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Original Article

Protective Effect of Cinnamon Aqueous Extract on Cypermethrin-Induced Hepatotoxicity in Albino Rats

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ABSTRACT

Cypermethrin is a synthetic pyrethroid with potent insecticidal property. Cinnamon is one of the oldest medicinal plants used in traditional medicine. The present work was carried out to evaluate the effect of cinnamon aqueous extract on liver injury induced by the cypermethrin in albino rats. Animals were divided into 4 groups. Group 1: control, Group 2: given cinnamon (200mg/kg b.w.), Group 3: given cypermethrin at a dose level 1/10 LD_{50} for 6 weeks, Group 4: given cypermethrin and cinnamon. Liver were obtained from these groups for histological, histochemical and immunohistochemical preparations. Liver tissues of cypermethrin-treated animals showed leucocytic infiltrations, rupture of sinusoids with hemorrhages, congested blood vessels, cytoplasmic vacuolation of the hepatocytes and fatty infiltration. Histochemical results revealed depletion of carbohydrates and protein. Expression of PCNA was increased in the liver of treated rats. Biochemical results showed that cypermethrin caused elevation in ALT and AST. Treating animals with cypermethrin and cinnamon revealed an improvement in the histological, histochemical and biochemical changes with decrease in expression of PCNA. In conclusion, the administration of cinnamon aqueous extract provided significant protection against cypermethrin-induced hepatotoxicity in albino rats by its antioxidant effects.

Keywords: Cypermethrin, Cinnamon, Hepatotoxicity, Histology, PCNA, Rats

INTRODUCTION

The use of pesticides in agriculture, animal husbandry, post-harvest technology is a treat to the natural water system, public health and welfare of mankind [1]. Cypermethrin is a synthetic pyrethroid insecticide used to kill insects on olive trees, cotton and vegetables, as well as, to kill cockroaches, fleas also termites in houses and other buildings. Likewise, some veterinary products are based on cypermethrin, which are popularly used for dipping or showering of food animals [2]. Cypermethrin residues have been found in milk from cows wearing cypermethrin-impregnated ear tags (as a horn fly control measure) [3]. In countries where agriculture is labor intensive, agricultural workers are exposed to cypermethrin. Chen [4] reported that over 25 percent of the workers in Chinese cotton fields exhibited symptoms of pyrethroid (including cypermethrin) poisoning. Long-term feeding studies with laboratory animals have shown that cypermethrin causes adverse effects. Cypermethrin administration causes inhibition of serum cholinesterase in rats [5]. In rabbit administration of sublethal dose of cypermethrin showed an increase in the activities of AST, ALT and ALP enzymes [6]. Also Mamun [7] reported that cypermethrin has injurious effect on public health especially on liver and kidney tissues but intensity of degree damage depend on exposure time and dose level. Patel [8] reported that cypermethrin induces systemic genotoxicity in mice as it causes DNA damage in vital organs like brain, liver, kidney, apart from that in the haematopoietic system.

Plants have a long history in the traditional medicine uses that are in a rising demand using their extracts and chemical bioactive compounds for producing drugs against many diseases. Cinnamon has been employed in traditional herbal medicine to treat a variety of health conditions [9]. Cinnamon is one of the oldest medicinal plants used in traditional medicine, also is used as flavoring pastries and foods. Cinnamon has anti-

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bacterial and anti-fungal properties [10]. It is also used to treat nausea, diarrhea and in wound healing [11]. Some studies also showed that the extracts and constituents from cinnamon possess antioxidant and anti-mutagenic activities [12], moreover, anti-inflammatory properties [13]. Eidi [14] reported that cinnamon extract acts as a potent hepatoprotective agent against CCl_4 induced hepatotoxicity in rats. Sakr and Al-barakati [15] indicated that cinnamon has main ameliorative effect against kidney damage induced by cypermethrin. The present work studied the effect of cinnamon on histological, histochemical and immunohistochemical alterations in liver of rats induced by cypermethrin.

MATERIALS AND METHODS

Cypermethrin: Cypermethrin is the ISO approved common name for [a-Cyano-(3-phenoxyphenyl) methyl(±)-cis/trans-3-(2,2dichlorovinyl) 2,2 dimethyl -cyclopropanecarboxylate] was used at a dose level of 1/10 LD50 (5.5mg/kg b.w.) dissolved in corn oil.

Cinnamon aqueous extract: The plant materials were obtained from the local market. For aqueous extract, 20 g of dried powdered plant material was soaked with 100 ml of distilled boiled water in a sterile conical flask for 48 hr with continuous shaking. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected and concentrated (in oven at 45°C) to make the final volume half of the original volume (stock solution).The filtrate was kept at 4°C in refrigerator till use.

Animals and treatments: In this study, adult male albino rats (*Rattus norvegicus*) weighting 150 ± 10g were used. Rats were breed in the Animal House of the Department of Biology / Faculty of Science, Zagazig University) and placed under constant temperature (24± 2°C) throughout the experimental work. They were maintained on a standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5 % salt mixture and 5% vitaminized starch. Water was available *ad libitum*. Maintenance of animals and experimental procedures was approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals. Animals were divided into 4 groups:

Group-1. Animals of this group served as a control group.

Group-2. Animals of this group were orally administrated cinnamon extract at a dose level of 200 mg/kg b. w. 5 days / week for 6 weeks.

Group-3. Animals of this group were orally given cypermethrin at a dose level of 1/10 LD_{50} (5.5 mg/kg b.w.) 5 days / week for 6 weeks.

Group-4: Rats were given 1/10 LD_{50} of cypermethrin and cinnamon extract 5 days/ week for 6 weeks.

