Curative and protective roles of *Eruca Sativa* M. leaves in ameliorating thyroid toxicity of malathion treated male rats

[a]Hussein KA Al-Mayali, [b] Safa MK Hasan,
[a]Asst. Prof., Physiol., Dept. Biology, College of Education, University of Al-Qadisiya, Iraq.
[b] MSc student, Dept. Biology, College of Education, University of Al-Qadisiya, Iraq.

Abstract

The present study was conducted to find out the curative effect of aqueous extracts of *Eruca Sativa* M. leaves in reducing the toxicity of the malathion phosphoric pesticide in thyroid gland. Fifty mature male rats, aged 10-11 weeks, were randomly divided into five equal groups (10 each), orally drenched with 500 µl of corn oil for 4 weeks (C; control negative), 27 mg/kg bw of malathion solution for 4 weeks (M; toxicity control positive), 250 mg of E. sativa extract/ kg bw for 2 weeks followed by 27 mg/ kg bw of malathion solution for 2 weeks (ME group), 27 mg/kg bw of malathion and 250 mg/ kg bw of E. sativa extract together for 4 weeks (M+E group), and 250mg/ kg bw of E. sativa extract followed by 27 mg bw of malathion(EM group). After 24 hours of last treatment, males were weighted, sacrificed, thyroid was removed and weighted, and blood samples were obtained for serum TSH, T3 and T4 estimation. Samples of thyroid glands were fixed in 10% formalin for histopathological examination. The results revealed significantly decreased body weight and thyroil relative weight in M group than control whereas the three extract treated (ME, M+E, and EM) groups showed insignificant differences compared with control. In M group, TSH concentration increased significantly whereas T3 and T4 decreased significantly than control, while other experimental groups showed no significant differences in comparison with control. It can be concluded that aqueous Eruca sativa extract has potent curative and protective roles in ameliorating the toxicological effects of malation on thyroid functions.

KEYWORDS: Malathion, T3, T4, TSH, Eruca sativa.

INTRODUCTION

Various studies mention to the importance of traditional medicine by the use of medicinal plants as a natural source of pharmaceutical agents. Crude extracts of medicinal plants have been used for a long time due to its safety effects and availability as well as low price (American Botanical Council, 2005). It has been found that medicinal plants and its active compound have curative roles against various diseases of human being such as blood vessels diseases (Kuppusamy et al., 2008). Eruca sativa is one of the important medicinal plants, belong to the family Brassiceae. This plant has been cultivated in the neutral weather and short day light (Mohammed and Rafiq, 2009). E. sativa characterized by dark leaves color and 25-50 cm stem length (Heimler et al., 2007). The leaves characterized by its rich contents of strong antioxidant activity due to the high concentrations of intermediate metabolites, which have potent bioactivity, such as favanoidsand phenols as well as its high quantity of various minerals such as potassium, magnesium, calcium, manganese, iron, cupper, sodium, nickel, cadmium, zinc and nitrogen (Abdo, 2003; Bukhsh et al., 2007).

Malathion is an organo-phosphorous compound used as in a various fields as an insecticide to control and eradicate insects in the houses, fields and grain resources (Suresh et al., 2006). Chemically, malathion is known as [O, O- dimethyl- S- (1, 2-dicacethoxyethyl) Phosphorodithionate] and its chemical formula is $C_{10}H_{19}O_6PS_2$ (WHO, 2003; EPA, 2012). Malathion is usually manufactured as a colorless fluid with strong odor similar to that of garlic (HSDB, 2005). Many researchers pointed out that malathion causes hepatotoxicity (Moore et al., 2010; Josse et al., 2014), leading to acute hepatitis with high secretion of cytokins at the onset of the inflammation which followed later by hepatomegaly and increase of the severity of the inflammation which could be foolowed by liver cirrhosis (Li et al., 2012). Because there is no trials to find out the effect of malathion toxicity on thyroid function, the present study aims to investigate the toxicological effect of malathion of thyroid gland as well as the curative and protective role of S. sativa aqueous extract in ameliorating these effects.

MATERIALS AND METHODS

Preparation of E. sativa extract: E. sativa aqueous extract has been prepared according to Harborne (1984).

