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AMELIORATIVE EFFECT OF PUMPKIN SEED OIL ON ALTERATIONS IN THYROID GLAND FUNCTIONS INDUCED BY CHLORPYRIFOS IN ADULT MALE RATS

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ABSTRACT

This study carried out in College of Veterinary Medicine /University of Basrah to evaluate the effect of different doses of pumpkin seed oil (PSO) on Thyroid functions in Chlorpyrifos (CPF) treated male rats. Fifty adult male rats were used and randomly divided into five equal groups, 10 rats per each group: **Group 1**(control) administered orally corn oil(1ml /kg.bw) , whereas first treated group administered CPF 1/20th LD50 (6.7 mg/kg bw). **Group 3, 4** and **5** were administered CPF (6.7mg /kg.bw) plus PSO(20, 40, and 80) mg/kg.bw respectively. The treatments were given once daily by oral gavages for 8 weeks. At the end of the experiment, animals were sacrificed and blood samples were obtained for evaluation of thyroid hormones (T4 and T3) and tissue samples from thyroid gland kept in 10% neutral formalin for histopathological examination.

The results indicated that male rats treated with Chlorpyrifos demostreated a significant increase ($P<0.05$) in thyroid stimulating hormone (TSH) and a significant decrease ($P<0.05$) in thyroxin(T4) and triiodothyronine (T3) hormones compared with control group. Histopathological examination of CPF treated males showed that thyroid gland revealed destruction of thyroid follicles with infiltration of macrophages on the site of follicular destruction. On other hand groups treated with 20,40 and 80 mg pumpkin seed oil induced

improvement in above cited parameters and histological changes in dose dependent manner.

INTRODUCTION

The environment is a heavily contaminated with many chemicals, which interact with each other in such a way that modify their toxic response in humans and animals(1). According to WHO, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (2). Pesticide such as Chlorpyrifos (CPF) can cause adverse effects by interfering with the body's hormones or chemical messengers. These substances (pesticides) are therefore called hormone disruptors or endocrine disruptors (3). Organophosphate (OP) compounds are the most widely used insecticides accounting for 50% of global insecticidal use (4). Chlorpyrifos (CPF), an organophosphate insecticide (OP) that is widely used in agriculture, horticulture and public health has been shown to disrupt the activities of endocrine organs(5). The thyroid gland is the most commonly affected endocrine gland as it is considered a sensitive target to organophosphate insecticide (OPI) leading to affection of thyroid function (6).Thyroid hormones are important in several physiological processes such as metabolism and normal growth development and any imbalance in their levels could lead to a wide range of clinical conditions (7).

Pumpkin seed oil is used as nutritional supplements for natural source of proteins, essential fatty acids, polyunsaturated fatty acids, omega 3, 6 and 9, Beta-carotenes, lutein, vitamins such as carotenoids and β- and γ-tocopherols, phytosterols, chlorophyll, and trace elements, such as zinc and selenium (8). The essential trace mineral zinc in pumpkin seeds acts as an antioxidant which is attributed to its ability to neutralize free radical generation or directly engross the iron or copper binding sites of lipids, proteins, and DNA molecules(9). However, pumpkin seed extracts have been used as an adjuvant for immunomodulation, reproductive health and therapeutic purposes for wide ranges of disease conditions(10). In addition, omega 3 and 6 essential fatty acids in pumpkin seed oil are important for healthy brain and body functioning as well as preventing and improving bladder and prostate problems(11). The aim of this study carried out to investigate the effect of different doses of pumpkin seed oil on thyroid hormones levels and histological changes in chlorpyrifos- induced thyroid dysfunction of adult male rats.

MATERIALS AND METHODS

Experimental Animals:

Forty adult male rats weighting (280- 290 gm) and twenty healthy adult fertile female rats weighting (270-280 gm) were used in this study. The animals were kept in the animal house for acclimatization for fifteen days before the beginning of the experiments. The animals were maintained under optimum conditions (25±2°C) and (12/12 hours light/dark) cycle throughout the study, with standard pellets and tap water *ad libitum*.

