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A Protective Effect of Propels and Vitamin C against Cypermethrin Toxicity on Liver of *Clarias gariepinus*

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Abstract: *The present study investigated the protective role of propolis (10 ppm) in comparision with vitamin C (50 mg / Kg) against the toxicity of cypermethrin (.05 and 0.1 ppm) .Exposure of Clarias gariepinus for 15 days and 30 days to cypermethrin induced histopathological changes in the liver of the fish.These changes included proliferation of hepatocytes , lymphocytic aggregation , necrosis , increase of kuffer cells, congestion of blood vessels ,migration of macrophages , nuclear pyknosis and sinusoids enlargement. These cypermethrin induced lesions were significantly improved with supplementation of propolis and /or vitamin C by reducing the harmful effects of cypermethrin on the normal structure of liver.*

Keywords: *Cypermethrin, propolis, vitamin C,hepatocytes*

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I. Introduction

Clarias gariepinus is valuable food of commercial importance(Marioghae, 1991) and it is very hardy in nature (Verma *et al*., 2011 and Kumar *et al*., 2012).Exposure of aquatic organisms to very low levels or sublethal concentration of pesticides in their environment may result in histological changes in some tissues (Rao, 2006a andVelmurugan *et al*., 2007). Liver is a suitable organ for histological study in order to determine the effect of tissue damage (Korkmaz *et al*., 2009).Several researchers have proved the effects of natural therapeutics on cypermethrin damage in fish (Koru *et al.,* 2007 and Kanbur *et al.,*2009).Kolankaya *et al*.(2002) and Sforcin (2007) believed that propolis is one of these natural agents. Propolis (bee glue) is a natural dark-coloured, resinous sticky substance produced by honey bees and it has been used since ancient times as a medicine owing to those biological properties as an antifungal, antiprotozoal, antimicrobial and antiviral agent (Sforcin *et al*., 2000 and Moreira *et al*., 2008).Ascorbic acid is an important intracellular antioxidant and it works as an antitoxic agent against pesticides (Guha *et al.,*1993) and protectaquatic species from poisoning of various hazardous chemicals including pesticides and domestic wastes (Ambali *et al.,* 2007).

II. Materials and methods

Specimen's collection and treatment manipulation

One hundred and twenty healthy fish of the Nile catfish, *Clarias gariepinus* (200-300 g) in weight were caught from the River Nile at Qena, Egypt in November , 2015. Fishes immediately were transported to the fish laboratory in the Department of Zoology, Faculty of Science, South valley University.The experimental fishes were reared in aerated glass tanks (160 L capacity) and divided into 12 groups (10 fish /tank) and acclimatized for two weeks before being used in the experimental study. The experimental fish fed pellets at a rate of 3% of wet weight twice daily. The water temperature, pHand dissolved oxygen (DO) concentration were measured daily (19±.2 ºC, 7.5±.21 pH and 7.18±.1 mg L-1 DO).

Preparation of propolis extractive solution.

Propolis was collected from a farm , then it was dissolved to 30 % in ethanol (Mani *et al.,* 2006). Obtained solution was protected from light and moderately shaken for 1 day at room temperature. Also, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until using (Mani *et al.,* 2006). According to Talas and Gulhan (2009) 10ppm of propolis will used.

Experimental design

Fishes were weighed, measured and classified randomly into 12 groups (10 fish/tank) according to doses of cypermethrin, Propolis, vitamin C and their combinations (Table 1). The diets were pelleted after addition of vitamin C and propolis doses for the treated groups and the addition of suitable amounts of molasses and water. The diets were dried at room temperature and stored in small bags for fish feeding.

Stock solution (0.05 ppm and 0.1 ppm) of cypermethrin was prepared and stored in clean glass bottles . Such doses was chosen according to levels monitored by Akinrotimi *et al*. (2012). Cypermethrin doses were prepared and added constantly to the aquarium for four weeks. The test water was replaced daily with the required amount of stock solution to prevent deterioration of water quality and replenish cypermethrin levels. Propolis was added to the diet in concentration of 10 ppm.Dose response of propolis was described previously by Talas and Gulhan (2009) . Also, vitamin C was supplemented in 50 mg/kg such vitamin C concentration was chosen according to levels monitored by Vani *et al*.(2011).