Histological and histochemical study: For histological study, animals were sacrificed after 6 weeks, liver were immediately removed and fixed in 10% neutral formalin for 24 hours**.** After fixation, specimens were dehydrated in ascending series of ethyl alcohol, cleared into two changes of xylene, infiltrated in three changes of molten paraffin wax with melting point of 58- 60°C and then embedded in molten paraffin blocks. Sections of 5 microns thickness were cut by using rotary microtome and mounted on clean slides. For histological examination, sections stained with Ehrlich's haematoxylin and counter stained with Eosin. For histochemical demonstration of total carbohydrates periodic acid Schiff's technique (PAS) was used [16]. Total proteins were detected using the mercury bromophenol blue method [17].

Immunohistochemical study:

Immunohistochemical detection of PCNA was performed using an avidin biotin complex immunoperoxidase technique on paraffin sections. Formalin-fixed slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 minutes, antigen retrieval for 15 minutes using Biogenex Antigen Retrieval Citra solution in 90ºC water bath for 30 minutes. The slides were allowed to cool for 20 minutes before continuing. Slides were then blocked by normal horse serum for 5 minutes at 37°C. Sections were incubated overnight at 4ºC with the antibodies against PCNA polyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark).The immunohistochemical reaction was then developed and stained with diaminobenzidine chromogen solution "DAB" (Sigma). The sections were counterstained with hematoxylin, dehydrated, cleared and mounted with DPX. For the negative controls, PBS was used in place of the primary antibody [18].

Image analysis: Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images). The immune-stained sections were analyzed in 10 microscopic fields under high-power field (×400) microscope. In each field, the immunopositive (brown) area was recorded. Percentage of positive stained area (%) was calculated as mean of 10 fields / slide.

Biochemical assays: For biochemical analysis, blood samples were collected in clean centrifuge tubes. Blood samples left to clot in room temperature and then serum separated by

centrifugation at 3000 rpm for 20 minutes. The collected serum stored at 18 -20 °C until analysis. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to the method of [19].

Statistical analysis: Data were expressed as mean values ± SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® 4 Inc., USA).

RESULTS

Histological observation: Liver of a control rat consists of hepatic lobules; each lobule consists of hepatocytes arranged in strands around the central vein. The spaces between these strands are called sinusoids which are irregularly dilated blood vessels that contain phagocytic cells of the mononuclear phagocytes system called Von Kupffer cells derived from blood monocytes, these cells found on the luminal surface of the endothelial cells (Fig. 1a). The space between hepatic lobules known as portal tract containing a branch of the portal vein, a branch of the hepatic artery and a bile ductule, which bound together by connective tissue.

Examination of liver of rats treated with cypermethrin for 6 weeks revealed many histopathological alterations when compared with control group. Disruption of normal structure of hepatic architecture; a characteristic sequence of events of inflammation including a congested central vein, winded sinusoids with large number of activated Kupffer cell and intensive leuckocytic infiltration were observed (figs. 1b & 1c). Moreover, cell injury and degenerated changes such as cytoplasmic vacuolation with pyknotic nuclei was observed in most hepatocytes, as well as the presence of fatty infiltrations (figs. 2a, 2b). Marked histological improvement of hepatic structure was clearly observed after treatment rats with cypermethrin and cinnamon for 6 weeks. examination of liver sections after this periods showed that the hepatic cells began to be arranged in normal strands with no cytoplasmic vacuolation, Kupffer cells became smaller and sinusoids appeared narrow but with slight leucocytic infiltrations (fig. 2c).

Histochemical results: Examination of liver section of a control rat showed deeply pink granules of a strong **PAS** reaction in the pole of cytoplasm of hepatocytes (glycogen flight) while the nuclei are not exhibited any reaction (negative stained) (fig. 3a). Examination of liver of rats treated with cypermethrin for 6 weeks showed a decrease of the glycogen content (fig. 3b), while treatment rats with cypermethrin and cinnamon for 6 weeks revealed an increase in the glycogen content (fig. 3c).

Examination of liver section of a control rat showed normal protein content in the hepatocytes as dense blue granules in cytoplasm, cell membrane, nuclear membrane, chromatin bodies, nucleoli and Kupffer cells (fig. 4a). Liver sections rats treated with cypermethrin for 6 weeks showed reduction of the protein content (fig. 4b), while rats treated with cypermethrin and cinnamon for 6 weeks revealed improvement in the protein content (fig. 4c).

Immunohistochemical results: Expression of PCNA appeared in a few nuclei of the hepatocytes of control rats and rats given cinnamon extract (fig. 5a, b). Rats treated with cypermethrin for 6 weeks showed a strong expression of PCNA in many nuclei of the hepatocytes (fig. 5c). Liver sections obtained from rats treated with cypermethrin followed by cinnamon for 6 weeks showed slight expression of PCNA in the nuclei of scattered hepatocytes (fig. 5d). The results in figure (6), showed the area percentage of PCNA positive nuclei of the hepatocytes in the different experimental groups after 6 weeks. The obtained data showed that there were no statistical differences between control group and cinnamon group. The area percentage of PCNA positive nuclei of the hepatocytes showed highly significant elevation ($P<0.05$) in rats treated with cypermethrin for 6 weeks. Treatment of rats with cypermethrin and cinnamon resulted in a significant decrease ($P<0.05$) in the area percentage of PCNA positive nuclei of the hepatocytes.

Biochemical results: Cypermethrin administration significantly increased the serum levels of liver transaminases (ALT and AST) over the normal group (Figs.7&8). On the other hand, animals treated with cypermethrin and cinnamon showed a decrease level of ALT and AST.

