extract against atrazine hazardous effects on the thyroid gland of albino rats.

METHODS

Materials:

Atrazine was acquired from Sigma, Aldrich, Germany and transported by Cairo Chemical Company, Egypt. It was dissolved in corn oil, while ethanolic ginger extract (Z. officinale Roscoe) rhizomes were purchased from markets in Zagazig. It was cleaned, washed with water, cut, dried and finally powdered. The extract was made by maceration of 100 g of this powder in 1000 ml of ethanol for twelve hour at room temperature and finally filtered, 750 mg/ml was its final concentration. 1 ml of the final aqueous extract was given orally for each animal [11], Rabbit anti-cleaved-caspase-3 p17 polyclonal antibody, marker for cell apoptosis, purchesd from Abcam, Cambridge, Massachusetts, USA.

Methods:

Sample size:

It was calculated as 40 rats; 10 rat in each group using online sample size calculator (https://www.calculator.net/sample-size-calculator.html?type=2&cl2=95&ss2=40&pc2=60&ps2=&x=59&y=23#findci). They weighted about (200-225 grams) and were purchased from the laboratory animal house of faculty of medicine, zagazig university, Egypt. They were fed on a standard diet and given free access to water.

Ethical Approvals:

Accordance to the National Institutes of Health (NIH) animal care guidelines all animals were cared, and the Institutional Animal Care and Use Committee at Zagazig University, Egypt (ZU-IACUC/3/F/196/2021) approved the experiment.

Experimental animals:

Forty (40) male adult albino rats were subdivided into four equal subgroups [12]. Each one contains 10 rats as follows:

Group I (control-ve group): each rat was given 5ml of water once daily orally for thirty days. Group II (control+ve group): each rat received (750 mg/kg body weight) of ginger extract by oral gavage once daily for thirty days [13]. Group III: (atrazine treated group): each rat received (100 mg/kg body weight) of atrazine by oral gavage for thirty days [14]. Group IV: (atrazine + ginger extract treated group): each rat received (100 mg/kg body weight) atrazine and 750mg/kg body weight of ginger aqueous extract by oral gavage once per day for thirty days.

One day after the last dose, venous sample collection were done from retro-orbital plexus of veins from each rat for assessing of triiodothyronine (T3), tetraiodothyronine (T4) and TSH levels then anesthetization of animals with ether were done and neck dissection were performed, exposure of trachea and excision of the thyroid gland were done. Dissection of thyroids into two parts one part for histological examination and the other for tissue homogenate for preparation of level of MDA.

The histological study:

The samples of thyroid tissue were immersed in neutral-buffered formalin (10%) and later on the tissues were processed for paraffin wax, Preparation of sections and staining them with Hematoxylin and Eosin stain (H&E), Masson's trichrome stain and PAS stain and the slides were examined by light microscope at pathology department faculty of medicine zagazig university [15,16,17,18,19].

The immune-histochemical study:

Cutting of formalin fixed paraffin tissues into 5-µm thick sections and then they were incubated overnight with the primary antibody (activated caspase-3 antibody), then Incubated with secondary antibody biotinyle peroxidase for one hour and a chromogen was used as 3, 3'diaminobenzidine (DAB) for immunoreaction site localization. The counter stain that was used is Mayer's hematoxylin and washing of the slides with distilled water and PBS. [20& 21].

The hormonal assay:

Blood samples were taken for analysis of The Tri-iodothyronin (T3), Tetra-iodothyronin (T4) and thyroid stimulating hormones (TSH) by using (ELISA) enzyme-linked immunosorbent assay

The Oxidative assay:

Levels of Malondialdehyde (MDA) of thyroid gland tissues were assessed as a lipid peroxidation byproduct, MDA was assessed by spectrophotometer and expressed as nmol/g tissue according to the processed data [22].

The Morphometric study:

The image analysis system (Leica Q 500 MC program) at the faculty of medicine, zagazig university, for examination, five different microscopic fields from every studied group were taken at magnification of X400 and they were used to measure: In slides stained with H&E →The size of the follicles, in slides stained with Mallory's trichrome → collagen fibers percentage area, in slides stained with PAS →The area percentage of

colloid and in caspase stained slides →The intensity of immune- reaction

Statistical Analysis:

The (SPSS) statistical package for social science, version 19, was used for performance of all statistical analysis. All results obtained were presented by using (ANOVA) one-way analysis of variance test and the results were presented as means ±SD (standard deviation) then followed by least significant difference test (LSD), When Probability value (P) ≤ 0.05, the statistical significance was considered [23].

RESULTS

Light microscopic results:

The control negative and control positive groups showed nearly similar histological and immunohistochemical results. So, we compared the other groups with control negative group.

Hematoxylin and eosin (H &E) staining:

As regarding the thyroid gland histological examination of both controls I & II treated groups indicated normal thyroid gland parenchyma, which composed of multiple variable sized and shaped thyroid follicles that had cubical follicular cells with rounded basophilic nuclei at their lining epithelia. The lumens of the follicles were engorged with colloid, which was acidophilic and homogenous with low vacuoles at its periphery (Figures 1A&B).

In atrazine treated thyroid sections, it showed disturbance of normal architecture of the gland. The size of many thyroid follicles was reduced with vacuolation of follicular cells, vacuolation of colloid and exfoliation of the epithelial cells of follicles, some thyroid follicles showed disturbance of its lamina and communicate with its neighboring follicle (Figure 1 C).