Preparation of malathion: malathion has been prepared by adding 10 ml of malathion stock solution to 990 ml of distilled water and 400 ml of corn oil as described by Kamrin (1997) and Gallo and Lawryk (1991). This dilution is considered as a stock solution in the experiment.

Experimental animals: Adult male Wistar rats (aged 10-11 weeks and weighted 165±8.3 g) have been housed in a controlled ambient temperature (22-25 ºC) and 12:12 dark and light. The animals were fed on standard food and drinking water ad libitum.

Experimental design: Fifty adult male rats have been assigned randomly to five equal groups (10 for each) and treated as follow:

1. Negative control (C group): daily drenched with 0.5 ml of corn oil for 4 weeks.
2. Positive control (M group): daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks.
3. Treated 1 (EM group): daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks.
4. Treated 2 (M+E group): daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks.
5. Treated 3 (ME group): daily drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

Body weights have been measured at the beginning and end of experiment. After 24 hours of the last treatment, male rats have been sacrificed, thyroid glands were removed and weighted, and blood samples were obtained for TSH, T3, and T4 assessment in the serum. Samples of thyroid glands were fixed in formalin (10%) for histopathological examination.

Hormones assessment: TSH, T3, and T4 have been assessed using ELISA technique according to the manufacturer instructions (Monobind Inc.).

Histological examination: Histological sections have been prepared, stained and examined according to Humason (1997).

Statistical analysis: All values have been presented as Mean±Standard Error of the mean. One way analysis of variance (ANOVA-1) was used to test the presence of significant differences at the level of 0.05% . Least significant difference was used to test the significant difference between the means (Schielfer, 1980).

RESULTS

Body weight (g):Initial body weight of the rats was similar in all groups. At the end of the treatment period (4 weeks), all rats gained weight but M group reported the lowest gain ($P<0.05$) among experimental groups, whereas C, EM and E+M groups reported the highest body weight (figure 1).

Relative thyroid gland weight (g%): thyroid gland weight (gL100 g of bw) decreased significantly ($P<0.05$) in M group than control and ME groups whereas EM and E+M reported insignificant difference compared with control (figure 2).

Serum TSH concentration (mcIU/ml): serum TSH concentration of M group increased significantly ($P<0.05$) among experimental groups. Although EM group was significantly ($P<0.05$) lower than M group, but its mean was significantly higher than C, M+E and ME groups, which showed insignificant differences between each other (figure 3).

Serum T3 and T4 concentration: serum T3 and T4 concentrations of M group reported the lowest levels among experimental groups. Serum T4 level of EM group was significantly ($P<0.05$) lower than C, M+E and ME groups, which showed insignificant differences between each other (figure 4). Serum T3 level of M+E group was significantly ($P<0.05$) lower than C, EM and ME groups, which reported insignificant differences between each other (figure 5).

Histopathological changes: microscopic examination of thyroid glands revealed marked histopathological changes in malathion treated male rats, represented by numerous hyperplasia of thyroid tissue with the appearance of scattered follicular lining cells in the form of papillary projections extended to lumen of the follicles and disappeared colloid materials from the lumen, as well as the presence of necrotic and degenerative changes in the stroma and follicular lining cells (figure 6-M) compared with control section (figure 6-C). In EM group, the sections showed that treatment with E. sativa results in proliferative follicles filled with colloid materials with increased proliferation of cuboidal cells in the lining of the follicles (figure 6-EM). Same picture has been shown in the male rats treated with malathion and the extract together (figure 6-M+E), whereas ME group showed high number of small thyroid follicles filled with colloid materials as well as high proliferative changes of follicular lining (figure 6-ME).

DISCUSSION

The observed changes in body weight, in the present study, could be attributed to the low consumption of water and food due to the toxicity induced by malathion administration as well as the atrophic effect of toxic substance on muscular tissues by inhibiting acetyl cholinesterase and/ or ATPase (Hariri et al., 2011). This result was in agreement with reported by Espinoza-Navarro and Bustos-Obregon (2014) Sief et al. (2015), and Geng et al. (2015). On other side, treatment of male rats with E, sativa extract improved the body weight which could be due to improvement of appetite, since the extract contain many valuable nutritional compounds as well as its role to increase salivery and bile secretion (Mustafa et al., 2006).