DISCUSSION

Cypermethrin is a synthetic pyrethroid pesticide used to control ectoparaside including moth pests, cockroaches, fleas, and termites of cotton, fruit and vegetable [2]. Cypermethrin is primarily absorbed from gastrointestinal tract and may also be absorbed by inhalation of spray mist and only simply through the intact skin. Due to its lipophilic nature, cypermethrin has been found to accumulate

in body fat, skin, liver, kidneys, adrenal glands, ovaries and brain [20]. Exposing rats to cypermethrin in the present work was found to induce different histopathological alterations in the liver. Leucocytic infiltrations, congestion of blood vessels, cytoplasmic vacuolation of the hepatocytes and fatty degeneration were observed in the treated liver. These observations are in agreement with Grewal [21] who reported that cypermethrin intoxication in rats resulted in necrosis of hepatic cells with pyknotic nuclei, disorganization of hepatic laminae and dilatation of sinusoids in hepatic structure. Oral administration of cypermethrin to Wistar rats caused intralobular vein dilatation, cytoplasmic vacuolization, multinuclear cells, nuclear polymorphisms, nuclear vacuolization, hepatocyte membrane damage, nuclear division, nuclear eccentricity, necrosis, pyknosis and karyorrhexis in the liver [22]. Mamun [7] observed that liver of mice treated with cypermethrin showed enlargement in the sinusoidal space, vacuole formations in hepatocytes, leucocytic infiltrations and congestion of blood vessels with hemorrhage.

Treating rats with cypermethrin induced elevation in ALT and AST. These enzymes are considered as a specific indicator for liver damage. An increased ALT and AST were detected in sera of female rats intoxicated with cypermethrin [23]. Orally administration of cypermethrin in albino rats resulted in an increase of AST, ALT, ALP, LDH, total lipids, phospholipids, glycerides, total proteins, cholesterol and bilirubin [22]. In addition, Bhatti [24] reported that daily oral administration of cypermethrin caused significant elevation in the activities of liver marker enzymes such as serum ALT, AST and LDH.

Histochemical results revealed depletion of glycogen and total proteins contents in liver of cypermethrin-intoxicated rats. Similarly, Bhushan [22] reported that hepatic proteins and glycogen decreased in liver of rats treated with cypermethrin. Sakr [25] showed that inhalation of tetramethrin caused decrease of glycogen and proteins. Disturbances in carbohydrate metabolism were observed in a variety of animals under the effect of different insecticides and were suggested to be achieved through modifying the activities of the enzymes of glycolytic pathway, TCA cycle, glucogenesis and the oxidative phosphorylation [26]. The reduction in protein content may be attributed partially to the decreased level of hepatic protein synthesis in the cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes [27]. PCNA expression was increased in liver of cypermethrin-exposed rats. In agreement with this result, Marouani [28] reported that strong positive PCNA staining was observed in liver cells of DDT-treated rats. The authors added that the increase in PCNA expression may be associated with an increase in the number of cells that accumulate in the S phase of the cell cycle. Also, the increase in hepatocyte proliferation may be at least related to regenerative liver response to pesticide, since during liver growth, histological signs of necrosis and vacuolated cytoplasm were present.

Accumulation of pesticides in tissue is associated with induction of oxidative stress and production of ROS [29]. Cypermethrin is reported to induce free radicals in the liver of rabbits [6] as well as lipid peroxidation in rat erythrocytes [30] thus induces oxidative stress. Cypermethrin-induced oxidative damage is also evident from the findings of Manna [31] who observed increase in malondialdehyde level and decrease in the activities of catalase, superoxide dismutase in liver of rat. Thus, the hepatotoxicity of cypermethrin observed in the current work may be due to oxidative stress induced by cypermethrin.

The present results showed that cinnamon has a protective effect against hepatotoxicity of cypermethrin. Treating rats with cypermethrin and cinnamon caused an improvement in the liver architecture, increased glycogen and proteins and decreased expression of PCNA. Moreover, the liver enzyme markers, ALT and AST were decreased in sera of treated animals. In agreement with these results, Eidi [14] reported that cinnamon ethanolic extract had hepatoprotective and antioxidant effects against CCl_4-induced liver injury in rats. Kanuri [32] reported that alcoholic extracts of cinnamon bark protect against acute alcohol induced liver steatosis in mice by attenuating the alcohol-dependent induction of MyD88 and the subsequent formation of reactive oxygen species and induction of TNFa. Lamfon [12] showed that cinnamon aqueous extract ameliorated deltamethrin induced histological and biochemical alterations in liver of rats. Iqbal [33] reported that cinnamon had hepatoprotective effect against cholesterol-induced fatty changes in rats. Administration of cinnamon extract was found to improve the histopathological alterations and ALT and AST in rats treated with paracetamol [34].

The potential mechanism underlying the hepatoprotective effect of cinnamon could be attributed to the antioxidant activity of its constituents [35]. Kim [36] reported that cinnamon stimulates the increase of antioxidant enzymes activities, including SOD and CAT in rat's liver. Su [37] indicated that cinnamon may serve as potential dietary source of natural antioxidants for improving human nutrition and health. Ethanolic extract of

cinnamon has potent hepatoprotective action against CCl4 by lowering the MDA level and elevating antioxidants enzymes activities (SOD and CAT) [38]. Morgan [39] reported that, pretreatment with cinnamon extract provided a protective antioxidant role against adverse effects of bisphenol. They added that treatment with cinnamon caused decrease in lipid peroxidation and increase in the antioxidant enzymes, CAT and SOD. Cinnamon bark was found to include the antioxidant compounds, polyphenol and flavinoids [40] [41]. Thus, the hepatoprotective of cinnamon recorded in this study may be due to the presence of these compounds.