Histological and Histopathological examination

For microscopic preparations, after intervals of 15 and 30 days, 4 surviving fish of each group were removed and sacrificed. Small pieces of the liver, were taken and immediately fixed in 10% neutral buffered formalin.Fixed tissues were processed routinely for paraffin embedding technique. Embedded tissues were sectioned at 7µ in thickness and then stained by the following stains: Harris's hematoxylin and eosin stain (HE) (Bancroft and Steven, 1982).

Table 1.The fish groups exposed to cypermethrin (ppm) and propolis (10 ppm) and vitamin C(50 mg/kg) and their combinations.

Treatments	C	VC	PRO	+VC PRO	CYP1	CYP1+ VC	CYP1+ PRO	CYP1+PRO+VC	CYP2	CYP2+VC	CYP2+ PRO	CYP2+ PRO+ VC
cypermethrin (ppm)	0	0	0	0	0.05	0.05	0.05	0.05	0.1	0.1	0.1	0.1
Vitamin C (mg/kg)	0	50	0	50	0	50	0	50	0	50	0	50
Propolis (ppm)	0	0	10	10	0	0	10	10	0	0	10	10

C= control, VC= vitamin C, PRO= propolis and CYP= cypermethrin doses

III. Results

Control liver

The liver of the control fish *Clarias gariepinus* appears as a continuous mass of hepatic cells (hepatocytes) that exhibit cord-like pattern interrupted by blood vessels and sinusoids. The cords of hepatocytes are arranged around the central vein. The hepatocytes are large in size, polygonal in shape with centrally located nuclei. The hepatocytes have homogenous eosinophilic cytoplasm. The sinusoids are seen as communicating channels occupied by blood cells (Fig.1a)

Treatment of fish *Clarias gariepinus* with (10ppm) propolis and/or (50 mg/kg) vitamin C .

Histological examination of liver sections of fish administered propolis and /or vitamin C for 15 or 30 days of exposure showed normal hepatic cells with numerous blood sinusoids similar to the control (Figs.1b, 1c ,1d).

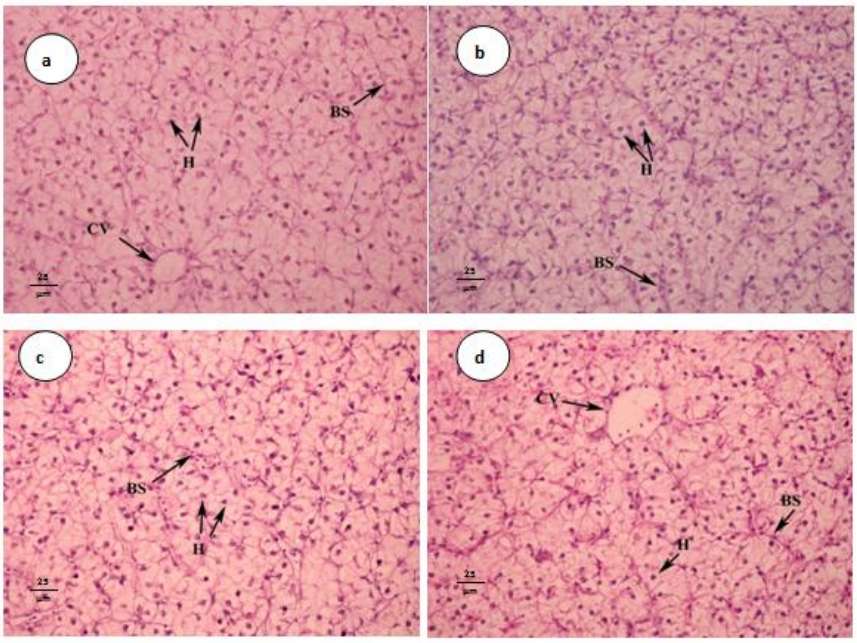

Fig.1. Sections of fish liver for 30 days showing the general structure of the liver ,Blood sinusoids(BS),central vein (CV),hepatocytes (H).(HE. 400 X).(a)control,(b) propolis treatment,(c)vitamin C treatment (d) propolis and vitmin C treatment.

Treatment of fish *Clarias gariepinusv*with 0.05 ppm and 0.1 ppm cypermethrin.

