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Toxic Effects of Detlamethrin on Histological structure of Urogenital System of Adults Male Rabbits and Antioxidants Role of Folic Acid

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ABSTRACT: Introduction: Deltamethrin insecticide has been widely applied for agriculture and veterinary purposes. However, exposure to it attributed to several deleterious impacts to animals and human health. **Aim of the Work:** The study aimed to evaluate the negative impacts of deltamethrin on the normal architecture of kidney and testes of adult male rabbits on long term exposure and manifested the possible protective effect of folic acids against this insecticide toxicity. **Materials and Methods:** Twenty adult male rabbits were divided into four groups; group 1 (control group), group 2 administrated 1.28mg/Kg of deltamethrin, group 3 received 5mg/kg BW of folic acid and group 4 was given a combination of deltamethrin and folic acid. The treatment was applied once daily for 12 weeks. The specimens were processed and stained with H&E and PAS satin for light microscopic examination. **Results:** Deltamethrin treated rabbits showed tubular injury in the form of loss of brush border, dropping off nuclei and karyolysis of tubular cells. In addition, flattening of tubular epithelium and dilatation of renal tubular were also observed. Glomerulus appeared with mesangial cells expansion and capillary tuft congestion. The peritubular lymphocytic infiltration and areas of hemorrhagic were noticed in the interstitium. Kidney sections stained with PAS demonstrated a marked decrease of carbohydrate contents of tubular epithelium and disruption of membrane structures which are induced by deltamethrin toxicity. The same finding were also seen in combination group indicating no role of folic acids as antioxidants. Regarding to testicular tissue, deltamethrin induced hydropic degeneration of seminiferous tubules leading to destroy of stratified germinal layers and series of spermatogenia with focal loss of spermatid production. The interstitial cells of Leydig between tubules revealed a marked proliferation. In spite of treated animals, combination group showed a focal improvement of spermatogensis with returning back to the normal length spermatgonia layers. **Conclusion:** Deltamethrin induced obvious structural changes to kidney and tests of rabbits, therefore it should be carefully limited at farms and animal houses to avoid serious impacts on human health. Application of antioxidants such as foilc acid could be useful to ameliorate testes damage while showed on beneficial effect on renal structures.

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Key Words: Kidney, testicular tissue, deltamethrin toxicity, rabbits and folic acid.

INTRODUCTION

Application of insecticides in public health, forestry and in farms have increased recently because of their roles enhancing quality of vegetable crops and their effects eradicating white fly, mites and ant (1-3). However, the intensive use of these insecticides are negatively impacts on environmental and human lives as they can pollute soil and reach water resources (4). There are different types of insecticides vary in their toxicity and potency, among common pesticides are synthetic pyrethroids, which are hugely introduced since it is less toxic to human and highly potent against unwanted pests (5-7). Deltamethrin (DLM) is the most accepted type of synthetic pyrethroids (class II) that commonly utilize for agriculture purposes (8, 9). While, several publications have highlighted its negative effects on human body causing nephrotoxicity(10), immunosuppression (6), genital inability and neurological symptoms (11, 12). DLM is lipophilic substance acquiring its ability to attach the lipid membranes leading to lipid peroxidation and subsequently overwhelming free radicals production

(ROS)(5, 13). the later put the body under oxidative stress and consuming of natural antioxidants enzymes, all these events are participating in the main mechanism of deltamethrin toxicity. Poonam had reported that exposure to deltamethrin lead to decrease in the organ weight of Wister rats including testes and epididymis as well as affected on the morphological activities of the sperm(14). Others, revealed severe degenerative changes on renal tubules and glomerular tuft necrosis in rats after oral administration of DLM (15). Deltamethrin similar to other synthetic pyrethroids in its oxidative toxicity as seen after exposure to cytpermethrin which induce DNA damage and block normal cell divisions (16). Some experimental studies have confirmed that dietary supplementations of antioxidants like vitamin C or Vitamin E play a vital role in preventing lipid peroxidation, oxidative stress and protein oxidation in rat tissue that exposed to insecticides (8, 17, 18). Folic acid which is synthetic form of folate and considered vitamin B group member (B9) that usually recommended to enhance male and female fertility (19, 20). It stimulates DNA synthesis and promote a proper nuclear cell division as well as stimulate cellular redox hemostasis (21) it is involved in converting toxic accumulation of hemocysteine into methonine (22). It commonly prescribed to reduce the risk of neural tube defect in newborns (23, 24). Thus the aim of this study was to determine the antioxidant activity of folic acid on kidney and testicular tissue of male rabbits induced by deltamethrin toxicity.