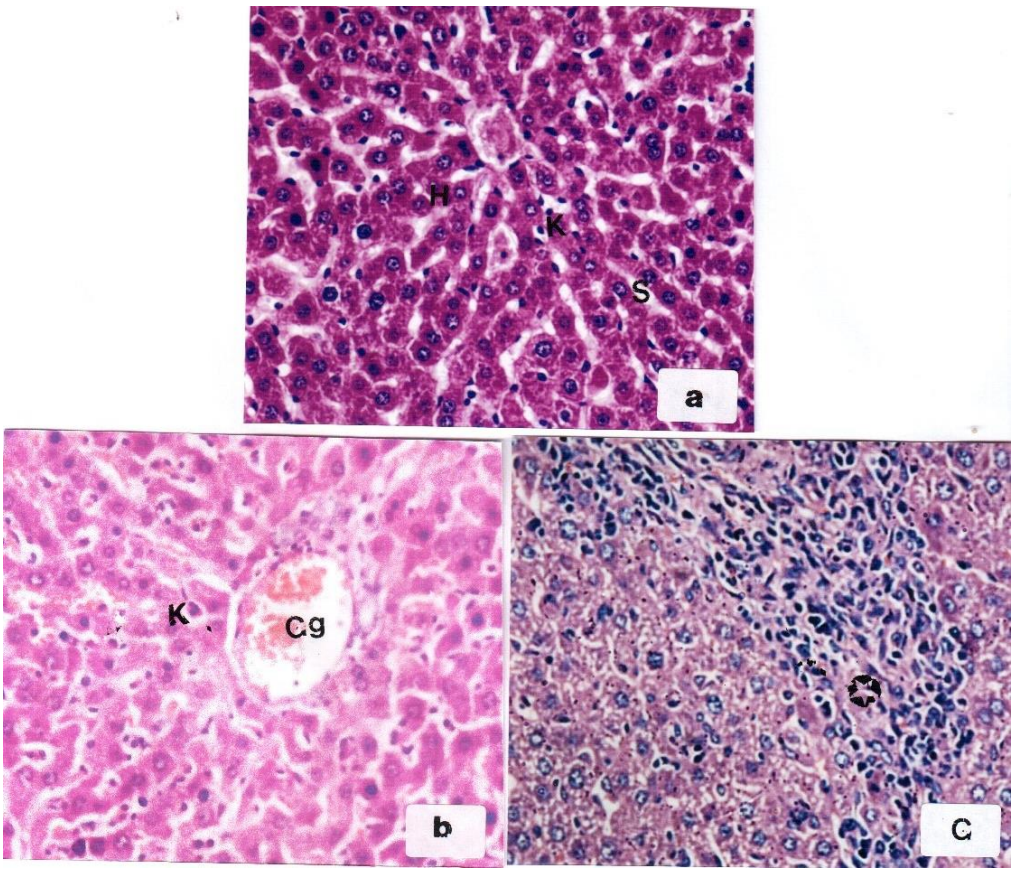

Figure (1)a: Liver section of a control rat showing the normal hepatic architecture, the hepatocytes (H) arranged in strands around the central vein and find between them sinusoids (S) with Kupffer cell (K), (x 400).

Figure (1)b: Liver section of a rat treated with cypermethrin showing congestion of central vein with blood (Cg), winded sinusoids with large number of activated Kupffer cell (K), (x 400).

Figure (1)c: Section in the liver of a rat treated with cypermethrin showing intensive leuckocytic infiltration (*), (x 400).

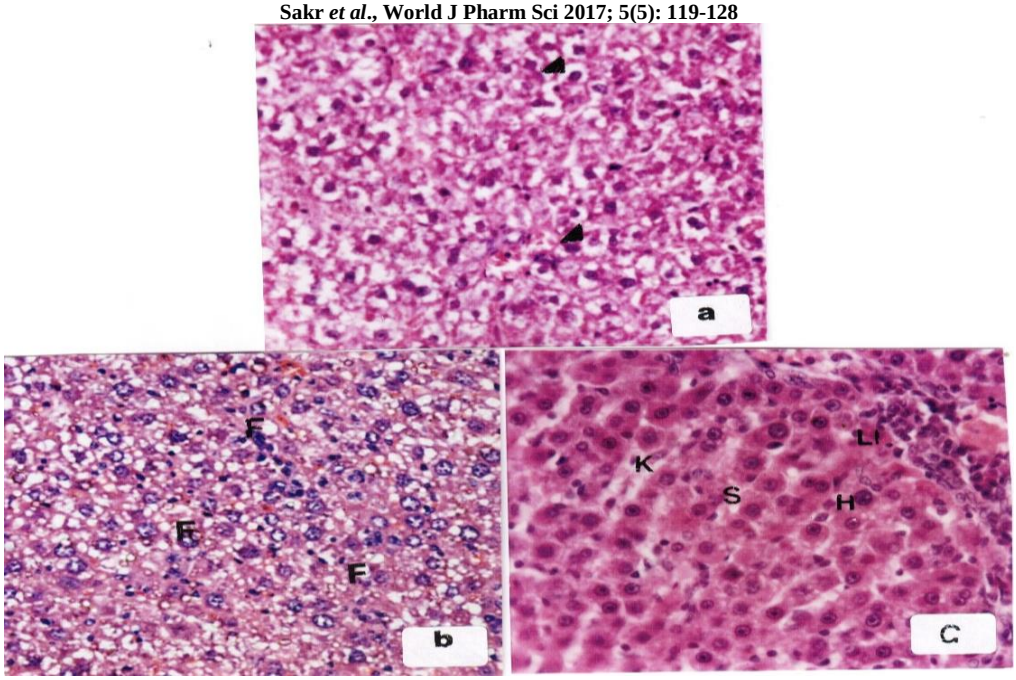

Figure (2)a: Liver section of a rat treated with cypermethrin showing loss of characteristic hepatic strands arrangement, cytoplasmic vacuolation of the hepatocytes with pyknotic nuclei (arrow head), (x 400).
Figure (2)b: Section in the liver of a rat treated with cypermethrin showing disruption of normal structure of hepatic architecture, the presence of fatty infiltrations (F), (x 400).
Figure (2)c: Liver section of a rat treated with cypermethrin and cinnamon showing restoration of normal organization of hepatic lobule, sinusoids (S) with Kupffer cell (K) between hepatocytes (H), slight leucocytic infiltrations, (x 400).