Examination of liver sections after exposure to .05 ppm cypermethrin for 15 days showed that hepatocytes lost their normal polygonal shape .The lymphocytic aggregation was observed close to dilated blood vessel with congestion of blood vessels .Marked necrosis among the hepatic tissue and hypertrophy of kupffer cells were also observed (Fig2a) .After 30 days of exposure to the same dose of cypermethrin ,marked hepatocytes delimited by ruptured cell membrane with marked proliferation of hepatocytes.Thickening in the wall of some blood vessels and lymphocytic aggregation close to the blood vessels .There was also hypertrophy in kupffer cells (Fig2b).

Examination of liver sections after exposure to 0.1 ppm cypermethrin for 15 days showed that hepatocytes lost their normal polygonal shape. Thickening in the wall of some blood vessels and melanomacrophges were observed close to the blood vessels, Also marked proliferation was observed (Fig2c).After 30 days of exposure to the same dose of cypermethrin.Marked necrosis among the hepatic tissue was observed. Thickening in the wall of some blood vessels ,lymphocytic aggregation and melanomacrophges close to the blood vessels were observed (Fig2d).

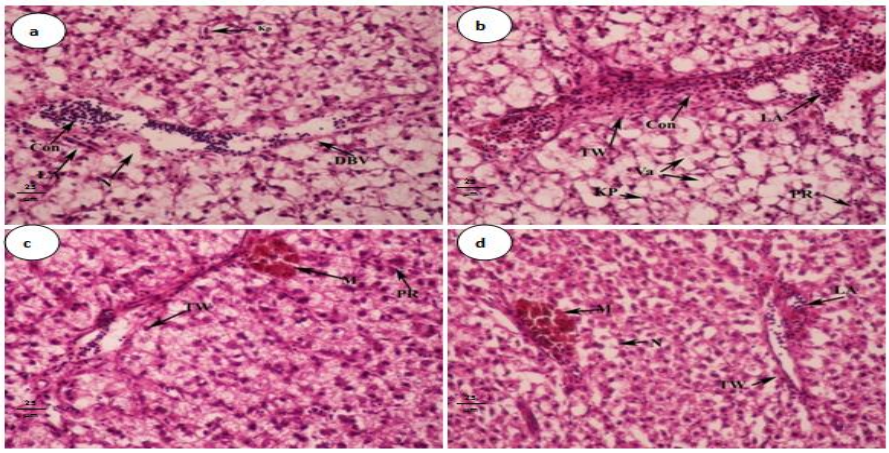

Fig.2. Sections of treated fish liver exposed to (.05 ppm and 0.1 ppm) of cypermethrin.(a) Exposure to .05 ppm of cypermethrin for 15 days showing deliated central vein lining (RCV) with blood congestion (Con) in central vein (CV) , lymphatic aggregation (LA) beside the central vein (CV) , necrotic zone (N),hypertrophy of küpffer cells (Kp) and thickened wall (TW) of blood vessel.
(b) Exposure to .05 ppm of cypermethrin for 30 days showing lymphocytic aggregation (LA) ,vacuolation (Va) ,prolifetration (PR) and thickened wall (TW) of blood vessel
.(c) Exposure to 0.1 ppm of cypermethrin for 15 days showing melanomacrophages (M) in the hepatic tissues,prolifetration(PR), and thickened wall (TW) of blood vessel.
(d) Exposure to 0.1 ppm of cypermethrin for 30 days showing lymphocytic aggregation (LA) ,melanomacrophages (M) in the hepatic tissues,necrosis(N) and thickened wall (TW) of blood vessel.

Treatment of fish *Clarias gariepinus* with (0.05 ppm and 0.1 ppm) cypermethrin plus 10 ppm propolis.
After 15 days of exposure to 0.05 ppm cypermethrin plus propolis. Marked necrosis among the hepatic tissue was observed with vaculation in hepatocytes .Also hepatocytes showed pyknosis (Fig.3a) . After 30 days of exposure the same dose of cypermethrin plus propolis hepatocytes lost partly their normal shape but nuclei still clear. Also marked proliferation and necrosis in liver tissue were observed (Fig.3b). After 15 days of exposure to 0.1 ppm cypermethrin plus propolis.The liver tissue began to return to the normal structure with some necrosis(Fig.3c). After 30 days of exposure the same dose of cypermethrin plus propolis ,marked hepatocytes delimited by ruptured cell membrane and congestion in blood vessels (Fig.3d).