MATERIALS AND METHODS

Chemicals

Commercial formulation of deltamethrin (25% EC), of high purity (98%) product of DuPont, was obtained from Kafr El-Zayat Pesticides & Chemicals Company, Egypt. Folic acid (pteroylglutamic acid) tablets (5mg) which is water soluble vitamin was purchased from Sigma Aldrich, India.

Animals

The animal work followed the guide lines of " laboratory animal care principles " (nih publication no. 85- 23, revised 1985). Twenty adult male rabbits aged nearly 6 months weighting (1.891 ± 27.6) Kg were involved in this works, they were divided into four groups with five animals in each. All animals were housed in a separate cages and they acclimatized for five days in laboratory environment before experiment under a standard laboratory conditions with free access to the food and water. Cleanliness and hygiene of the rabbits was checked regularly and cages were also cleaned from wastes every day.

Experimental design

Animals were divided into four groups as follow;

1. **Control group** animals were given only distilled water.
2. **Deltamethrin treated group** was orally treated with 1.28mg/Kg body weight (BW) of insecticide (12, 25).
3. **Folic acid group** which received only folic acid 5mg/kg BW.
4. **Combination group** was co-administrated deltamethrin (1.28kg/mg BW) and folic acid (5mg/Kg BW).

Treatments were applied once daily in the morning after food supplement for 12 weeks via gastric gavage.

Histological Examination:

At the end of experiment animals were anesthetized by chloroform, sacrificed and kidney and testes organs were removed from each animal , then immersed in 10% of buffered formalin and dehydrated with absolute ethanol (70-100%), cleared with xylene and finally embedded in paraffin blocks (26). The prepared sections of 5µm thickness were stained with Harris Hematoxyline and Eosin (H&E) and Periodic acid Schiff's (PAS). The slides then examined for histopathological changes in renal and testes.

RESULTS

Control section of kidney rabbit's stained with H&E showed normal architecture in term of epithelium of renal tubules; proximal and convoluted tubules (PT&DT) as well as normal structure of renal corpuscles with two layered bowman capsule and glomerular capillaries (Fig. 1A). Examination of kidney of deltamethrin treated rabbits showed tubular injury in the form of loss of brush border, dropping off nuclei and karyolysis. In addition, flattening of tubular epithelium and dilatation of renal tubular were also observed. The renal glomeruli exhibited hypercelluarity revealing mesangial cells expansion and capillary vascular congestion were also noticed.Interstitium showed peritubular lymphocytic infiltration along with peritubular congestion (Fig. 1B and C). Similarlythe same changes seen were also manifested in folic acid treated animals with prominent of leukocytic infiltration and vascular congestion(Fig.1D).