Thyroid gland weight loss in malathion treated male rats may be a result of huge free radical productions, where malathioncaused increased lipid peroxidation (Halliwell and

Gutterifos, 1985), which may damage the cell membrane of follicular lining cells, which could lead to thyroid gland weight loss. Whereas the potent antioxidant activity of E. sativa extract (Ambali et al., 2010) could ameliorates the toxic effect of oxidative stress caused by malathion. This results was in agreement with that reported by Islam et al. (2008).

Increased serum TSH concentrations, in M group, could be a response to the decreased T3 and T4 production in the thyroid gland, where this decrement will stimulates pituitary thyrotrophs by feedback action to increase TSH secretion (Rathore et al., 2002). The cellular toxic effect of malathion, shown inthe follicular lining, could be the cause of low T3 and T4 secretion, since it may results in the decrease of the activity of thyroid peroxidase responsible for chelating of iodine from blood circulation and attached it with tyrosine. On the other hand, the result could be attributed to the down-regulation of TSH receptors on the surface of thyroid secretory cells(Junquaira and Carneiro, 2003). The decrement of T4 could be due to the inhibition of 5-deiodinase (type 1) activity, which is responsible for the conversion of T3 to T4 (Maiti et al., 1996). Kale (2007) mentioned that T3 and T4 secretion is in negative correlation with the level of oxidative stress. On other side, the antioxidant activity of E. sativa extract due to its constituents of flavonoids, saponin, and vitamin-C (Gauthaman et al., 2003).

Histopathological changes, observed in the present study, could be attributed to the toxic effect of malathion, where it causes destruction of secretory epithelial lining of follicle due to the necrotic and degenerative changes. These changes could lead to lower secretion of T3 and T4 and colloid material production inside the lumen of the follicles (Yu et al., 2008; Mahjoub-Samet et al., 2005). The hyperplasia could be due to the high levels of TSH, where the repeated stimulation leads to mitotic activity of follicular secretory cells (Hood et al., 1999; Capen et al., 2004). The potent antioxidant activity of E. sativa extract resulted in decreased histopathological changes, which is mainly due to its free radical scavenging activity (Khalaf et al., 2002).

RETERENCES

Abdo, M., and Zeinab, A. (2003).Using Egyptian *Eruca-sativa* seed meal in broiler ration with or without microbial phytase. Egypt. J. Nutr. and Feeds, (6) special Issue., 97-114.

Ambali S.F., J.O. Ayo, S.A. Ojo, and K.A.N. Esievo, (2010) “Vitamin C protects rats from chlorpyrifos-induced increased erythrocyte osmotic fragility in Wistar rats,” Food and Chemical Toxicology, vol. 48, pp. 3477–3480.

American BotancialCouncil(2005). Integrative Medical Communication Monographs Austin (Internet).http://www.abc@herbalgram.com.

Bukhsh, E.; Malik, S. A. and Ahmad, S. S. (2007). Estimation of nutritional value and trace elements content of *Carthamusoxyacantha*, *Eruca sativa* and *Plantagoovata*. J. Bot. 39(4): 1181-1187.

Capen, C (2004). Pathophysiology of Thyroid Gland .In: Veterinary pathophysiology (1st ed). by: Dunlop, R. H. and Malbert, C.H.(eds.) Blak Well Publishing. USA.pp:67-44.

Environmental Protection Agency (EPA). Pesticide News story: *The EPA publishes amended Azinphos-Methyl cancellation order allowing use of existing stocks through September 2013.* 2012. Available in: WWW. epa.gov/pesticide/chemical search/

Gallo, M J and Lawryk, NJ (1991): Organic phosphorus pesticides. Handbook of Pesticide Toxicology; Hayes Jr., W. J.; Laws Jr., E. R.,Eds.; Academic Press, Inc.: San Diego,917-1123.

Gauthaman,K.; Gansean, A, P. and Parasad, R.N.(2003)Sexual effects of puncture vine (*Tribulusterrestris*) extract protodioscin an evaluation using a rat model . J. Altern. Complement Med. 9(2): 257-65.