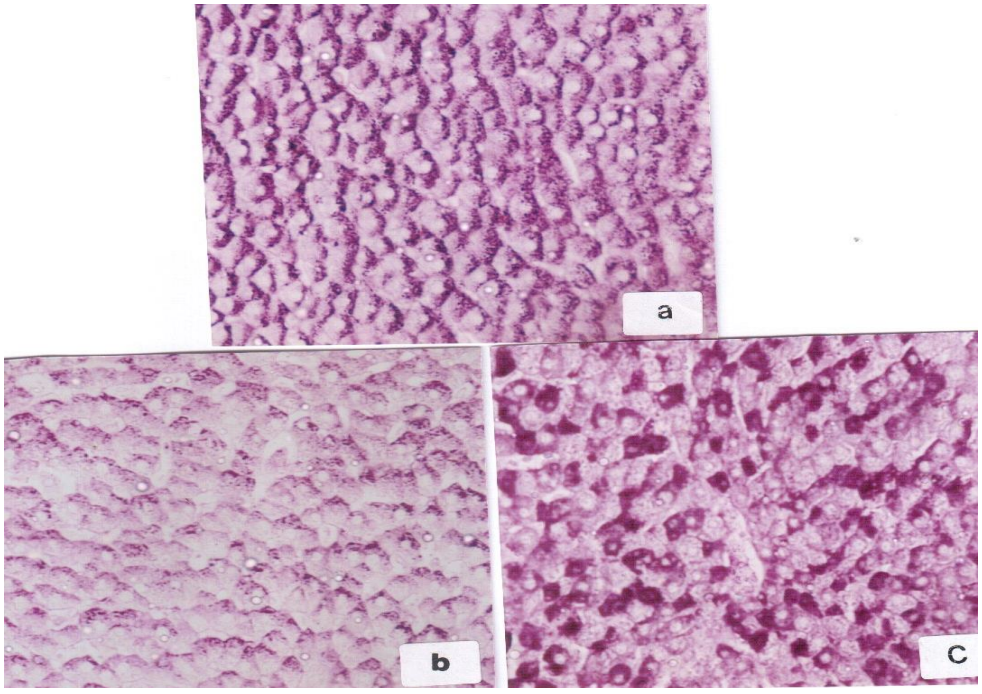

Figure (3)a: Liver section of a control rat showing strong PAS reaction in the pole of cytoplasm of hepatocytes (glycogen flight) with negative stained nuclei, (x 400).
Figure (3)b: Section in the liver of a rat treated with cypermethrin showing a decrease of the glycogen content, (x 400).
Figure (3)c: Liver section of a rat treated with cypermethrin and cinnamon showing an increase in the glycogen content, (x 400).

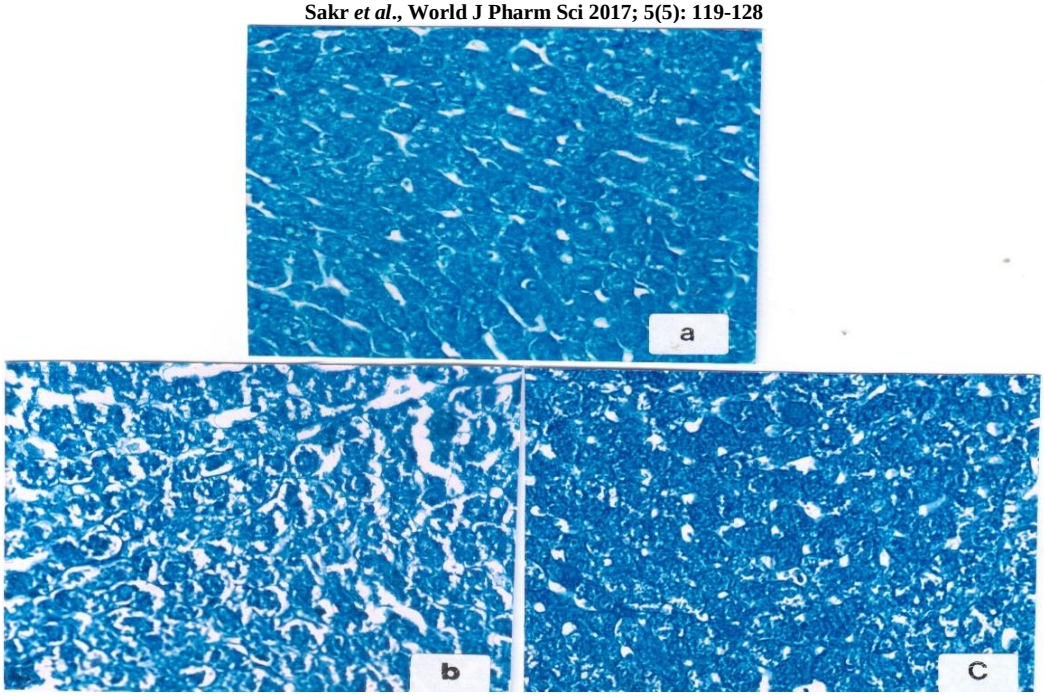

Figure (4)a: Liver section of a control rat showing normal protein content in the hepatocytes as dense blue granules in cytoplasm, cell membrane, nuclear membrane, chromatin bodies, nucleoli and Kupffer cells, (x 400).
Figure (4)b: Section in the liver of a rat treated with cypermethrin showing reduction of the protein content, (x 400).
Figure (4)c: Liver section of a rat treated with cypermethrin and cinnamon showing improvement in the protein content, (x 400).