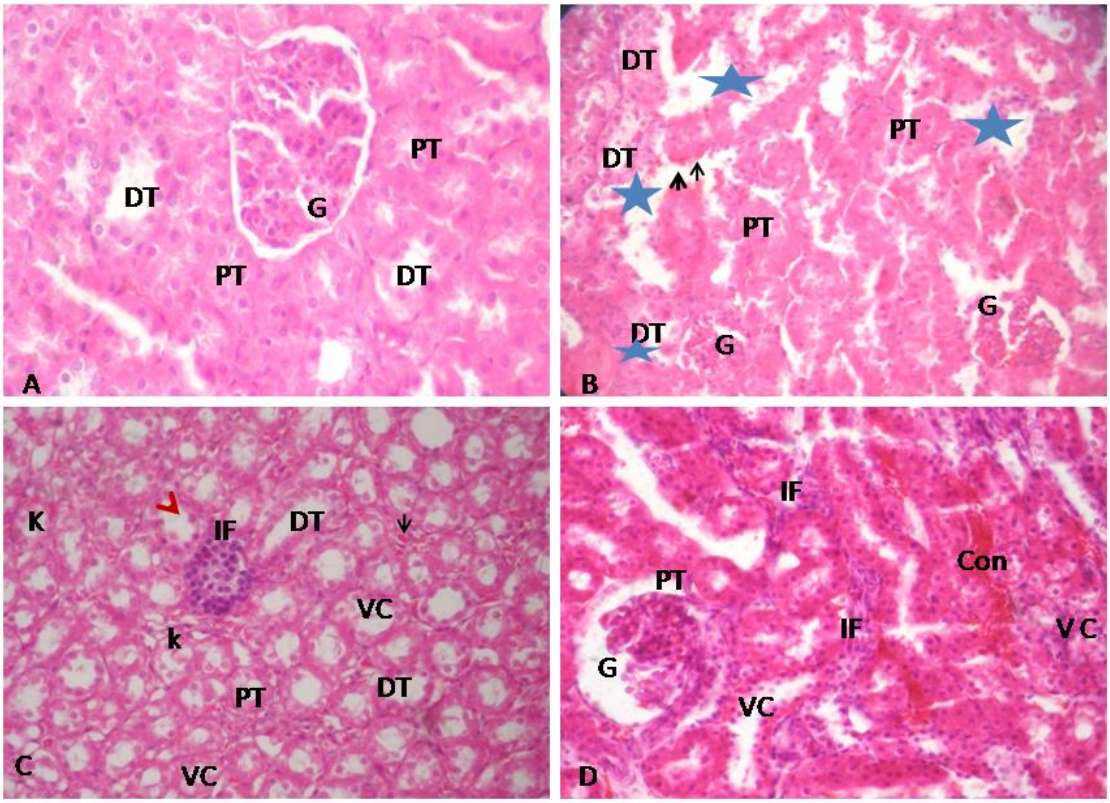

Fig. 1: Light micrographs of kidney's rabbits showing: A). Normal renal corpuscles of control group with tuft of glomerular capillaries(G), cytoplasm and nuclei of epithelium lining proximal and distal tubules appeared in normal morphology (PT&DT). B&C). DLM treated group reveals tubular injury (Star) in the form of loss of brush border, dropping off nuclei (Red arrow), vacuolated cytoplasm (VC), karyolysis (K) and peritubular congestion (black arrow). Interstitium showed lymphocytic infiltration (IF). The similar pathological changes were also manifested in combination group with prominent of leukocytic infiltration and vascular congestion in section (D). (x400 H&E).

Kidney of treated rabbits by carbohydrates staining techniques (PAS reaction) demonstrated loss of brush border of tubular epithelium and disruption of membrane structures which are induced by deltamethrin toxicity (Fig. 2B). The same finding were also seen in combination group indicating no role of folic acids as antioxidants. (Fig. 2C).