Extraction and Evaluation of Pumpkin Seed Oil (PSO):

Pumpkin seeds (*Cucurbita pepo L.)* brought from local market in the province of Basrah . Seeds were selected according to their condition, damaged seeds were discarded and those seeds which were in good condition cleaned. After cleaning, the sand and foreign materials were removed. The dried pumpkin seeds were ground to a fine powder using a grinder. The oil was extracted with n-hexane (1:4 w/v) by soxhlet device and agitation in a shaker at room temperature in the dark for 36 h. The solvent was evaporated at 40 °C to dry it . The extracted oil was stored in sealed and dark bottles until analysis(12). Pumpkin oil content was analyzed by GC- MS , Collage of Agriculture ,University of Basrah. GC-MS analysis showed 20 oil constituents, Linoleic acid,Oleic acid(omega-9 fatty acid,omega-3 fatty acid) ,Palmatic acid, Stearic acid , Ascorbic acid and tocopherol.

Experimental Design:

After acclimation period, 50 male rats were divided into 5 groups with 10 animals in each as the following: **Group 1 (Control group):** Animals of this group administered corn oil (0.2 ml/kg)/day orally by gavages. **Group 2 (CPF treated group):** Animals of this group administered orally CPF 1/20th LD50 (6.7 mg/kg BW),/day by gavages (13). **Group 3 (CPF + 20 PSO):** Animals of this group administrated orally CPF (6.7 mg) + pumpkin seed oil (20 mg)kg BW daily orally by gavages. **Group 4 (CPF + 40 PSO):** Animals of this group administer orally CPF (6.7 mg) + pumpkin seed oil (40 mg)/kg BW daily orally by gavages **Group 5 (CPF + 80 PSO):** Animals of this group administer orally CPF (6.7 mg) + pumpkin seed oil (80 mg)/kg BW daily orally by gavages. The experimental was continued for 8 weeks .

Blood and Specimens Collection :

At the end of the experiment, animals of each group were anaesthetized by chloroform and sacrificed. Blood sample were collected from the heart via the cardiac puncture of sacrificed animals by using 5cc sterile syringe and dropped in plain without anticoagulant tubes and serum samples were isolated from blood by centrifugation at 3000rpm for 15 min. and separated in eppendroff tubes and stored at -20°C until used for hormonal analysis. Thyroid gland was removed and kept in 10% formal saline for histological study.

Hormonal assay:

T_3 and T_4 concentrations were assayed by using ELISA kits from Monobind Inc.lake forest CA 92630; USA, Product code : l25-300. TSH also was measured by using ELISA kit from (Calbiotech Inc. a life science company, USA), Product Code: TS227T.

Histopathological study:

Samples of the thyroid gland were taken and fixed in 10% formalin and then embedded in paraffin and sectioned at thickness of 6 micrometers and then carried on glass slides, stained with hematoxylene-eosin and examined under a light microscope(14).

Statistical Analysis:

Data are expressed as mean values ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyzes were performed using SPSS statistical version 8 software package (SPSS Inc., USA).

RESULTS

The results of thyroid function tests revealed a significant increase ($P \leq 0.05$) in TSH concentration and significant decrease ($P \leq 0.05$) in serum concentrations of T3 and T4 in CPF treated group compared with control group, as seen in table (1). On the other hand the group of CPF treated with different doses of PSO revealed a significant decrease ($P \leq 0.05$) in serum TSH concentration compared with CPF treated group. The results showed a significant decrease ($P \leq 0.05$) in serum concentration of TSH in CPF group treated with 20 mg / kg. bw PSO compared with CPF group but still significantly higher ($P \leq 0.05$) than those of control group. Whereas no significant differences were observed in TSH concentrations in CPF groups treated with 40 and 80 mg / kg. bw PSO respectively compared with control group. A significant decrease ($P \leq 0.05$)

in T3 concentration was recorded in CPF group treated with 20 mg / kg. bw PSO compared with control group. While no significant differences were observed in T3 concentration in CPF groups treated with 40 and 80 mg/ kg.bw PSO compared with control. On the other hand, significant decrease ($P \leq 0.05$) in T4 concentration was noted in CPF groups treated with 20 and 40 mg / kg. bw PSO compared with control group, however no significant difference was recorded in T4 concentration in CPF group treated with 80 mg / kg. bw PSO compared with control group.