Geng, X. ; Shao, H. ; Zhang, Z. ; Ng, J. C. and Peng, C.(2015). Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats. environmental toxicology and pharmacology .39: 659–667.

Halliwell , B. and Gutteridge , J. M. C. (1985) . Free radicals in biology and medicine Clarendon Press .Oxford , New York .classification. Switzerland: Geneva.

Harborne JB (1984). Phytochemical Methods: A Guide to Modern Techniques of plant Analysis. Chapman and Hall, London, UK.pp. 1- 34.

Hariri AR, Manuck SB, Ferrell RE, NikolovaYS . (2011). Multilocus Genetic Profile for Dopamine Signaling Predicts Ventral Striatum Reactivity. Neuropsychopharmacology 36(9): 1940-1947.

Heimler D, Isolani L, Vignolini P, Tombllin S, Romani A.(2007).Polyphenol content and antioxidative activity in some species of freshly consumed salads. J. Agric. Food Chem., 55:1724-1729.

Hood, Y. P. Liu, V. H. Gattone, and C. D. Klaassen, (1999)"Sensitivity of thyroid gland growth to thyroid stimulatinghormone (TSH) in rats treated with antithyroid drugs," Toxicological Sciences, vol. 49, no. 2, pp. 263–271.

Hazardous Substances Databank (HSDB).(2005). Malathion; U.S. Department of Health and Human Services, National Institutes of Health, National Library of Medicine.http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen? HSDB (accessed Jan 2008), updated June 2005.

Humason,G.(1997).Humason animal tissue techniques.5thed.London.

Islam, S.; Yesmine, S.; Khan, S.A.; Alam, N.H. and Islam, S. (2008). A comparative Study of thyroid hormone Levels in diabetic and non-diabetic patients. Clin Diabetes. 39(5):913-916.

Josse R, Sharanek A, Savary CC, Guillouzo A. Impact of isomalathion on malathion cytotoxicity and genotoxicity in human HepaRG cell. Chem. BioI Interact 2014; 209: 68-76.

Junqueira, L.C. and Carneiro , J. (2003). Basic histology , 10th ed. Lange medical books McGraw- Hill , New York .pp:423 - 456 .

Kale , M.K .; umathe, S.N. and bhusari,K.P .(2007).oxidative stress and the Thyroid.PositIve Health .

Kamrin, MA (1997): Pesticide Profiles:Toxicity, Environmental Impact, and Fate; Lewis Publishers: New York: 191-195.

Khalaf, B .H .; Numan, N. A. and Wohaieb, S. A. (2002). The effect of vitamins C and E, and allopurinol on the antioxidant status in asthmatic patients .Iraqi . J. pharm., 2 : 30-39 .

Kuppusamy, K.; Panneerselvam, K. and Viswanathan, P. (2008).Antioxidant efficacy of flavonoid-rich fraction from Spermacoce*hispida in hyperlipidemic- rats*. J. Appl. Biomed., 6(1214- 0287): 165-176.

Li XH, McGrath KCY, Nammi S, Healther AK, Roufogalis BD. Attenuation of liver pro-inflammatory responses by Zingiberofficinale via Inhibition of NF-kappa B activation in high-fat diet-fed rats. Basic ClinpharmacolToxicol 2012; 110: 238-44.

Mahjoubi-Samet, H., Fetoui, L., Soussia, F., Guermazi, N. Zeghal.(2005).Dimethoateevects on thyroid function in suckling rats, Ann. Endocrinol.66 (2) 96-104.

Maiti PK, Kar A (1997). Dimethoate inhibits extrathyroidal 5'-mono- deiodination of thyroxine to 3,3',5-triiodothyronine in mice: the possible involvement of the lipid peroxidative process. ToxicolLett; 91: 1-6.

Mohammed; H.C. and Rafiq; A. (2009). Investigating possibility of using least desirable edible oil of *Eruca sativa* in bio diesel production . Pak. J. Bot.; 41 (1): 481 – 87 .

Moore PD, Yedjou CG, Tchounwou PB.Malathion –induced oxidative stress, cytotoxicity, and genotoxicity in human liver carcinoma ($HepG_2$) cells. Environ Toxicol 2010; 25:221-6.