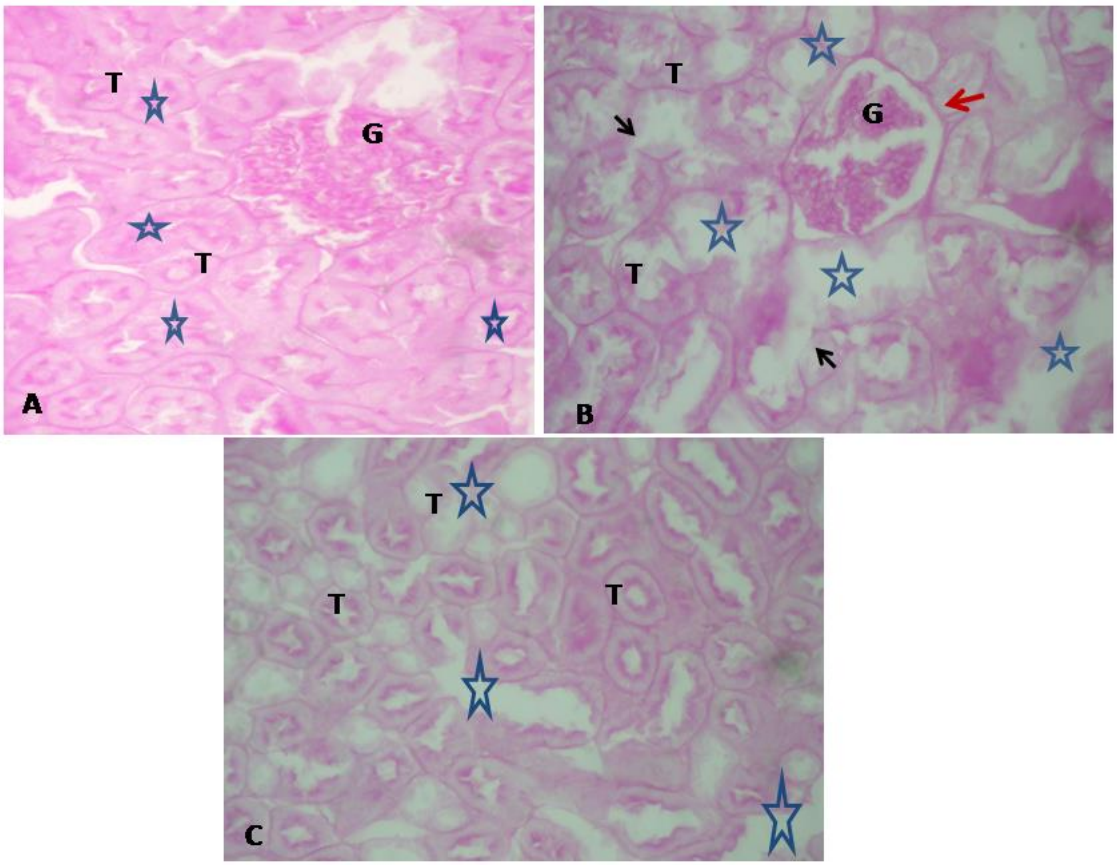

Fig. 2: Kidney tissue of rabbits with PAS reaction showing A). Control group appeared with normal carbohydrate contents of brush borders and intact cell membrane of tubular epithelium (star). B). DLM treated rabbits showed the adverse effect of insecticide in term of loss brush borders (star) and distortion of renal membranes tubules (T) (black arrow). C). DLM with folic acid group also presented of lack of carbohydrate content of renal tubules (star). (x400(PAS satin).

Light microscopic examination of control testes showed normal structure of seminiferous tubules with normal maturation stages of spermatogenic cells as well as interstitial cell Leydig (Fig. 3A). While deltamethrin induced hydropic degeneration of seminiferous tubules that leads to decrease of spermatogenic layers with focal loss of spermatid production. The interstitial Leydig cells revealed a marked proliferation (Fig. 2B). Interestingly, in combination group there was a focal improvement of spermatogensis with returning back to the normal length spermatgonia layers. (Fig.3 C)

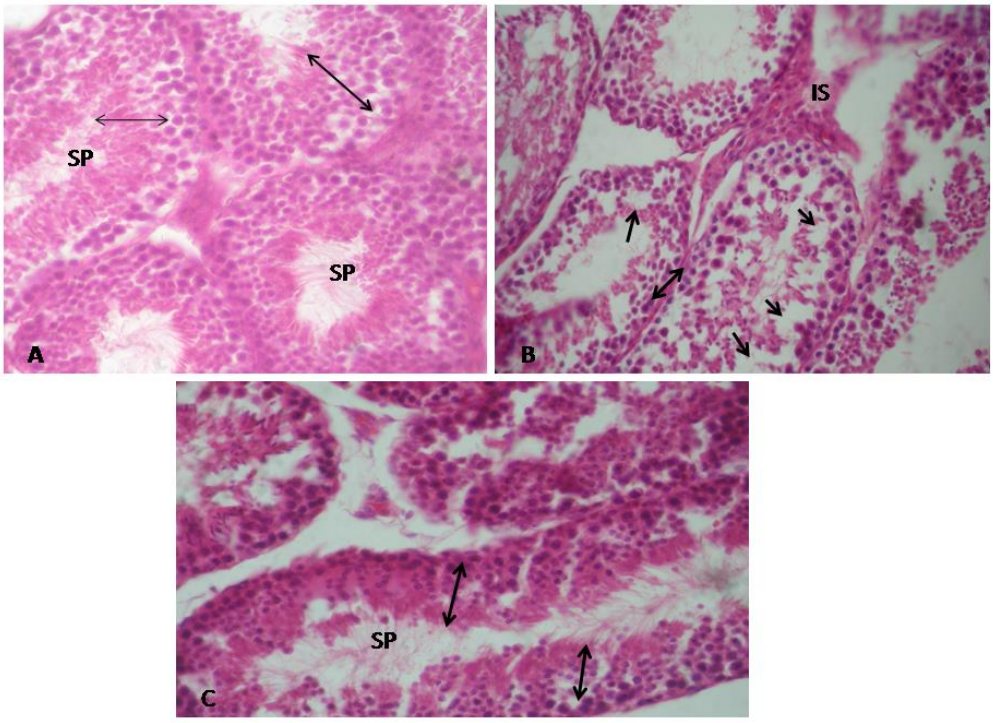

Fig. 3: A). Semniferous tubules of control rabbits with normal height of germinal epithelium and maturation layers of spermatogonia (arrow). B). Section of testicular tissue of after exposure DLM (arrow refers to the area of decreasing spermatogenic series) and proliferation of leydig cells were also seen (IS). C). Testicular tissue of rabbits giving folic acid a long with deltamethrin showing alleviation of insecticide toxicity and returning height of the germinal (arrow) and lumen filled with sperm (SP). (X400 H&E).