On the contrary, at the Atrazine+Ginger extract group the thyroid sections showed that the thyroid follicles extremely improved as there was decreasing in the vacuolation of colloid with disappearance of exfoliation but few follicles still showed follicular cytoplasm vacuolation (Figure 1D).

Mallory's trichrome staining:

Mallory trichrome stained sections of control groups showed minimal collagen fibers between thyroid follicles (Figures 2A&B), but atrazine treated group showed increased blue color collagen fibers between thyroid follicles (Figure 2C). On the other hand, in ginger extract treated group showed that collagen fibers was normal in relation to control group (Figure 2D).

Periodic Acid Schiff (PAS) staining:

In the PAS stained sections of control I&II groups, they showed strong colloidal PAS +ve reaction and strong reaction in the basement membrane (Figures 3 A&B). The PAS stained sections of atrazine treated group showed weak (-ve) colloidal PAS reaction and weak reaction in the basement membrane (Figure 3C). Also, sections of Atrazine+ginger received group showed moderate colloidal PAS reaction and moderate reaction in the basement membrane (Figure 3D).

Caspase-3 immunohistochemistry staining:

As regarding immunoreaction for activated caspase-3 stained sections in control I&II groups, the cells of thyroid follicles showed weak positive reaction in their nuclei and the cytoplasm (Figure 4A&B).

As regarding immunoreaction for activated caspase-3 stained sections in atrazine treated group, the cells of thyroid follicles showed strong positive reaction in their nuclei and the cytoplasm (Figure 4C).

As regarding immunoreaction for activated caspase-3 stained sections in Atrazine+ ginger extract treated group, the cells of thyroid follicles showed moderate positive reaction in their nuclei and the cytoplasm (Figure 4D).

Morphometric results:

The mean size of the thyroid follicles were significantly decreased in the group treated with atrazine in comparison to the control group, while there was a non-significant decrease in atrazine +ginger extract treated group in comparison to control groups.

The mean collagen fiber percentage area indicated a significant increase in the group treated with atrazine in comparison to the control group. On the contrary, there was a non-significant increase in atrazine +ginger extract treated group in comparison to control group

The mean colloid percentage area indicated a significant decrease in the group treated with atrazine in comparison to the control group. While there was a non-significant decrease in atrazine +ginger extract treated group in comparison to control groups.

Finally, there was a significant increase in color intensity of caspase-3 immunoreaction in group treated with atrazine in comparison to the control group. On the contrary there was a non-significant increase in atrazine +ginger extract treated group in comparison control groups

Hormonal assay:

T3 hormonal level indicated that there was a significant decrease in group treated with atrazine in

comparison to control group, while there was a non-significant decrease in Atrazine +ginger extract treated group in comparison to control group.

T4 hormonal level showed a significant decrease in group treated with atrazine in comparison to control group, while there was a non-significant decrease) in Atrazine +ginger extract treated group in comparison to control group.

TSH hormonal level, there was a significant increase in group treated with atrazine in comparison to control group, while there was a non-significant increase in Atrazine +ginger extract treated group in comparison to control group.

Oxidative assay:

In this current work, there was a significant increase in MDA oxidative activity in thyroid gland tissue of atrazine treated group as compared to control group. While there was a non-significant increase in Atrazine+Ginger extract as compared to control group.

Table1: morphometric analysis of different parameters in the thyroid tissue.

Groups	**Group 1**	**Group 2**	**Group 3**	**Group 4**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Mean follicle sizes	331.7 ± 28.5	314.5 ± 39.6	152.9 ± 38.6*	299.9 ± 35.6
Mean percentage area of Collagen	14.2± 3.6	12.4 ± 3.5	24.8± 4.8 *	13.56 ± 3.5
Mean percentage area of Colloid	25.23 ± 3.61	23.88 ± 2.71	10.30 ± 1.07 *	22.55 ± 4.12
Mean percentage of color intensity of caspase immunoreaction	17.13 ± 0.08	17.39 ± 0.28	28.15 ± 0.02 *	18.15 ± 0.05

Mean ± SD = standard deviation * significant, P < 0.05

Table 2: Serum hormones levels at different groups.

Groups	**Group 1**	**Group 2**	**Group 3**	**Group 4**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
T3(Tri-iodothyronin**) (ng/ml)**	62.4 ± 0.18	63.6±0.16	55.27 ± 0.89*	61.6 ± 0.18
T4(Tetra-iodothyronin**)(ng/ml)**	65.33 ± 0.18	67.3±0.18	57.71 ± 0.21*	64.53± 0.28
TSH(Thyroid stimulating hormone**) (pg/ml)**	0.67± 0.04	0.69±0.05	0.77 ± 0.01*	0.68 ± 0.04

mean ± SD =standard deviation * significant, P < 0.05

Table 3: Tissue MDA levels at different groups.