Mustafa; G., Fugen; G., Ome; C., Belkis; A. and Nesimi; E. (2006). Warfarin resistances interaction between Warfarin and *Eruca sativa* L. Swiss Med. Wkly. 136:327

Espinoza-Navarro, O. and Bustos-Obregón, E. (2014).Effects of Malathion on Cellularity and Sperm Differentiation in Testis and Epididymis of Adult Rats. Int. J. Morphol., 32(1):119-124.

Rathore M, Bhatnagar P, Mathur D, Saxena GN(2002). Burden of organochlorine pesticides in blood and its effect on thyroidhormones in women. Sci Total Environ, 295 : 207-215.

Schielfer,W.C(1980).Statistic for the biological sciences.2nded.Addison Wesley publcomp,California,London.

Sief, M. M. ; Khalil, F . A. ;Abou Arab, A. A. K. ; AbouDonia, M. A. ; El-Sherbiny, A. M. and Mohamed, S. R. (2015). Ameliorative Role of *Melissa officinalis* against Hepatorenal Toxicities of OrganophosphorusMalathion in Male Rats.MOJ Toxicology. 1 (3) :1-8

Suresh BN, Malik JK, Roa GS, et al.(2006). Effects of subchronicmalathion exposure on the pharmacokinelic disposition of pefloxacin. Environ ToxicolPharmacol 22:167-171.

WHO.(2003). Malathion.Genava,Report No.12.Lu, J. ; Lin, P.H. ; Yao, Q. and Chen, C.(2010). Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. J. Cell Mod. Med.14(1):840 – 860.

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

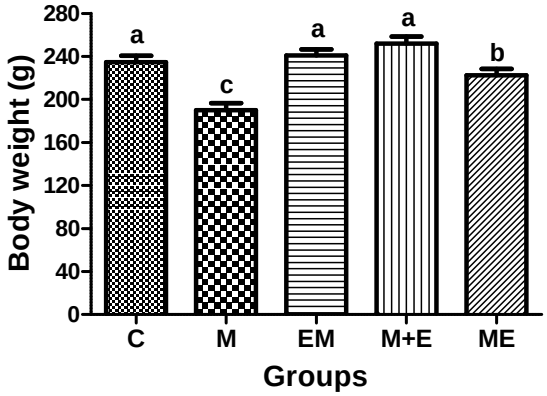

Figure (1): effect of Eruca sativa aqueous extract on body weight (g) in malathion treated male rats.

Values represent mean ± standard error. Different small letters represent significance (P<0.05) between groups. C: male rats daily drenched with 0.5 ml of corn oil for 4 weeks. M: male rats daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks. EM: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks. M+E: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks. ME: male rats daily drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

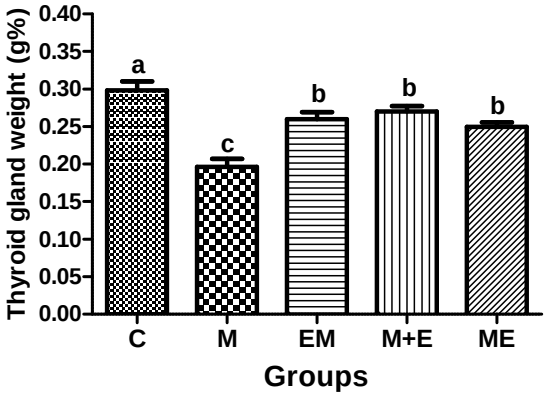

Figure (2): effect of Eruca sativa aqueous extract on relative thyroid gland weight (g/ 100 g bw) in malathion treated male rats.

Values represent mean ± standard error. Different small letters represent significance (P<0.05) between groups. C: male rats daily drenched with 0.5 ml of corn oil for 4 weeks. M: male rats daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks. EM: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks. M+E: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks. ME: male rats daily

drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

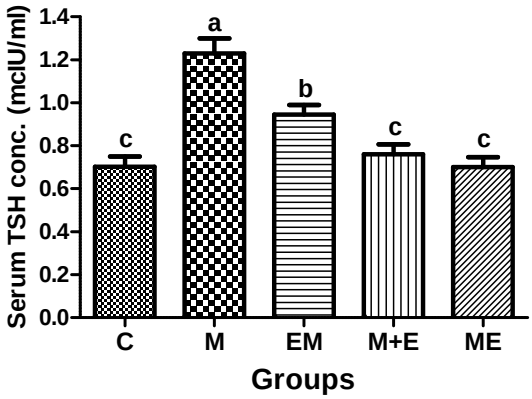

Figure (3): effect of Eruca sativa aqueous extract on serum TSH concentration (mcIU/ ml) in malathion treated male rats.