DISCUSSION

The use of insecticides could have an inhibitory effect on the cell function and promote the organ damage. Our study found that exposure to deltamethrin resulted in pathological alterations in kidney tissue of rabbits including signs of tubular cells injury, flattening of tubular epithelium ,dilatation and peritubular congestion. Interstitium showed lymphocytic infiltration spread between renal tubules. These findings are in corroboration with other researches that manifested degenerative changes on the cells of the renal tubules with glomerular congestion and focal leukocytic infiltration induced by toxic effect of methomyl on kidney of rats (22). Likewise, other studies reported noxious effect of DLM on kidney's cortex of Wister rats (27). The degenerative changes observed on glomerulus and tubules due to the deltamethrin cytotoxicity inducing cellular dysfunction and further caused bio-accumulation of ROS, depletion of glutathione (GSH) and other antioxidants enzymes(11, 28, 29). Oxidative stress is attributed to an increase in cell permeability leads to hydropic cytoplasmic appearance of the damaged cells (5, 22). Additionally, mesangial cells expansion was exhibited in our findings which may result from cell proliferation. This coincide with Abdel Razik and et al works on effects of Lufenuron and Profenofos insecticide on kidney of albino rats (30) as well as reported by other investigators (3). Destructive renal system is strongly related to impaired of renal excretion and their function which assessed through marked elevation of blood urea and creatinine in the experimental modules and this coincided with results obtained by Ali Khaled in his research (8, 16). Moreover, leukocytes are attracted to the site of tubular injury promoting inflammatory reaction and induce inflammatory cascade. El-Gerbed et al had confirmed elevation of cytokines primarily IL6,IL1β and TNFα) in kidney rats exposed to DLM (15, 31). Renal toxicity had been reported in the previous studies through elevation of kidney Malondilaldyde (MDA) tissue level, which is indicating the oxidation of polyunsaturated fatty acid of lipid membrane and perturbation of kidney antioxidant activity (8, 22, 32). Nearly similar changes were also seen in group that co administrated of folic acid and deltamethrin indicating that folate has no protection effect against nephrotoxicty induced by DLM.

Regarding to kidney tissue with PAS reaction, the use of DLM led to a marked decrease in polysaccharides contents in kidney tissue. Roa elucidated that reduced of glycogen contents in kidney tissue probably reflects consuming of carbohydrates in response to the toxic effects on pesticides exposure mainly by oxidative stress

mechanism (30, 33, 34). Others, explained that depletion of carbohydrates under insecticides therapy is related to the lysosomal destructions and rupture of hydrolytic enzymes (35).

Our finding also showed that deltamethrin inhibit spermatogensis and sloughed off the germinal epithelium which is strongly supported by other works reported a decrease in testes weight and elevated of testosterone levels perhaps DLM destroy germinal layers leading incomplete series of spermatogenic cells with distortion of seminniferous tubules (14, 36). Abdel-Rahman and et al reported that administration of deltamethrin caused inhibition of hypothalamus releasing hormone mainly LHRH which in turn block release of LH and subsequently affecting testosterone production. (37). In addition, hydrophilic nature of DLM owing its harmful effect on testes structure since the sperm is coated with high levels of fatty acids thus it is simply enter peroxidation processes. On contrary, there was an improvement in the histological features of seminniferous tubules in combination group (deltamethrin plus folic acid) in comparison with deltamethrin group, indicating the critical role of folic acid in supplying one methyl group for DNA methylation and preventing genetic defects during neonatal life(20). Others highlighted its role in scavenging of reactive oxygen species through induction of apoptosis related gene (22). A group of workers noted that folic acid alleviate reproductive toxicity through elevation of serum testosterone level in combination group when compared to treatment group (carbufuran)(38). In the vitro animal studies on deleterious impact of carbamate on testicular tissue showed that this pesticide cause over expression of apoptosis genes and inhibit proliferation related gene at spermatgonia level, also led to down regulate of leydig cells function through inhibition of testosterone producing (22, 39). However, damage of the testicular cell was diminished to a certain degree after oral administration of folic acid which is in agreement with our works (22).