Group	**Group 1**	**Second 2**	**Group 3**	**Group 4**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
MDA: Malondialdehyde; (nmol/g/tissue)	2.52± 1.07	2.55±1.09	3.45±1.28*	2.58 ± 1.12

mean ± SD =standard deviation * significant, P < 0.05

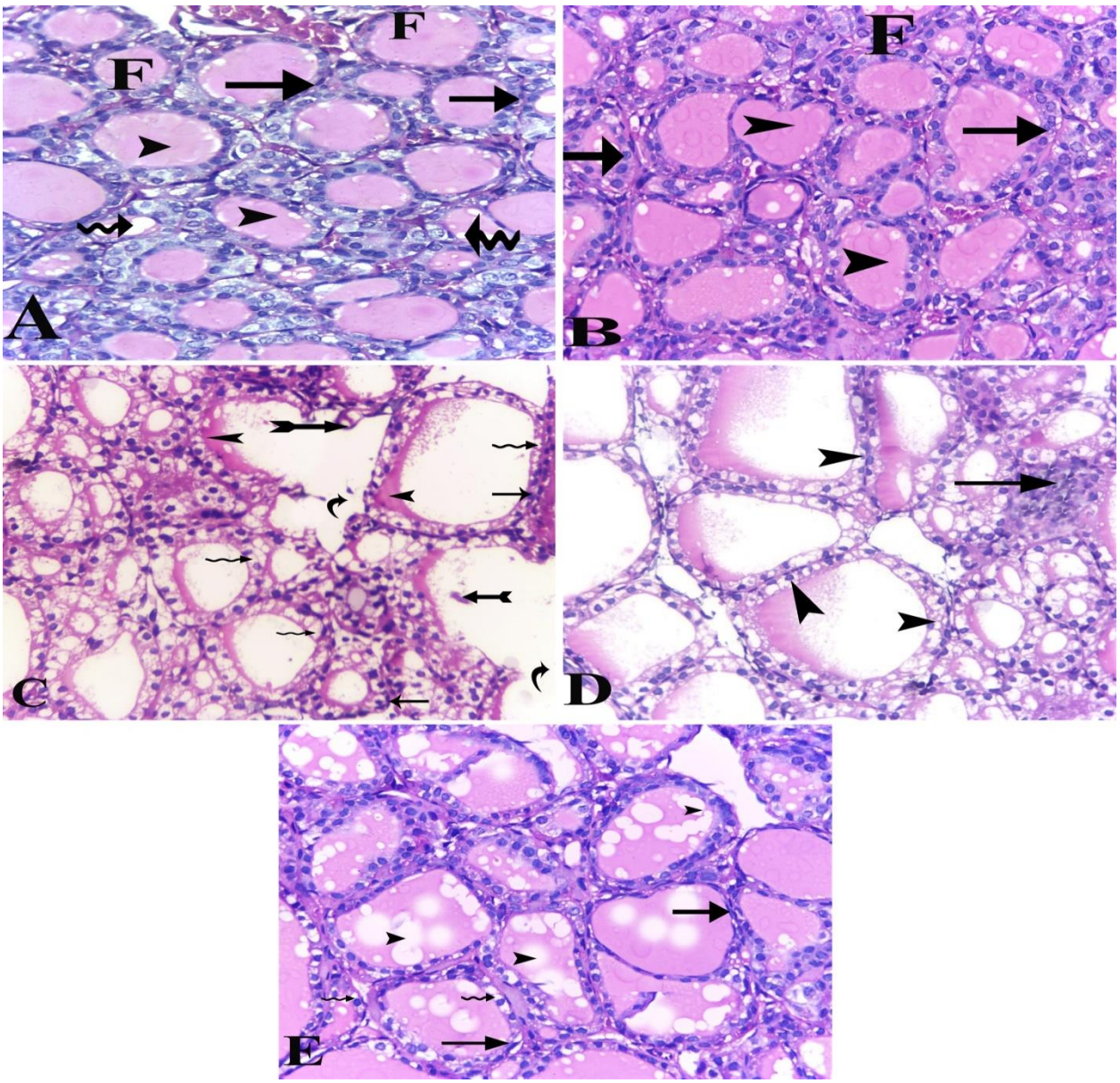

Figure 1 (**1A&B**): A Photomicrographs of control groups indicating that thyroid follicles (F) are normally organized with different sizes lined by follicular cells (arrow) filled with homogenous colloid (arrow head) in their Lumina and few colloid vacuolation are seen at the periphery of the follicles (wavy arrow). (1c)a photomicrograph of Atrazine received group indicating loss of normal thyroid follicles appearance lined by follicular cells with vacuolated cytoplasm(wavy arrow) and deeply stained nuclei (arrow) and the follicles appear empty of colloid and some colloid aggregate at the periphery (arrow head). exfoliated cells are showed in lumens of Some follicles (double head arrow). Some follicles showing disruption of thyroid lamina and communicate with neighboring follicle (curved arrow) (1D) a photomicrograph of Atrazine received group indicating follicles lined by vacuolated follicular cells (arrow head) with many cellular infiltrates(arrows). (1E)) a photomicrograph of atrazine +ginger extract treated group showing restoration of normal shape of thyroid follicles, they are lined by flattened squamous epithelium (arrow)with vacuolated cytoplasm (wavy arrow), the follicles contain homogenous colloid with some vacuolation (arrow head) (H&E, x400).