Table (1): Effect of PSO on TSH, T4 and T3 Concentrations in Male Rats treated With CPF.

Parameter / Group	TSH µIU/ml	T3 ng/dl	T4 µg/dl
Control	0.37 ± 0.04c	2.78 ± 0.31 a	9.57 ± 0.53 a
G1 CPF treated	2.19 ± 0.59 a	1.38 ± 0.71 b	5.06 ± 0.77 c
G2 CPF+20 mg / kg PSO	1.55 ± 0.64b	1.91 ± 0.41 b	5.71 ± 1.04 bc
G3 CPF+40 mg / kg PSO	0.67 ± 0.17 c	2.48 ± 0.55 ab	6.12 ± 1.01 b
G4 CPF+80 mg / kg PSO	0.46 ± 0.09 c	2.87 ± 0.55 a	9.56 ± 0.84 a
LSD	0.64	0.86	1.06

- Values expressed as Mean ±SD (n=8)
- Different small letters denote significant defferences (P≤0.05) between expermintal groups.

Results of Histopathological Examination:

Thyroid gland of control male rats showed normal architecture composed of normal thyroid follicles with normal epithelium as shown in figure (4-1). While histopathological section in thyroid gland of adult male rats treated with CPF for 8 Weeks (fig. 4-2) revealed marked destruction of thyroid follicles with infiltration of macrophages on the site of follicular destruction. Figure (4-3) Thyroid gland of CPF+20 mg PSO treated group , preservation of thyroid follicles with infiltration of macrophages in the follicles in which most of the contain no colloid. Figure (4-4) Thyroid gland of CPF+40 mg PSO treated group, reveal preservation of

thyroid follicles in which most of follicles contain colloid secretion of the gland, while histopathological section in thyroid gland of adult male rats treated with CPF + 80 mg PSO for 8 Weeks (fig. 4-5) reveal well preserved thyroid follicles (F) in which the follicles contain colloid secretion of the gland.

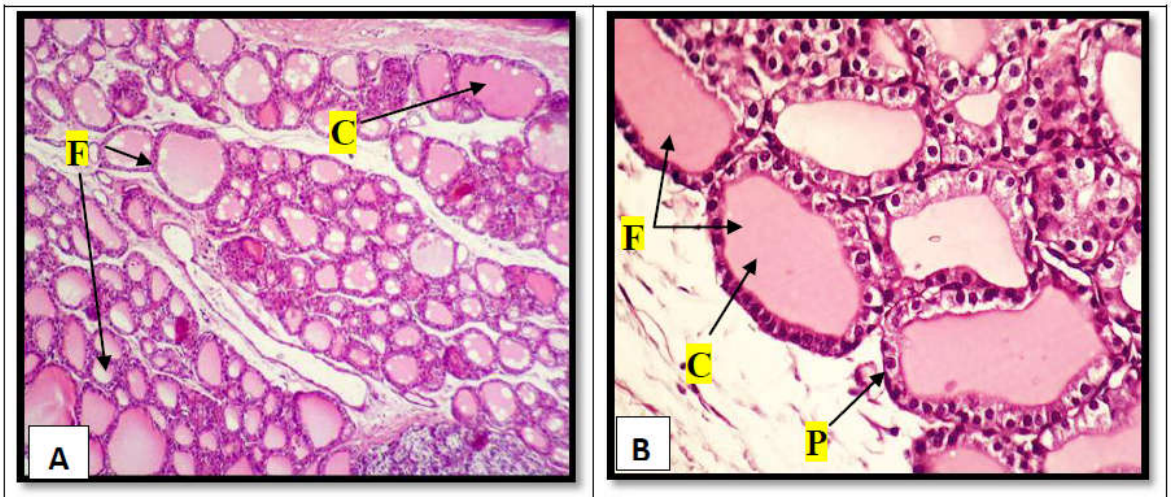

Fig .4-1:Thyroid of control rat. Showing normal architecture of different sizes thyroid follicle(F) filled with homogenized colloid(C) surrounded by normal parafollicular cells (P). H&E (A)125X (B) 500X