CONCLUSION

Undoubtedly , that deltamethrin toxicity participated in organ dysfunction and cellular damage through oxidative stress and depletion of natural antioxidants activity of the most of body tissues especially kidney and testes. Our result indicated that folic acid can alleviate the destructive effect of deltamethrin on histological architecture of testes while failed to encounter oxidative injury on kidney tubules.

CONFLICT OF INTERESTS

There is no conflict of interest

REFERENCES

[1]. Galeb Luciana do Amaral Gurgel FAC, Fam DAmico , Rocha Rita Maria Mangrich,Kirschnik Peter Gaberz , Weber Saulo Henrique , Pimpão Cláudia Turra Determination of lethal and sub-lethal concentrations of deltamethrin in Jundiá (Rhamdia quelen). Rev Agrar Acad. 2013;11(2):125-30.

[2]. ULLAH M. S AM, AHMAD N, KHAN M. Z, AHMAD I. Toxic Effects of Cypermethrin in Female Rabbits. Pakistan Vet J. 2006;26(4):193-6.

[3]. Bouzar A.C. BY, A Bitam. A low-dose protective effect of phycocyanin on the toxicity of deltamethrin to vital organs in rats: in vivo study. J Fundam Appl Sci. 2020;12(1):149-66.

[4]. Korkmaz N, Erdoğan K, Örün GN, Erkmen B, Doğru Mİ, Doğru A, et al. Determination of acute toxicity of sodium pyrithione on common carp and its effects on some hormones and hematological parameters. 2022:1-22.

[5]. Chargui I, Grissa I, Bensassi F, Hrira MY, Haouem S, Haouas Z, et al. Oxidative Stress, Biochemical and Histopathological Alterations in the Liver and Kidney of Female Rats Exposed to Low Doses of Deltamethrin (DM): AMolecular Assessment. Biomed Environ Sci. 2012; 25(6):672-83.

[6]. Tewari A GJPS. Assessment of hemato-biochemical parameters on exposure to low level of deltamethrin in mouse model. Vet World. 2014;7 (3):152-7.

[7]. Ananda S Sathanandam BVJ, Hainesc T Wendy, Muralidhara,Srinivasa, Fisher W Jeffrey, Padilla Stephanie Characterization of deltamethrin metabolism by rat plasma and liver microsomes. Toxicol Appl Pharmaco 2006;212:156-66.

[8]. Amin KA, Hashem KS. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias gariepinus): antioxidant defense and role of alpha-tocopherol. BMC Veterinary Research 2012;8(1):1-8.

[9]. ATAMANALP Muhammed YT. Alteration in hematological parameters of rain bow Trout (Oncorhynchus Mykiss)exposed to Mancozeb. Turk J Vet Anim Sc 2003;27:1213-7.

[10]. Mansour SA, Mossa A-TH. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pesticide Biochemistry and Physiology. 2010;96(1):14-23.

[11]. MM A-D, SM A, SM H. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. PLoS One. 2013;8(9):e72991.