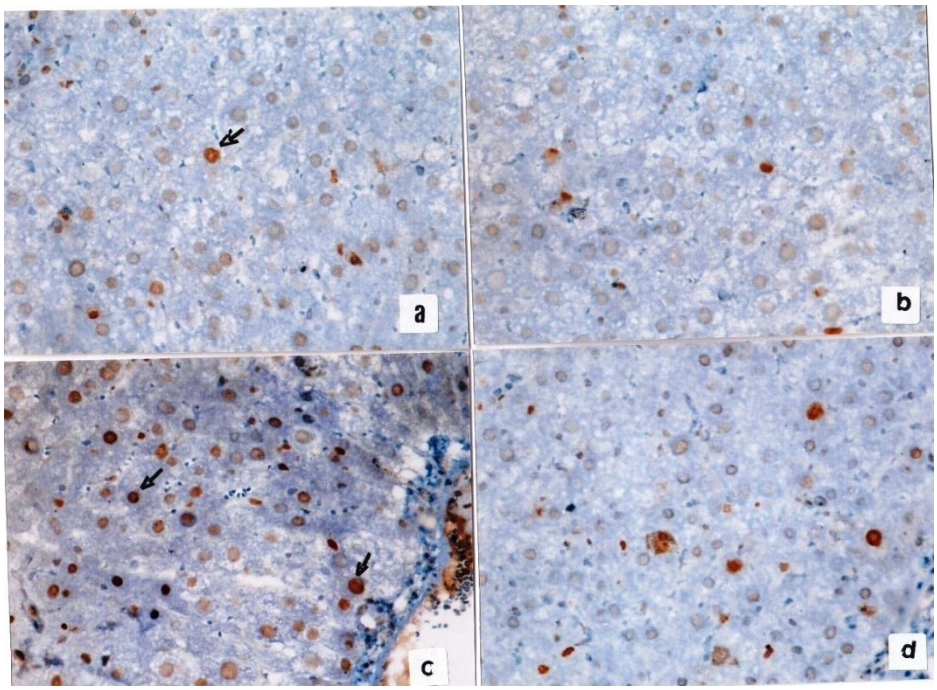

Figure (5)a. liver section of a control rat showing few expression of PCNA (arrow) (Immunostain, X 400)
Figure (5)b. Few expression of PCNA in hepatocytes of a rat treated with cinnamon (Immunostain, X 400)
Figure (5)c. liver section of a rat treated with cypermethrin showing an increase in expression of PCNA (arrows) (Immunostain, X 400)
Figure (5)d. A decrease in expression of PCNA in hepatocytes of a rat treated with cypermethrin and cinnamon, (Immunostain, X 400).

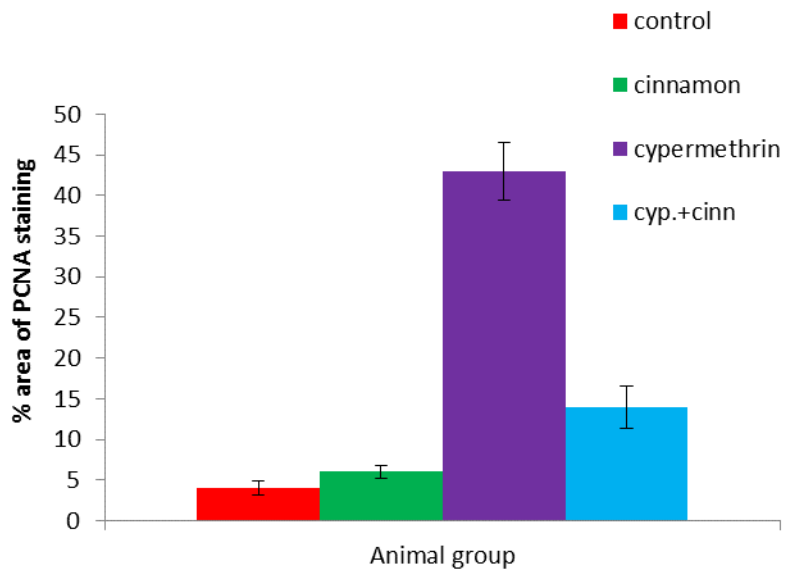

Figure 6. change in % area of PCNA staining in different animal group.

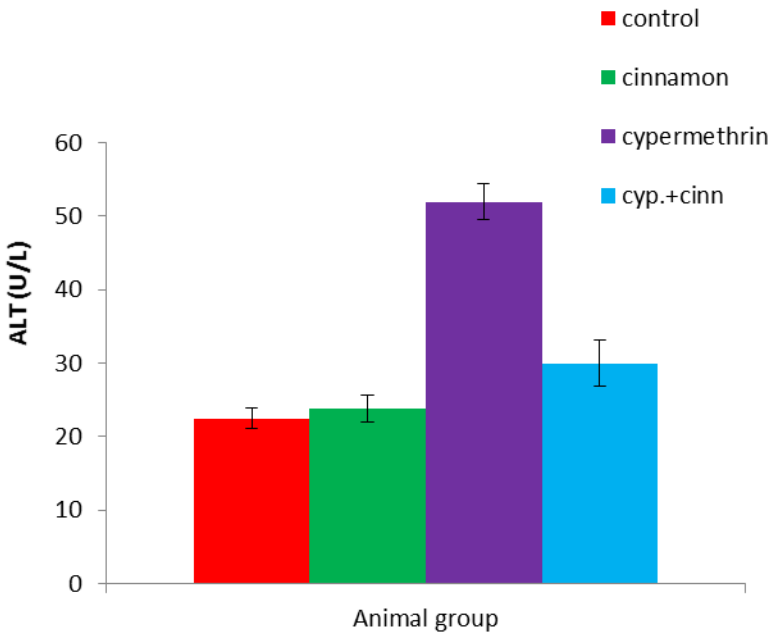

Figure 7. Effect of different treatments on serum ALT.