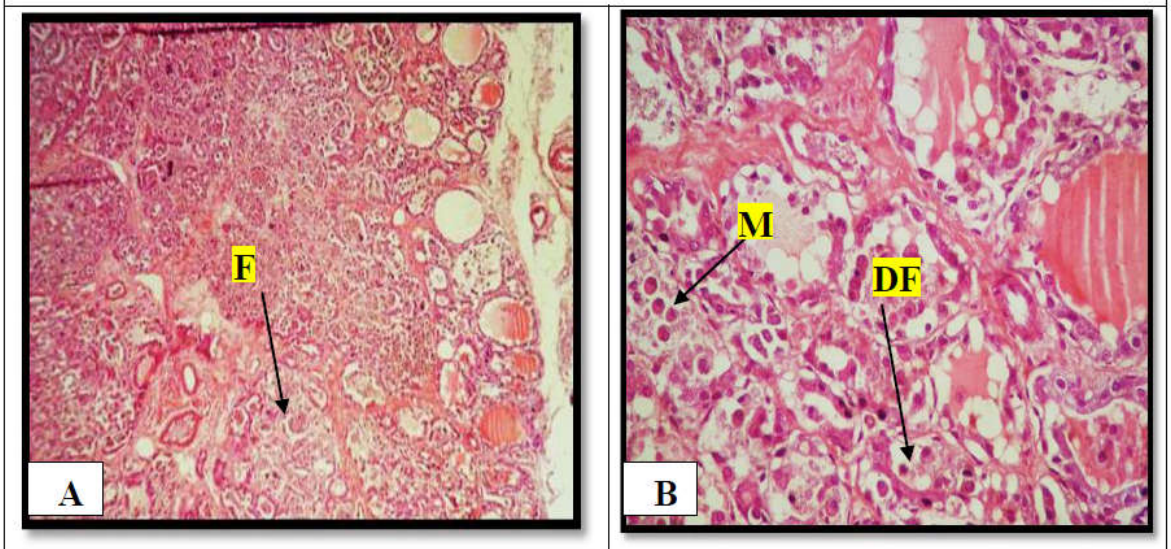

Fig 4-2 : Thyroid of rat treated with CPF showed marked destruction of thyroid follicles (DF) infiltration of macrophages on the site of follicular destruction (M) . H&E (A)125X (B) 500X

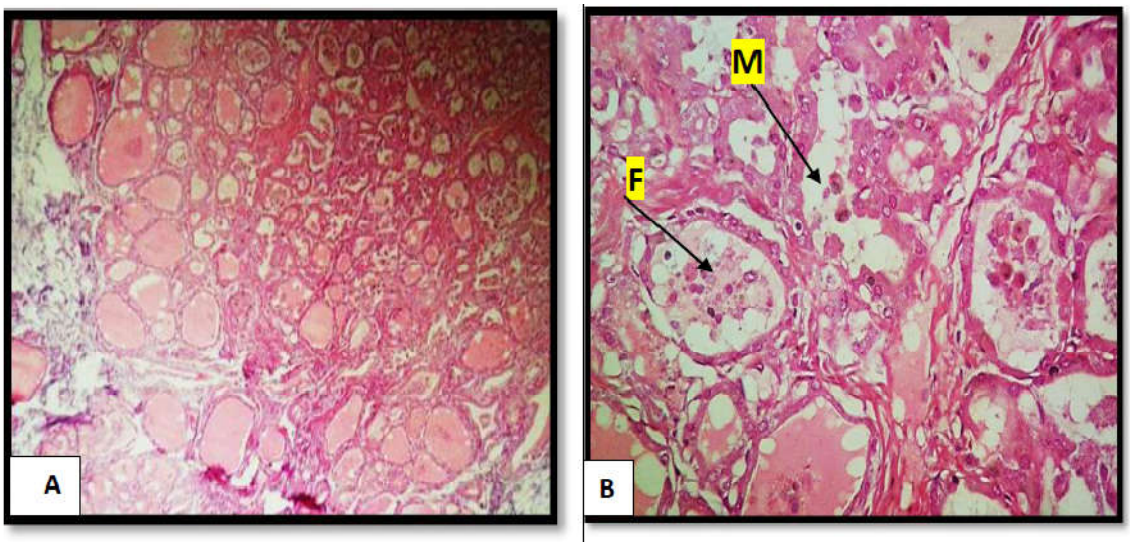

Fig.4-3:Thyroid of rat treated with CPF+20 mg PSO: showed preservation of thyroid follicles (F) with infiltration of macrophage(M) in the follicles in which most of the contain no colloid . H&E (A)125X (B) 500X