Values represent mean ± standard error. Different small letters represent significance (P<0.05) between groups. C: male rats daily drenched with 0.5 ml of corn oil for 4 weeks. M: male rats daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks. EM: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks. M+E: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks. ME: male rats daily drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

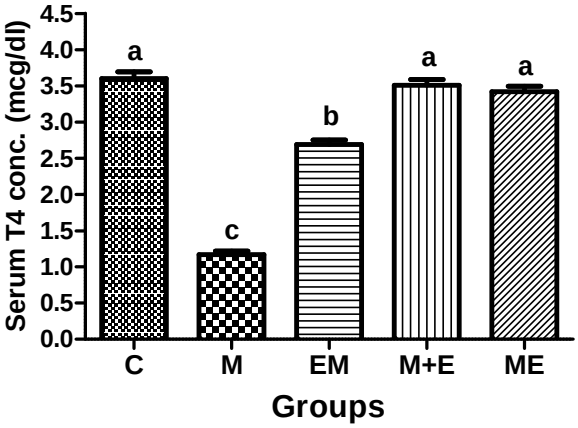

Figure (4): effect of Eruca sativa aqueous extract on serum T4 concentration (mcg/ ml) in malathion treated male rats.

Values represent mean ± standard error. Different small letters represent significance (P<0.05) between groups. C: male rats daily drenched with 0.5 ml of corn oil for 4 weeks. M: male rats daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks. EM:

male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks. M+E: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks. ME: male rats daily drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

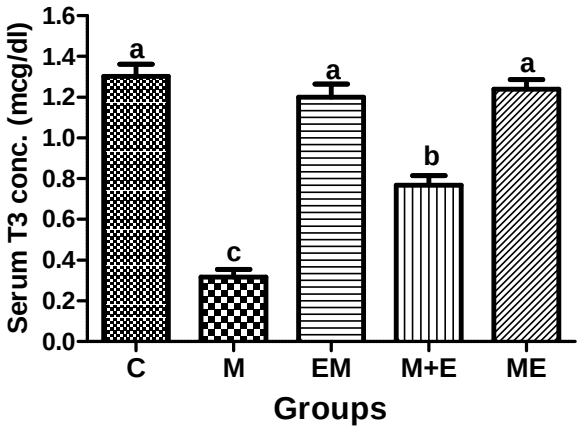

Figure (5): effect of Eruca sativa aqueous extract on serum T3 concentration (mcg/ ml) in malathion treated male rats.

Values represent mean ± standard error. Different small letters represent significance ($P<0.05$) between groups. C: male rats daily drenched with 0.5 ml of corn oil for 4 weeks. M: male rats daily drenched with 0.5 ml of malathion (27 mg/kg bw) for 4 weeks. EM: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks and followed by daily drenching of 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks. M+E: male rats daily drenched with 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) and 0.5 ml of malathion (27 mg/ kg bw) for 4 weeks. ME: male rats daily drenched with 0.5 ml of malathion (27 mg/ kg bw) for 2 weeks followed by drenching 0.5 ml of E. sativa aqueous extract (250 mg/ kg bw) for 2 weeks.