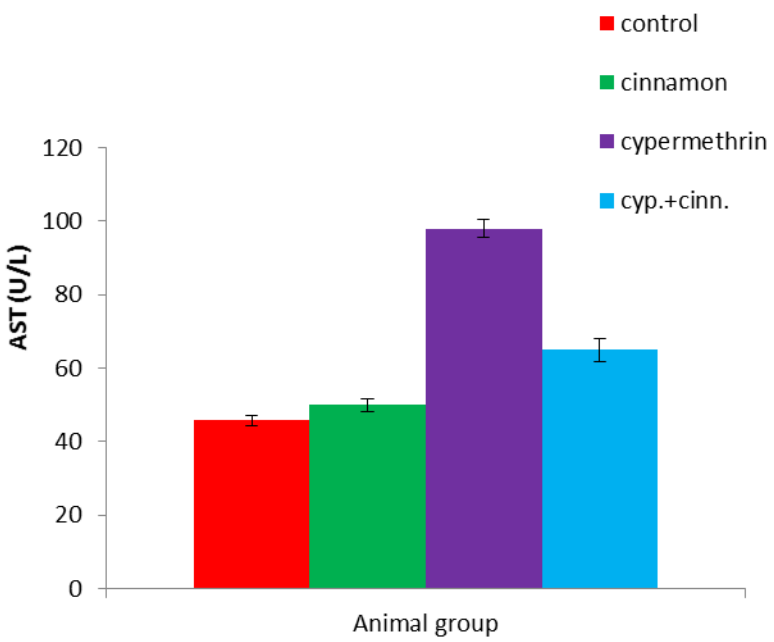

Figure 8. Effect of different treatments on serum AST.

REFERENCES

1. Tilak KS, Veeraiah K, Butchiram MS. Effect of Phenol on Haematological Components of India Major Carps Catla, Labeorohita and Cirrhinusmrigala. J. Environ. Biol., 2007; 28: 177-179.
2. Shah MK, Khan A, Rizvi F, Siddique M, Sadeequr-Rehman. Effect of cypermethrin on Clinico-hematological Parameters in Rabbits. Pakistan Veterinary Journal, 2007; 27 (4): 171-175.
3. Braun H, Frank R, Miller l. Residues of Cypermethrin in Milk from Cows Wearing Impregnated Ear Tags. Bull. Environ. Contam. Toxicol., 1985; 35: 61-64.
4. Chen S, Zhang Z, He F, Yao P, Wu Q, Sun J, Liu L, Li Q. An Epidemiological Study on Occupational Acute Pyrethroid Poisoning in Cotton Farmers. Brit. J. Occup. Med., 1991; 48: 77-81.
5. Latuszyńska J, Luty S, Raszewski G, Tokarska-Rodak M, Przebirowska D, Przylepa E, Haratym-Maj A. Neurotoxic Effect of Dermally-appliedchlorpyrifos and cypermethrin in wistar rats. Ann. Agric. Environ. Med., 2001; 8: 163-170.
6. El-Demerdash FM, Yousef MI, Al-Salhen KS. Protective Effects of Isoflavone on Some Biochemical Parameters Affected by Cypermethrin in Male Rabbits. J. Environ. Sci. Hlth. B., 2003; 38: 365-378.
7. Mamun MA, Illa IJ, Haque KMF, Ferdousi Z. Histological Study of the Effects of Cypermethrin on Liver and Kidney Tissues of Mice Model. Iosr Journal of Pharmacy and Biological Sciences, 2014; 9(5): 121-128.
8. Patel S, Pandey A, Bajpayee M, Parmar D, Dhawan A. Cypermethrin- Induced DNA Damage in Organs and Tissues of the Mouse: Evidence From The Comet Assay. Mutal. Res., 2006; 607: 176-183.
9. Gruenwald J, Freder J, Armbruester N. Cinnamon and health. Crit. Rev. Food Sci. Nutr., 2010; 50: 822-834.
10. Nir Y, Potasman I, Stermer E, Tabak M, Neeman I. Controlled Trial of the Effect of Cinnamon Extract on Helicobacter Pylori. Helicobacter, 2000; 5(2): 94-97.
11. Kamath J, Rana A, Chowdhury A. Pro-healing Effect of Cinnamon Zeylanicum Bark. Phytotherapy Research, 2003; 18(8): 970-972.
12. Lamfon N. Cinnamon Aqueous Extract Ameliorates Deltamethrin-Induced Hepatotoxicity in Albino Rats. R.J.P.B.C.S, 2014; 5(6): 898-905.
13. Tung Y, Chua M, Wang S, Chang S. Anti-inflammation Activities of Essential Oil and its Constituents from Indigenous Cinnamon (*Cinnamomum osmophloeum*) twigs. Bioresour. Technol., 2008; 99: 3908-3913.
14. Eidi A, Mortazavi P, Bazargan M, Zaringhalam J. Hepatoprotective Activity of Cinnamon Ethanolic Extract Against ccl4-Induced Liver Injury in Rats. Excli. J., 2012; 11: 495-507.
15. Sakr SA, Al-barakati AY. Effect of Cinnamon on Cypermethrin-Induced Nephrotoxicity in Albino Rats. Int. J. Adv. Res., 2014; 2(7): 578-586.
16. Kiernan JA. Histological and Histochemical Methods, Theory and Practice. Pergamon Press. New York, USA, 1981.
17. Pearse AGE. Histochemistry, Theoretical and Applied, 3rd end.,vol. 2. Churchill Livingstone. London,1972.
18. Ramos-Vara JA. Principles and methods of immunehisto-chemistry. Methods Mol Biol., 2011; 691: 83-96.
19. Reitman S, Frankel S. A colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. Am. J. Clin. Pathol., 1957; *28*, 56–63.
20. Tao TY, Wei LZ, Yang Y, Tao Z, Zhwo Y. Affects of Alpha and Theta Cypermethrin Insecticide on Transient Outward Potassium Current in Rat Hippocampal Ca3 Neurons. Pesticide Biochem. Physiol., 2008; 90: 1-7.
21. Grewal KK, Sandhu GS, Ranjitkaur RS, Sandhu HS. Toxic Impacts of Cypermethrin on Behavior and Histology of Certain Tissues of Albino Rats. Toxicol. Int., 2010; 17: 94-98.