[12]. Yousef M. I , Awad I T, Mohamed. E .H. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. J Toxicol. 2006;227(3):240-7.
[13]. Rehman H AA, Saggu S, Abba ZKs, Mohan A, Ansari AA. Systematic review on pyrethroid toxicity with special reference to deltamethrin. J Entomol Zool Stud 2014;2(6):60-70.
[14]. Sharma P SR, Jan M. Dose-Dependent Effect of Deltamethrin in Testis, Liver, and Kidney of Wistar Rats. Int J Toxicol. 2014;21(2).
[15]. Gündüz E ÜBV, İbiloğlu İ, Ekinci A, Dursun R, Zengin Y, Mustafa İ, Uslukaya Ö, Ekinci C, Güloğlu C. Glutamine Provides Effective Protection against Deltamethrin-Induced Acute Hepatotoxicity in Rats But Not Against Nephrotoxicity. Med Sci Monit. 2015;21:1107-14.
[16]. Alwan AK. Toxic Effects of Cypermethrin on Liver and Kidney of Male Domestic Rabbits. Gaza: The Islamic University of Gaza; 2015.
[17]. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium Journal of Trace Elements in Medicine and Biology. 2004;18(1):113-21.
[18]. Raina R, Verma PK, Pankaj NK, Kant V. Ameliorative effects of alpha-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats. Int J Med Sci. 2009;1:396-9.
[19]. Yousef MI, M E-DF, Kamil KI, Elaswad FA. Ameliorating effect of folic acid on chromium (VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. Reproductive Toxicology. 2006;21(3):322-8.
[20]. Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL. Impact of folate and homocysteine metabolism on human reproductive health. Human Reproduction Update. 2007;13(3):225-38.
[21]. Shalaby M, El Zorba H, Ziada RM. Reproductive toxicity of methomyl insecticide in male rats and protective effect of folic acid. Food and Chemical Toxicology. 2010;48(11):3221-6.
[22]. Sakr S, Hassanien H, Bester MJ, Arbi S, Sobhy A, El Negris H, et al. Beneficial effects of folic acid on the kidneys and testes of adult albino rats after exposure to methomyl. Toxicology research. 2018;7(3):480-91.
[23]. Barrett JR. Folic Acid and ASDs: A Preventive Measure against Potential Effects of Pesticide Exposures? Environmental Health Perspectives. 2017;125(10):104006.
[24]. Ulaiwi HK. Hemato-biochemical and histopathological alterations induced by acute cypermethrin toxicity in rabbits AL-Qadisiya Journal of Vet Med Sci. 2011;10:84-94..
[25]. Adil Mehraj Khan ND, Rajinder Raina, Gagandeep Singh, Shafayat Ahmad Beighe Jammu. Toxic Effects of Deltamethrin and Fluoride on Hematological Parametrs in Rats. Fluoride. 2013;46(1):34-8.
[26]. Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. Philadelphia: Churchill Livingstone Elsevier; 2008. 126-7 p.
[27]. Abbassy MA, AH. M. Hematobiochemical effect of formulated and technical cypermethrin and deltamethrin insecticides in male rats. J Toxicol Pharmacol. 2012;7(7):312-21.
[28]. Meng SL, Hu GD, Qiu LP, Song C, Fan LM, Chen JZ, et al. Effects of chronic exposure of methomyl on the antioxidant system in kidney of Nile tilapia (Oreochromis niloticus) and recovery pattern. Journal of Toxicology and Environmental Health, Part A. 2013;76(15):937-43.
[29]. Raina R, Verma PK, Pankaj NK, Kant V. Ameliorative effects of alpha-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats. International Journal of Medicine and Medical Sciences. 2009;1(9):396-9.
[30]. Abdel Razik H Farrag, Shalby S. Comparative Histopathological and Histochemical Studies on IGR, Lufenuron and Profenofos Insecticide Albino Rats. J Appl Sci Res. 2007;3(5):377-86.
[31]. El-Gerbed MSA. Protective effect of lycopene on deltamethrin-induced histological and ultrastructural changes in kidney tissue of rats. Toxicology and industrial health. 2014;30(2):160-73.
[32]. Sangha G. K KK, K. S. Khera, Balwinder Singh. Toxicological Effects of Cypermethrin on Female Albino Rats. Toxicology International 2011;18(1).
[33]. Rao JV. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline Wsh, Oreochromis mossambicus enkateswara. . Pesticide Biochemistry and Physiology ARTICLE IN PRESS. 2006.;86(2):78-84.
[34]. El-durssi ISH, El-Awami IOS, mahmoud GS, Benkhaial FA. Experimental Studies on the effects of chlorpyrifos on Rats-II.Histochemical changes. AL-Mukhtar. 2006;13:80-8.
[35]. Shalaby AA. Effect of cyolane on the cytology and histochemistry of the ileum of Clarias lazera. M Sc Thesis, Faculty of Science, Zagazig University. 1985.

[36]. Faddladdeen KAJ. Effect of liver fibrotic changes on testicular histological structure: An updated review. 2019;10(6):769-74.
[37]. A bdelrahman M ASM. Effect of deltamethrin on the release of catecholamines and its related effect on some sex hormones in adult male albino rats. ISOTOPE & RAD RES. 2005;37(1):89-102.
[38]. Kobeasy MI, El-Naggar AY, Abdallah AA. A novel methods for protective role against reproductive toxicity of carbofuren in male rats using palm pollen grains and vanadyl (II) folate as a new compound. J Chem Pharm Res. 2015;7(4):1142-8.
[39]. Lu M, Liu Y. Folic acid reduces methomyl insecticide damage to testicular cells by altering the DNA methylation environment. 2022.

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