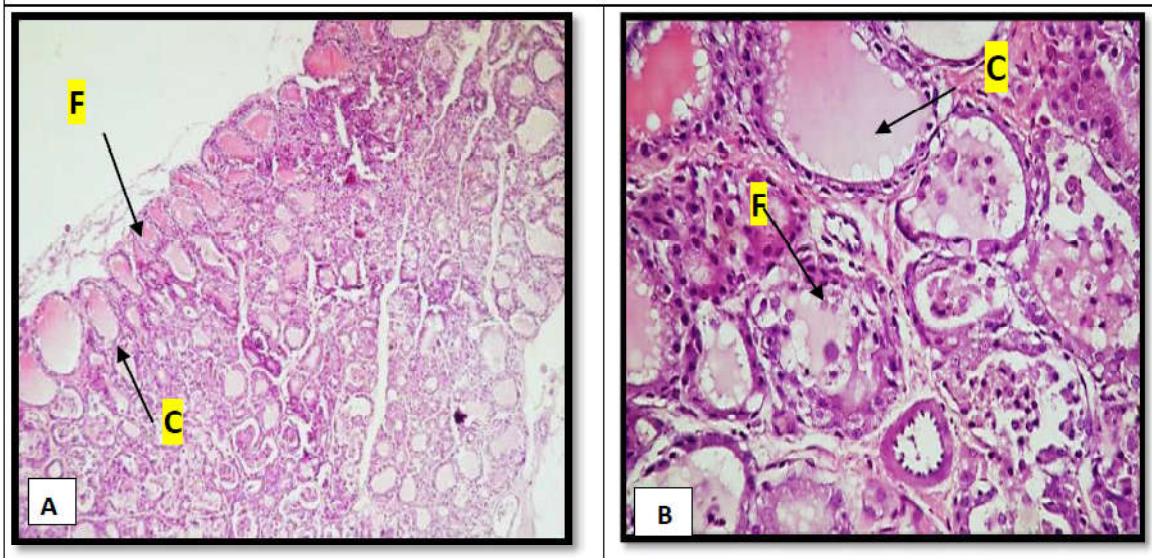

Fig.4-4 : Thyroid of rat treated with CPF+40 mg PSO: showed preservation of thyroid follicles (F) in which most of follicles contain colloid secretion of the gland (C) . H&E (A)125X (B) 500X

DISCUSSION

The present study demonstrated that CPF treated rats showed hypothyroidism which was evidenced biochemically by a significant decrease in serum T3 and T4 levels. Hypothyroidism in CPF treated rats was reported also by some investigators (15) ; (16) and (17). Some OPs disrupt endocrine system, some thyroid disruptors inhibit thyroperoxidase; thereby they change ability of follicular cell in producing T4 and then T3, even at sufficient iodine concentration(18). Many chemical compounds have high structural similarity to T4 and T3thereby they disrupt the binding of thyroid hormones to their receptors or transfer proteins, resulting in subclinical hypothyroidism(19). (20) showed that CPF administration to rats for 12 weeks induced reduction in thyroid hormones which may be attributed to the excessive production of reactive oxygen spices and free radicals in the central nervous system and related glands, including the hypothalamic–pituitary axis (21). The elevation in TSH in CPF group reflects the response of this hormone to the lowered T3 and T4 concentrations. TSH stimulates the thyroid glands to increase the elaboration of T3 and T4. However, the failure of the thyroid glands to respond to increased TSH stimulation for the elaboation of T3 and T4 in the CPF group may have been due to peroxidative damage to the gland (17).