22. Bhushan B, Saxena N, Saxena N. Biochemical and Histological Changes in Rat Liver Caused by Cypermethrin and Beta-Cyfluthrin. Arh. Hig. Rada, Toksikol., 2013; 64: 57-67.
23. Mossa AH, Abou Baker DH, Ibrahim F, El Gengaihi S, Mohafrash SM. Protective Effect of Ethanolic Extract of Grape Pomace Against the Adverse Effects of Cypermethrin on Weanling Female Rats. Evidence-Based Complementary and Alternative Medicine, 2015; Doi.Org/10.1155/2015/381919.
24. Bhatti GK, Sidhu IpS, Saini NK, Puar SK, Singh G, Bhatti JS. Ameliorative Role of Melatonin Against Cypermethrin Induced Hepatotoxicity and Impaired Antioxidant Defense System in Wistar Rats. Journal of Environmental Science, 2014; 8 (1): 39-48.
25. Sakr SA, El-Mesady FA, El-Desouky NI. Pyrethroid Inhalation Induced Histochemical Changes in The Liver of Albino Rats. The Sciences, 2002; 2(1): 24-28.
26. Shakosri AR, Ali SS, Saleem MA. Effect of Six Months Feeding of Cypermethrin on the Blood and Liver of Albino Rats. J. Biochem.Toxicol.,1988; 3: 59-72.
27. Sivaprasada K, Sombasiva KR, Ramana KV. Effect of Parathion on Tissue Ionic Changes in Fish *Channa Punctatus* Geobios. Jodhpur, 1983; 10: 60-62.
28. Marouani N, Hallegue D, Sakly M, Benkhalifa M, Ben Rhouma K, Tebourbi O. Adverse Haemato-Biochemical Effects of Chlorinated Insecticide in Adult Male Rats. Int. J. Advanced Res., 2016; 4(7): 959-967.
29. Kale M, Rathore N, John S, Bhatnagar D. Lipid Peroxidative Damage on Pyrethroid Exposure and Alterations in Antioxidant Status in Rat Erythrocytes: A Possible Involvement of Reactive Oxygen Species. Toxicol. Lett., 1999; 105:197-205.
30. Gabbianelli R, Falcioni G, Nasuti C, Cantalamessa F. Cypermethrin-Induced Plasma Membrane Perturbation on Erythrocytes from Rats: Reduction of Fluidity in the Hydrophobic Core and in Glutathione Peroxidase Activity. Toxicology, 2002; 175:91-101.
31. Manna S, Bhattacharyya D, Mandal TK, Das S. Repeated Dose Toxicity of Alfa Cypermethrin in Rats. J. Vet. Sci., 2004; 5: 241-245.
32. Kanuri G, Weber S, Volynets V, Spruss A, Bischoff S, Bergheim I. Cinnamon Extract Protects Against Acute Alcohol-Induced Liver Steatosis in Mice. J. Nutr., 2009; Doi:10.3945/Jn.108.100495.
33. Iqbal Z, Iqbal K, Mudassar M. Hepatoprotective Effect of Cinnamon on Cholesterol Induced Fatty Changes in Albino Rats. Isra M. J., 2015; 7(4): 225-227.
34. Elkomy A, Aboubakr M, Soliman A, Abdeen A, Abdelkader A, Hekal H. Paracetamol Induced Hepatic Toxicity and Amelioration by Cinnamon in Rats, Int. J. of Pharmacol. and Toxicol., 2016; 4 (2):187-190.
35. Roussel AM, Hininger I, Benaraba R, Ziegenfuss TN, Anderson RA. Antioxidant Effects of A cinnamon Extract in People with Impaired Fasting Glucose That are Overweight or Obese. J. Am. Coll. Nutr., 2009; 28:16-21.
36. Kim H, Hyun SH, Choung SY. Antioxidative Effects of Cinnamon Cassia and Rhodiola Rosea Extracts in Liver of Diabetic Mice. Biofactor, 2006; 26: 209-219.
37. Su L, Yin JJ, Charles D, Zhou K, Moore J, Yu L. Total Phenolic Contents, Chelating Capacities and Radical-Scavenging Properties of Black Peppercorn, Nutmeg, Rosehip, Cinnamon and Oregano Leaf. Food Chem., 2007; 100: 990–997.
38. Moselhy SS, Ali HH. Hepatoprotective Effect of Cinnamon Extracts Against Carbon Tetrachloride Induced Oxidative Stress and Liver Injury in Rats. Biol. Res., 2009; 42: 93-98.
39. Morgan AM, El-Ballal SS, El-Bialy BE, EL-Borai NB. Studies on the Potential Protective Effect of Cinnamon against Bisphenol A-and Octylphenol-induced Oxidative Stress in Male Albino Rats, Toxicology Reports, 2014; 1: 92-101.
40. Rice-Evans CA, Miller NJ, Papanga G. Antioxidant Properties of Phenolic Compounds. Trends Plant. Sci., 1997; 2: 2152–2159.
41. Sekher A, Pannala-Chan TS, O'brien PJ, Rice-Evans CA. Flavonoid B-Ring Chemistry and Antioxidant Activity: Fast Reaction Kinetics. Biochem. Biophys. Res. Commun., 2001; 282: 116–118.