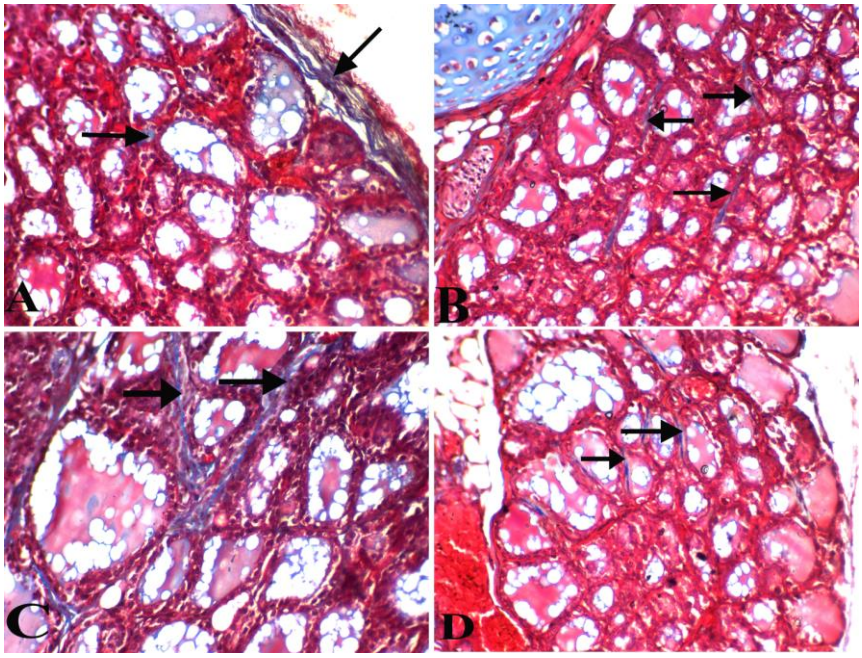

Figure 2 (2A&B): A photomicrograph of control groups showing thin connective tissue septa between thyroid follicles (arrow). (2C)A photomicrograph of atrazine treated group showing thick connective tissue septa between thyroid follicles (arrow). (2D) a photomicrograph of ginger extract treated group showing very thin connective tissue septa between thyroid follicles (arrow) (Mallory trichrome, x400).

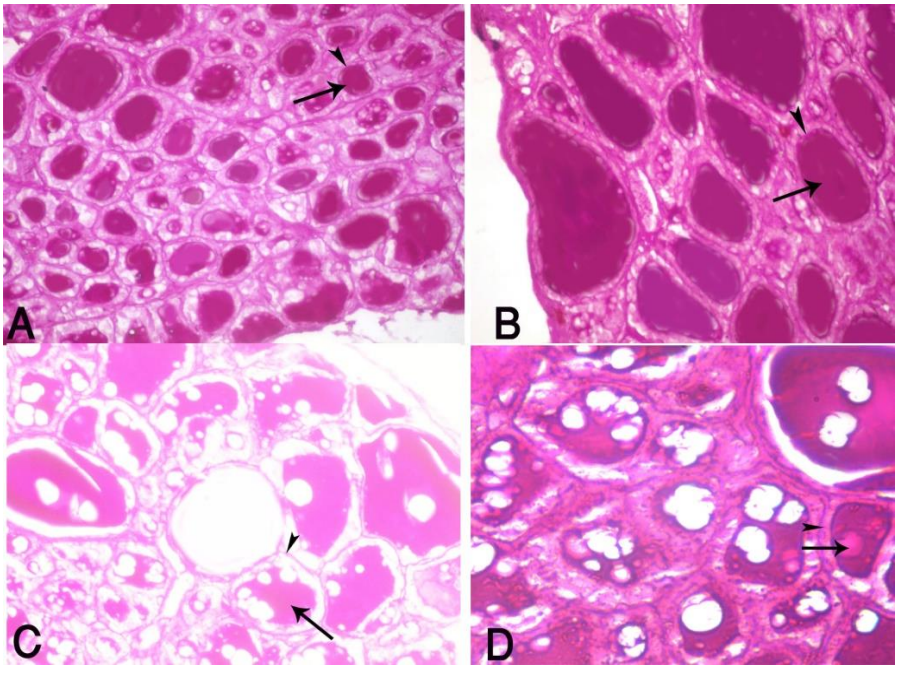

Figure 3 (**3A&B**): A Photomicrograph of control groups indicating strong +ve PAS colloidal reaction in the lumen of thyroid follicle (arrow) and strong +ve PAS reaction in the follicular basement membrane (arrow head). (3C)A Photomicrograph of atrazine treated group indicating marked reduction in PAS colloidal reaction of thyroid follicle (arrow) and weak PAS reaction in the follicular basement membrane (arrow head). (3D)A Photomicrograph of ginger extract group, indicating moderate PAS colloidal reaction in the lumen of thyroid follicles (arrow) and moderate PAS reaction in follicular basement membrane (arrow head) (PAS x400).