A significant improvement in the levels of thyroid hormones was observed in animal groups treated with different doses of pumpkin seed oil which may be attributed to vitamin E and phenolic compound found in pumpkin oil which acts as a potent antioxidant by removing free radicals and inhibition fat peroxidation and thus preserving the cell membrane. (22). showed that the supplementation with Iron, Zinc, Vit.E and Vit.C significantly increased the concentration of thyroid hormones and also caused a significant decrease in TSH level compared with control positive group. The marginal decrease in serum T3 and T4 with a significant increase in the concentrations of TSH in the group co-administered with CPF were ameliorated by Vitamin .C partly due to its antioxidant properties (1). A combination with vitamin C and E has been demonstrated to protect against CPF-induced toxicity(23). Since it has the ability to augment the activities of other antioxidants. (24), reported that administration of antioxidant vitamin C or E partially attenuated the toxicity induced by exposure to CPF. The results of above studies are consistent with the results of current study because the PSO contains both vitamin E and C and

the essential mineral elements that play an important reducing the oxidative stress resulted from exposure to CPF.

Histopathological examination of thyroid gland in CPF treated male rats for 8 weeks showed marked destruction of thyroid follicles with infiltration of macrophages on the site of follicular destruction (Fig.4-2). These results are correlated with those reported by (25) who found that degenerated follicles with decrease colloid, obvious exfoliation of the follicular epithelial cells and vascular congestion in CPF treated rats as compared with control. Similarly (13) reported that male rats treated with CPF showed decrease in the size of follicles and amount of colloid, degeneration of follicular cells and congestion of blood vessels .In addition ,(20) showed that male rats treated with 6.75 mg/kg b.w. CPF for 12 weeks revealed damaging effects in thyroid tissue, thickened collagen fibers between the follicles. (17) reported that CPF treated rats showed hypothyroidism that was evidenced by decreased amount of colloid. The present results confirmed with the reduction of serum T3 and T4 and elevation of serum TSH in CPF-treated rats.

CONCLUSION

The results of this study revealed that oil extracted from pumpkin seeds is an important source rich in many active substances such as vitamin C and vitamin D and metal elements such as zinc which play an important role as antioxidants relieving the harmful effects of exposure to chlorpyrifos.

REFERENCES

1. **Ambali, S.F.; Orieji, C.; Abubakar, W.O.; Shittu, M. and Kawu, M.U. (2011a).** Ameliorative Effect of Vitamin C on Alterations in Thyroid Hormones Concentrations Induced by Subchronic Coadministration of Chlorpyrifos and Lead in Wistar Rats**.** *Journal of Thyroid Research ,* Article ID 214924,P: 6

2. **Yang CC1, Deng JF.(2007).** Intermediate syndrome following organophosphate insecticide poisoning. J Chin Med Assoc;70(11):467-72

3. **Khatun N and Mahanta R(2014).** A Study on the Effect of Chlorpyrifos (20% EC) on Thyroid Hormones in Freshwater Fish. The Science Probe 2 (2): 8-16

4. **Casida JE, Quistad GB. (2004).** Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. Chem Res Toxicol 17:983–998

5. De Angelis, S., Tassinari, R., Maranghi, F., Eusepi, A., Di Virgilio, A., Chiarotti, F., Ricceri, L., Pesciolini, A. V., Gilardi, E., Moracci, G., Calamandrei, G., Olivieri, A. and Mantovani, A. (2009). "Developmental Exposure to Chlorpyrifos Induces Alterations in Thyroid and Thyroid Hormone Levels without Other Toxicity Signs in Cd1 Mice," Toxicological Sciences 108(2):311-9.

6. Meeker JD, Barr DB,and Hauser R (2006) Thyroid hormones in relation to urinary metabolites of non-persistent insecticides in men of reproductive age. Reprod Toxicol 22: 437-442.

7. Fliers E, Bianco AC, Langouche L,and Boelen A (2015). Thyroid function in critically ill patients. Lancet Diabetes Endocrinol 3: 816-825.

8. Gossell-Williams, M, Davis, A, and O'Connor, N.(2006). Inhibition of testosterone-induced hyperplasia of the prostate of sprague-dawley rats by pumpkin seed oil. Journal of Medicinal Food; 9(2): 284-286.