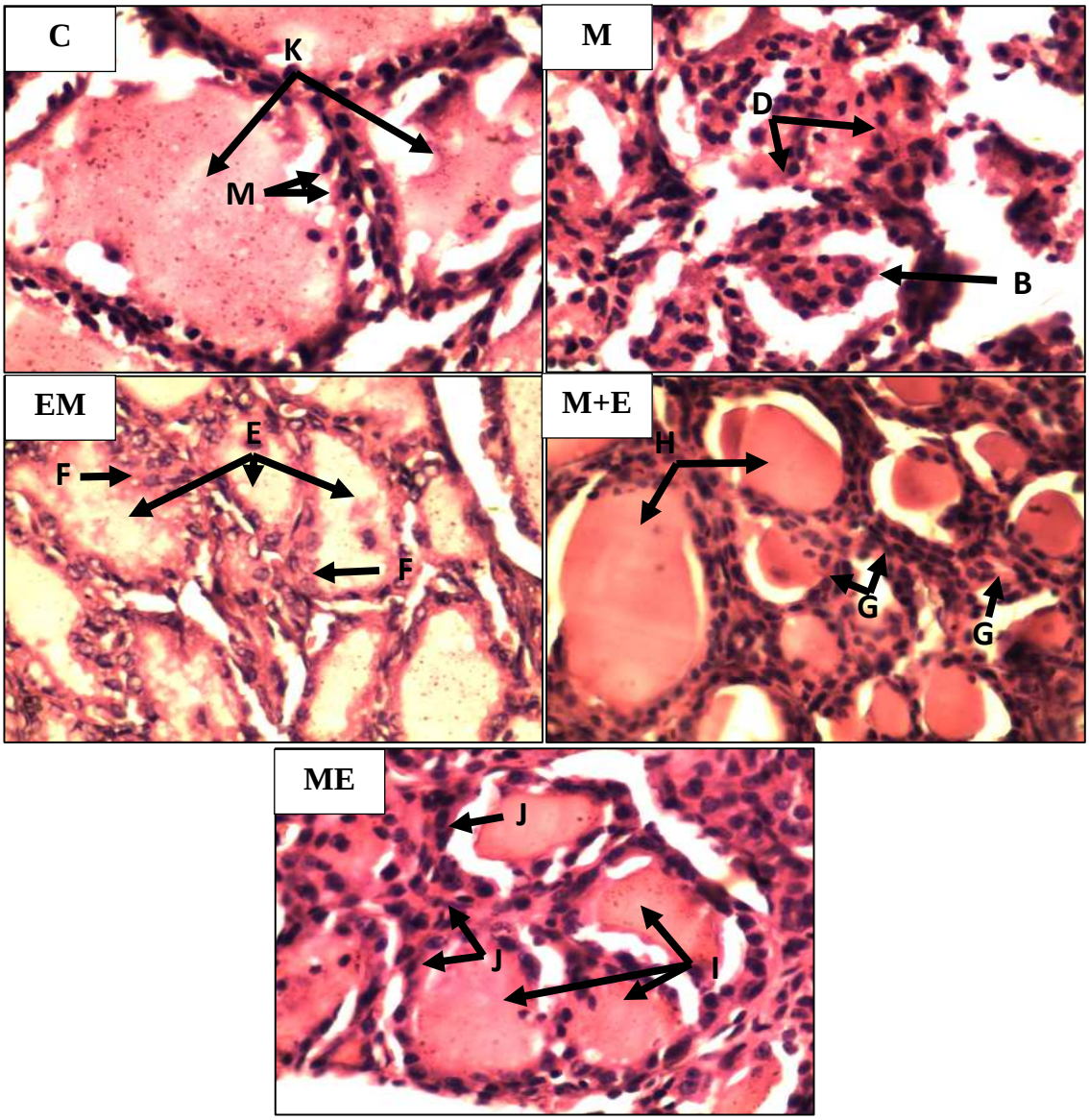

Figure (6): sections obtained from thyroid glands of control male rat treated with normal saline for 4 weeks (C), malathion (27mg/kg bw) for 4 weeks (M), E.sativa extract (250 mg/kg bw) for 2 weeks followed by malathion for 2 weeks (EM), malathion and E. sativa extract together for 4 weeks (M+E), and malathion for 2 weeks followed by E. sativa extract for 2 weeks (ME). K: thyroid follicles filled with colloids, M: normal cuboidal cells, B: proliferate cells of the lining of the follicles forming papillary projections, D: degenerate and nectrotic cells, E: thyroid follicles filled with colloid and numerous proliferative cells, F: proliferation and hyperplasia of lining cells, G: hyperplasia of thyroid follicular lining, H: thyroid follicles filled with colloid, I: numerous small follicles filled with colloid, and J: marked proliferation of follicle lining cells (E&H, 40X).