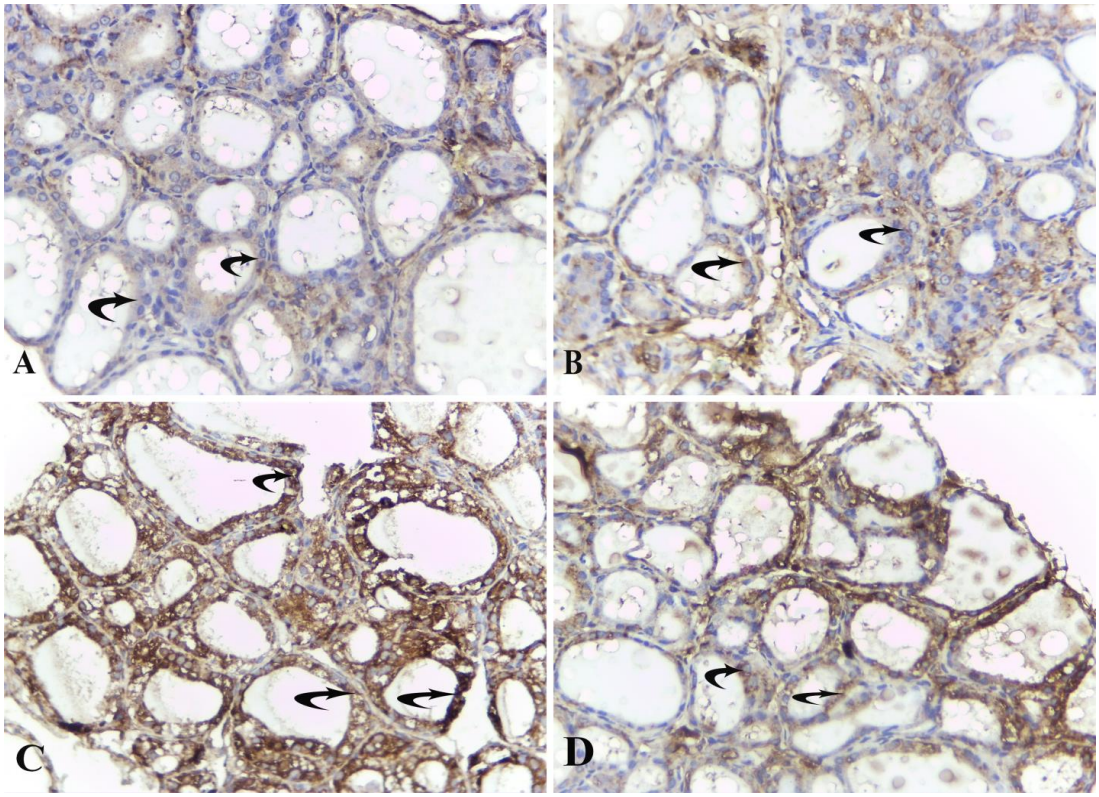

Figure 4 (4A&B): Photomicrographs of control groups indicating weak positive caspase-3 immunoreaction in nuclei of some follicular cells (curved arrows). (4C) a photomicrograph of sections from group treated with atrazine indicating strong positive caspase-3 immunoreaction in the nuclei of many follicular cells (curved arrows). (4D) a photomicrograph of ginger extract+atrazine-treated group indicating moderate positive caspase-3immunoreaction in the nuclei of few follicular cells (curved arrows) (Caspase-3 immunostaining X400).

DISCUSSION

Atrazine is a well-known pesticide commonly used in agriculture in Egypt. It is a chemical that cause disturbance of endocrine function and it is the cause of reproductive toxicity in mammals and other lower vertebrates. Moreover, no studies have been done for evaluation of the ameliorative role of ginger extract to counteract the toxicity of atrazine on thyroid tissue. The current study intended to evaluate the possible ameliorative effects of ginger extract against toxic effects of atrazine on the thyroid gland of albino rats by histological and biochemical investigations. The present work design was supported by [24] as the state of function of any organ was closely reflected by its structure and the glandular activity of the thyroid gland was sensitively indicated by histological examination of its structure rather than serum T3 and T4 levels.

In the current study, thyroid sections of atrazine treated group showed that the normal architecture of thyroid gland was lost in comparison to control groups. The follicles appeared with variable sizes lined by follicular cells with vacuolated cytoplasm and the colloid was vacuolated in some follicles and others with no colloid. Exfoliated cells are seen in lumens of some follicles and some follicles showed disruption of thyroid lamina and communicate with neighboring follicle and with many cellular infiltrates. Moreover, significant decrease in the follicle's mean size and mean percent area of colloid. Also, there was significant increase in deposition of collagen fibers between follicles and increased the color intensity of caspase-3 stained immune-histochemical sections were also found, these results were augmented by that the T3, T4 hormonal level which was significantly decreased and the TSH level was significantly elevated. These finding were in line with [25] whose results said that treating the rats with atrazine caused hypothyroidism in the form of decreased the T3 &T4 serum levels and increased TSH serum level and also these results are said by Scanlan et al. [26] who indicated that the cause of thyroid hormones

impairment was due to oxidative stress or due to follicular cell damage in thyroid gland. This result was due to liberation of thyroid hormone that begins with colloid endocytosis into vesicles then these vesicles transmitted to the follicular cells after that lysosomes bounded with these vesicles and released T3/T4.

Yu et al [27] denoted that apoptotic cell death was the cause of increased the intensity of caspase-3 immunoreaction in the epithelium of follicular cells which induced by organo-phosphorus exposure. The damaging effects of atrazine may be due to acceleration of collagen synthesis by lipid peroxidation which might cause increased the thickness of collagen fibers between thyroid follicles [28].

Previous reports recorded that herbicides caused decreased iodine binding proteins [29]. Many studies explained that herbicides exposure caused oxidative stress [30]. Also, the causes of the reduction of hormonal levels of thyroid hormones were the huge generation of reactive oxygen spices (ROS) and excessive liberation of free radicals in the CNS, excessive generation of free radicals and ROS in CNS adversely affected the hypothalamic-pituitary axis which was the principle factor in the process of ageing [31].The hypothalamic–pituitary axis aging causes progressive loss of function of the thyroid gland and endocrine deficiency [32].