9. Amara, S, Abdelmelek, H, Garrel, C, Guiraud, P,and Douki, T.(2008). Preventive effect of zinc against cadmiuminduced oxidative stress in the rat testis. J Reprod Dev;54(2):129-134.

10. Fruhwirth, GO, Hermetter, A.(2007). Seeds and oil of the Styrian oil pumpkin: components and biological activities. Eur J Lipid Sci Technol 2007; 109: 1128–1140

11.Taylor, SL, Eller, FJ, Jackson, MA & Stevenson, DG.(2006). Pressurized fluid extraction of pumpkin seed and pumpkin seed pressed cake. New crops and processing technology, Pittsburgh Conference, pp 2006: 1220–1225.

12. ArdabilI, G.; Farhoosh, R. and Khodaparast, M.H.(2011) . Chemical Composition and Physicochemical Properties of Pumpkin Seeds (Cucurbita pepo Subsp. pepo Var. Styriaka) Grown in Iran. J. Agr. Sci. Tech. (2011) Vol. 13: 1053-1063.

13. Lamfon, H.A.(2014). Effect of selenium on chlorpyrifos-induced thyroid toxicity in albino rats.*Publishing Research in Endocrinology*: 1-14

14. Luna L. G., (1968). Manual of histological staining methods of the armed forces institute of pathology. 3rd edition. New York, Mcgraw-Hill.

15. Venerosi, A.; Cutuli, D.; Colonnello, V.; Cardona, D.; Ricceri, L. and Calamandrei, G.(2008): Neonatal exposure to chlorpyrifos affects maternal responses and maternal aggression of female mice in adulthood. Neurotoxicol.*Teratol.* 30(6):468-474.

16. Shady, A.M. and Noor El-Deen, F.I.(2010). Effect of chlorpyrifos on thyroid gland of adult male albino rats. *Egypt J Histol* ,33(3):441-450.

17. **Shittu, M.;Aisha, O.O.;Ambali, S.F.;Oyedepo, I.T.;Umaru, K.M.; Yusuf, P.O.; Ishaku, K.P. and Isa, H.I.(2014).** Ameliorative Effect of *Hibiscus Sabdariffa Linn* on Subchronic Chlorpyrifos-induced Alterations in Sex and Thyroid Hormones in Male Wistar Rats. *American Journal of Pharmacology and Toxicology,* 9 (1): 96-106.

18. Miller, M.D.;Crofton, K.M.; Rice, D.C. and Zoeller, R.T.(2009). Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ Health Perspect,* 117:1033-41.

19. Farokhi, F. and Taravati, A.(2104). Pesticide exposure and thyroid function in adult male sprayers. Int J Med Invest; 3; 4; 127-132.

20. El-Sheikh,A.A. and Ibrahim, H.M.(2017). The Propolis Effect on Chlorpyrifos Induced Thyroid Toxicity in Male Albino Rats. *Journal of Medical Toxicology and Clinical Forensic Medicine,* 3(1):3-13.

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

22. **Rabeh, N.M. and El-Ghandour, H.A.(2016).** Effect of Iron, Zinc, Vitamin E and Vitamin C Supplementation on Thyroid Hormones in Rats with Hypothyroidism. *International Journal of Nutrition and Food Sciences ;*5(3):201-210.

23. Karaoz, E.; Gultekin ,F.; Akdogan, M.; Oncu, M. and Gokcimen, A. (2002). Protective role of melatonin and a combination of vitamin C and vitamin E on lung toxicity induced by chlorpyrifos-ethyl in rats. *Exp. Tox. Path.,* 54: 97-108.

24. **Baba, N.A.; Raina, R .; Verma, P.K.; Sultana, M.; Prawez, S. and Nisara, N.A.(2013).** Toxic effects of fluoride and chlorpyrifos on antioxidant parameters in rats: protective effects of vitamins C and E. *Research report Fluoride,* 46(2): 73-79.

25. Paules, M.G.; Zaher, J.F.; Hafez, E.M. and El-Tahawy, N.F.(2013). Toxicological Effects Induced by Chlorpyrifos Administration on Thyroid and Suprarenal Glands in Adult Male Albino Rats. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology January ,* 20:99-105