In this current work, The MDA activities level in group treated with atrazine indicated a significant increase in comparison to control group. These finding were in agreement with [33] who said that organo-phosphorus chloropyrifos caused increases level of MDA activities in rat thyroid tissue treated with it.

In addition, MDA formation produce oxidative stress, that leads to elongation factor 2 loss, which is responsible for synthesis of protein in the pituitary gland and hypothalamus, causing decrease the production of peptide hormones formation from the hypothalamic–pituitary axis [34].

In the current study, there is significant decrease in the MDA levels in the thyroid tissue of ginger extract group in comparison to group treated with atrazine this proved that ginger extract can ameliorate the oxidative stress produced by atrazine. The results of histopathological examination of thyroid of ginger extract group proved it's protective effect which were in agreement with results of [35] who proved the ginger extract antioxidant effect against toxicity of cypermethrin on thyroid gland.

Khaki et al. [36] revealed that ginger prevent free radicals generation and also possess a strong antioxidant effect. Ginger acts as strong antioxidant as it contain gingerols which is a phenolic compounds that elevated glutathione activity, increased superoxide dismutase activity and also decrease lipid peroxidation [37]. Histo-pathological examination of rats of atrazine and ginger extract group in the present study showed that the thyroid structure is markedly improved. Its normal architecture was nearly kept with persistence of some follicular epithelial vacuolations. These finding are in agreement with [38] who explained that ginger ethanolic extract decreased myocardial necrosis in rats which induced by isoproterenol. Moreover, [39] explained that ginger extract causes histological alterations of rat testes induced by exposure to deltamethrin.

Other researches confirmed that herbicide exposures can affect hormone levels of thyroid gland based on level of the exposure, the season and the agents used. [40]. The biochemical results of the present work of ginger extract group are in agreement with [41] who said that antioxidant effects of ginger extract could counteract the alterations of testis structure and the biochemical changes induced by metalaxyl.

It is concluded that atrazine has toxic effects on thyroid gland structure with resultant disturbance of hormonal level of the thyroid gland. So, limitation of atrazine utilization should be obliged. On the contrary, administration of ginger extract ameliorated the thyroid gland structure changes. It is recommended to use ginger extract to protect the thyroid gland structure against toxic effects of atrazine.

REFERENCES

1-Meeker JD, Barr DB, Hauser R: Thyroid hormones in relation to urinary metabolites of non-persistent insecticides in men of reproductive age. Reprod Toxicol. 2006**;** 22: 437-442. 9.

2- Fliers E, Bianco AC, Langouche L, Boelen A: Thyroid function in critically ill patients. Lancet Diabetes. Endocrinol. 2015**;**3: 816-825.

3-Sargazi, Z., Nikravesh, M. R., Jalali, M., Sadeghnia, H. R., & Rahimi Anbarkeh, F.: Apoptotic effect of organophosphorus insecticide diazinon on rat ovary and protective effect of vitamin E. Iran. J. Toxicol. 2016; 10(2), 37-44.

4-Abarikwu, S. O., Duru, Q. C., Chinonso, O. V., & Njoku, R. C.: Antioxidant enzymes activity, lipid peroxidation, oxidative damage in the testis

and epididymis, and steroidogenesis in rats after co-exposure to atrazine and ethanol. Andrologia. 2016; 48(5), 548-557.

5-Akunna, G. G., Saalu, C. L., Ogunmodede, O. S., Ogunlade, B., & Bello, A. J.: Aqueous extract of date fruit (Phoenix dactylifera) protects testis against atrazine-induced toxicity in rat. World J Life Sci Med Res. 2012; 2(2), 100-8.

6-Abass, M. A., Elkhateeb, S. A., El-baset, S. A. A., Kattaia, A. A., Mohamed, E. M., & Atteia, H. H.: Lycopene ameliorates atrazine-induced oxidative damage in adrenal cortex of male rats by activation of the Nrf2/HO-1 pathway. Environ. Sci. Pollut. Res.2016; 23(15), 15262-15274.

7-Abarikwu, S. O.: Protective effect of quercetin on atrazine-induced oxidative stress in the liver, kidney, brain, and heart of adult Wistar rats. Toxicology international. 2014;21(2), 148.

8-Hozyen, H. F., Eldebaky, H. A., Soror, A. H., Desouky, H. M., & Shalaby, S. I. A.: Protective Role of Selenium Against Adverse Effects of Atrazine Toxicity in Male Rats: Biochemical, Histopathological and Molecular Changes. Veterinaria. 2015;15(357), e365.

9- Rostamichijan M, Mosavat SH, Ghahramani L, et al.: Anorectal diseases in Avicenna's "canon of medicine". Acta Med Hist Adriat. 2015;103-14.

10- Daily JW, Yang M, Kim DS, et al.: efficacy of ginger for treating Type 2 diabetes: A systematic review and meta-analysis of randomized clinical trials. J Ethnic Foods. 2015;36-43.

11- **Kamtchouing P1, Mbongue Fandio GY, Dimo T, et al.**: Evaluation of androgenic activity of Zingiber officinale and Pentadiplandra brazzeana in male rats. Asian J Androl. 2002;299-01.

12 **ILAR (Institute of Laboratory Animal Resources): Laboratory animal management:** Rodents. Institute Of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, 1996 Washington, DC.

13-**Aljahawey MHO, Soeharto S, Sujuti H**.: The effect of ginger extract (zingiber officinale roscoe) on male leydig cell and testosterone Level in carbofuran induced rat. Int J Pharm Tech Res. 2015;879-88.

14- **Susan C. Laws, Janet M. Ferrell, Tammy E. Stoker, Judith Schmid, and Ralph L. Cooper:** The Effects of Atrazine on Female Wistar Rats: An Evaluation of the Protocol for Assessing Pubertal Development and Thyroid Function. Toxicol. Sci.2000; 58, 366–376.

15-**Clayden B:** Practical section cutting and staining. Fifth ed. Churchill Livingstone, Edinburgh and London. 1971**;**p: 270.

16- **Masson P:**Trichrome staining and their preliminary technique. J Tech Meth. 1929; 12:75-90.

17-**Prophet EB, Mills B, Arrington JB, Sobin LH:** Laboratory methods in histotechnology. Fourth ed. Washington, DC. 1992; 132: 53–58.

18-**McMANUS JF :** Histological demonstration of mucin after periodic acid. Nature. 1946; 158: 202.

19-**Bancroft DJ, Gamble M :**Theory and Practice of Histological Techniques. Sixth Edn. New York. 2008; 12: 121–123.

20- **Ramos-Vara JA, Beissenherz ME:** Optimization of immunohistochemical methods using two different antigen retrieval methods on formalin-fixed, paraffin-embedded tissues: Experience with 63 markers. J Vet Diagn Invest. 2000;12: 307–311.

21-**Wang YF, Biao L, Xiang-shan, F, Qiu R, et al**: Thyroid carcinoma showing thymus-like elements: A clinicopathologic, immunohistochemical, ultrastructural and molecular analysis. Amer J Clin Path. 2015; 143: 223-233.

22-**Ohkawa H, Ohishi N, Yagi K :** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-358.

23- **Dawson B, Trapp RG.** Basic & Clinical Biostatistics, 4th Edition, McGraw-Hill, 2004

24- **Zoeller RT, Tan SW, Tyl RW** General background on the hypothalamic-pituitary-thyroid (HPT) axis. Crit Rev Toxicol. 2007;p:11-53.

25-**Rajkovic V., Matavulj M., Johansson O.,**: Studies on the synergistic effects of extremely low-frequency magnetic fields and the endocrine-disrupting compound atrazine on the thyroid gland, Int. J. Radiat. Biol. 2010; 86, 1050-1060**..**

26-**Scanlan TS, Suchland KL, Hart ME, et al.**: 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat Med. 2004;638-42.

27- **Yu F, Wang Z, Ju B, Wang Y, Wang J, Bai D:** Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamins C and E. Exp Toxicol Pathol .2008;59(6): 415-423.

28-. **De Araújo-Ramos AT, Passoni MT, Romano MA, Romano RM, Martino-Andrade AJ**. Controversies on Endocrine and Reproductive Effects of Glyphosate and Glyphosate-Based

Herbicides: A Mini-Review. Frontiers in Endocrinology. 2021;12(627210).

29- **Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, et al.** Pesticide use and thyroid disease among women in the Agricultural Health Study. Am J Epidemiol. 2010; 171: 455-464.

30- **Jin YZ, hang X, Shu L, Chen L, Sun L, Qian H, Liu W, Fu Z** Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (Danio rerio). Chemosphe. 2010; 78: 846-852.

31-**McCann SM1, Mastronardi C, de Laurentiis A, Rettori V** The nitric oxide theory of aging revisited. Ann N Y Acad Sci. 2005; 1057: 64-84.

32-**Hertoghe T :** The "multiple hormone deficiency" theory of ageing: Is human senescence caused mainly by multiple hormone deficiencies? Ann NY Acad Sci. 2005; 1057: 448–465.

33- **Ahmed MM, Zaki NI :** Assessment the ameliorative effect of pomegranate and rutin on chlorpyrifosethyl-induced oxidative stress in rats. Nat. sci. 2009; 7: 49-61.

34- **Vitale G1, Salvioli S, Franceschi C:** Oxidative stress and the ageing endocrine system. Nat Rev Endocrinol. 2013;p 9: 228-240.

35-**Yousef DM, Abd El-Fatah SS, Hegazy AA.**: Protective effect of ginger extract against alterations of rat thyroid structure induced by cypermethrin administration. J Exp Med Biol. 2019;1(1):19-25.

36-**Khaki A, Fathiazad F, Nouri M, et al**.: The effects of ginger on spermatogenesis and sperm parameters of rat. Iran J Reprod Med. 2009;7-12. 29.

37-**Rodrigues SM, Ximenes CF, de Batista PR, et al**.: Tributyltin contributes in reducing the vascular reactivity to phenylephrine in isolated aortic rings from female rats. Toxicol Lett. 2014;378-85.

38-**Ansari MN, Bhandari U, Pillai KK. Ethanolic Zingiber officinale R**. extract pretreatment alleviates isoproterenol-induced oxidative myocardial necrosis in rats. Indian J Exp Biol. 2006;892-97.

39- **Sakr SA, Al-Amoudi WM.:** Effect of ginger extract on deltamethrin induced histomorphological and immunohistochemical changes in Testes of Albino Rats. Life Sci. 2012;771-78.

40-**Zaidi SS, Bhatnagar VK, Gandhi SJ, et al.:** Assessment of thyroid function in pesticide formulators. Hum Exp Toxicol. 2000;497-01.

41- **Sakr SA, Shalaby YS.**: Ginger extract protects metalaxyl-induced histomorphological and histochemical alterations in testes of albino mice. J Applied Pharmaceut Sci. 2011;36-42.

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Table s1: morphometric analysis of thyroid tissue:

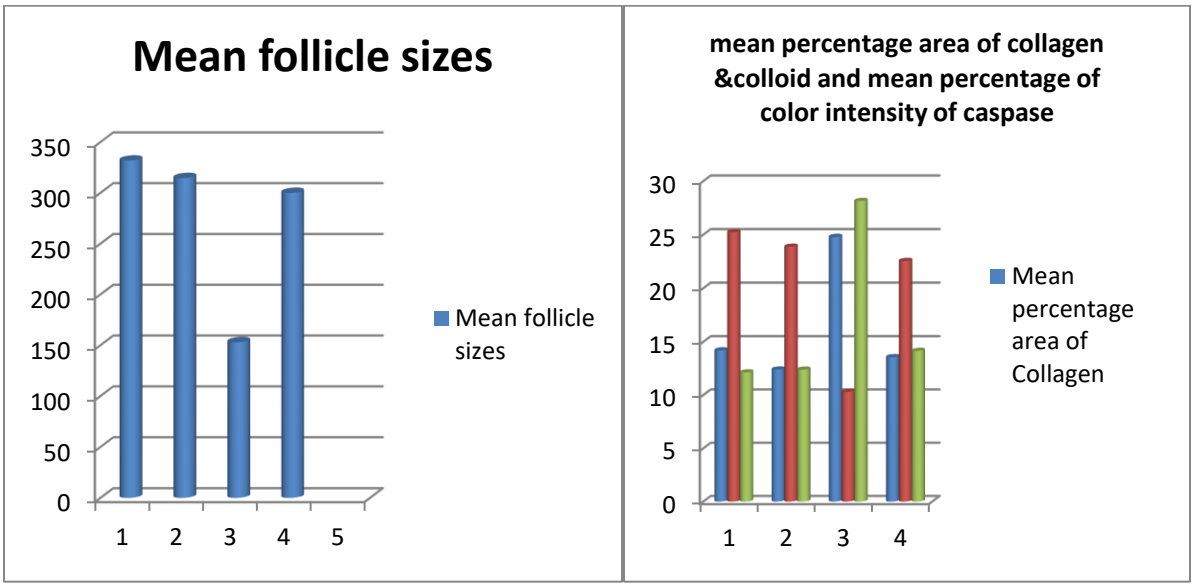

Table s2: serum hormones levels

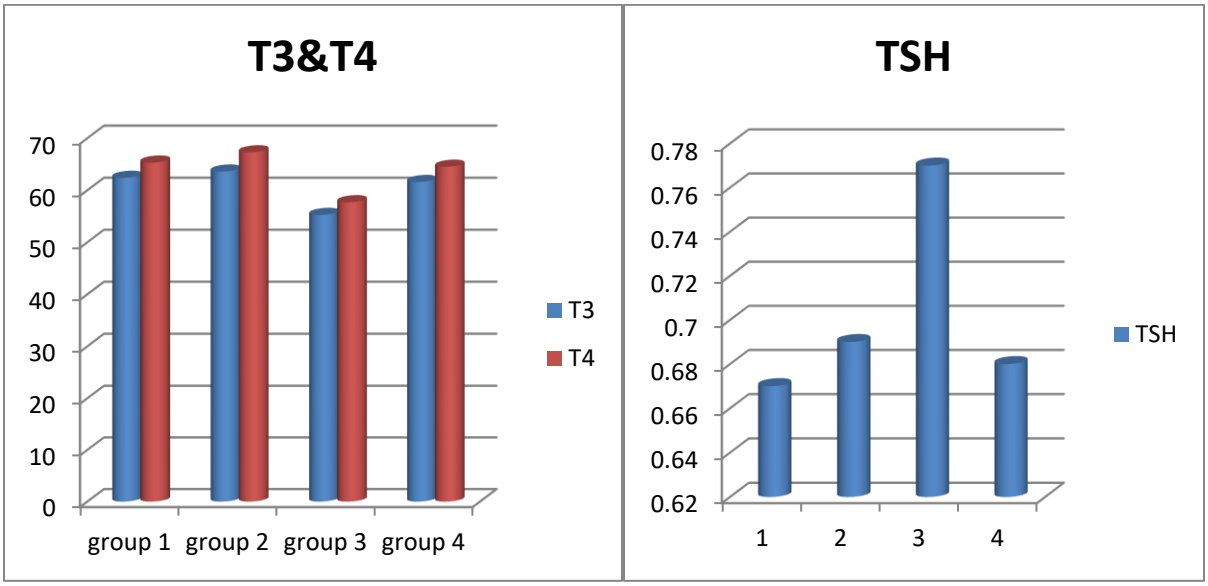

Table s3: Tissue MDA levels