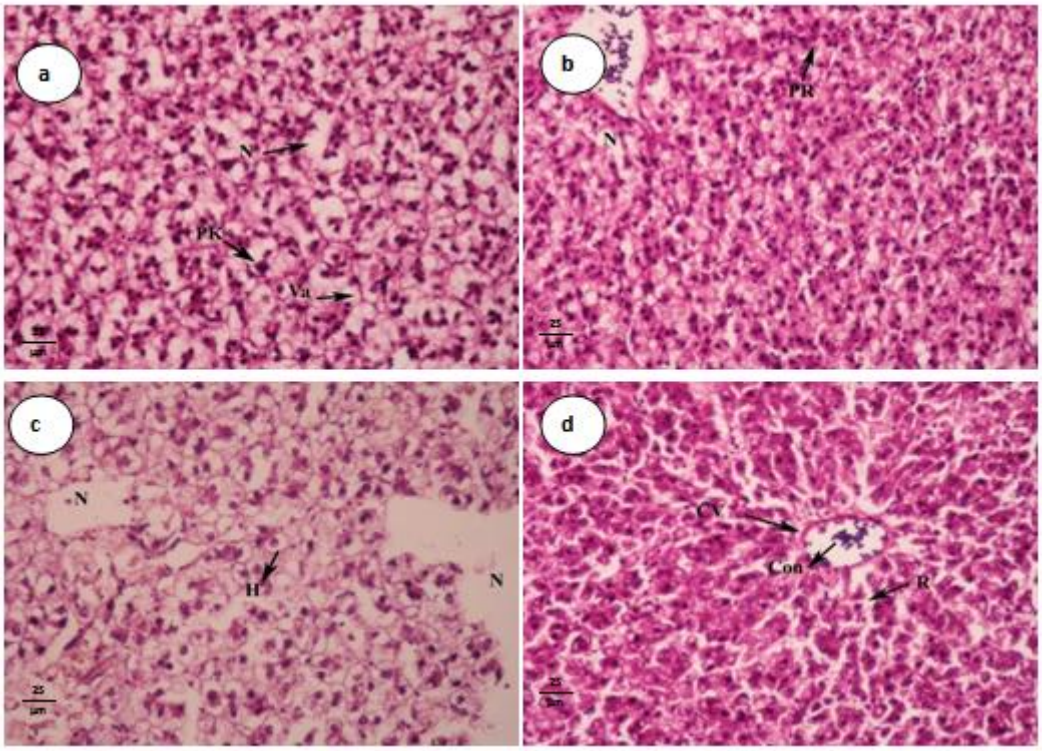

Fig.3. Sections of treated fish liver exposed to (.05 ppm and 0.1 ppm) of cypermethrin plus propolis. (a) Exposure to .05 ppm of cypermethrin plus 10 ppmpropolis for 15 days showing hepatocytes delimited by vacuolation (Va) ,pyknosis (PK) and necrosis (N).
(b) Exposure to .05 ppm of cypermethrin plus 10 ppmpropolis for 30 days showing showing improvement with necrosis (N) and proliferation (PR) of hepatocytes.
(c) Exposure to 0.1 ppm of cypermethrin plus 10 ppmpropolis for 15 days showing showing improvement with histological changes in the hepatic tissues such as necrosis (N)..
(d) Exposure to 0.1 ppm of cypermethrinplus 10 ppmpropolisfor 30 days showing improvement with histological changes in the hepatic tissues ,congested blood (Con) and rupture of hepatocytes(R).

Treatment of fish *Clarias gariepinus* with 50 mg/kg vitamin C plus 0.05 ppm and 0.1 ppm cypermethrin.

After 15 days of exposure to 0.05 ppm cypermethrin plus vitamin C ,the liver tissue showed improvement with little necrosis and pyknosis (Fig.4a) .After 30 days of exposure the same dose of cypermethrin plus vitamin C,liver tissue showed well improvement.A clear nuclei and cytoplasm and blood sinusoids were observed like normal hepatocytes with little congestion in blood vessels(Fig.4b).

Examination of liver sections after exposure to 0.1 ppm cypermethrin plus vitamin C for 15 days showed improvement with necrosis.Hepatocytes shape near to be as those in control (Fig.4c) . After 30 days of exposure the same dose of cypermethrin plus vitamin C,liver tissue showed well improvement with clear nuclei , cytoplasm ,blood sinusoids and normal hepatocytes (Fig.4d) .

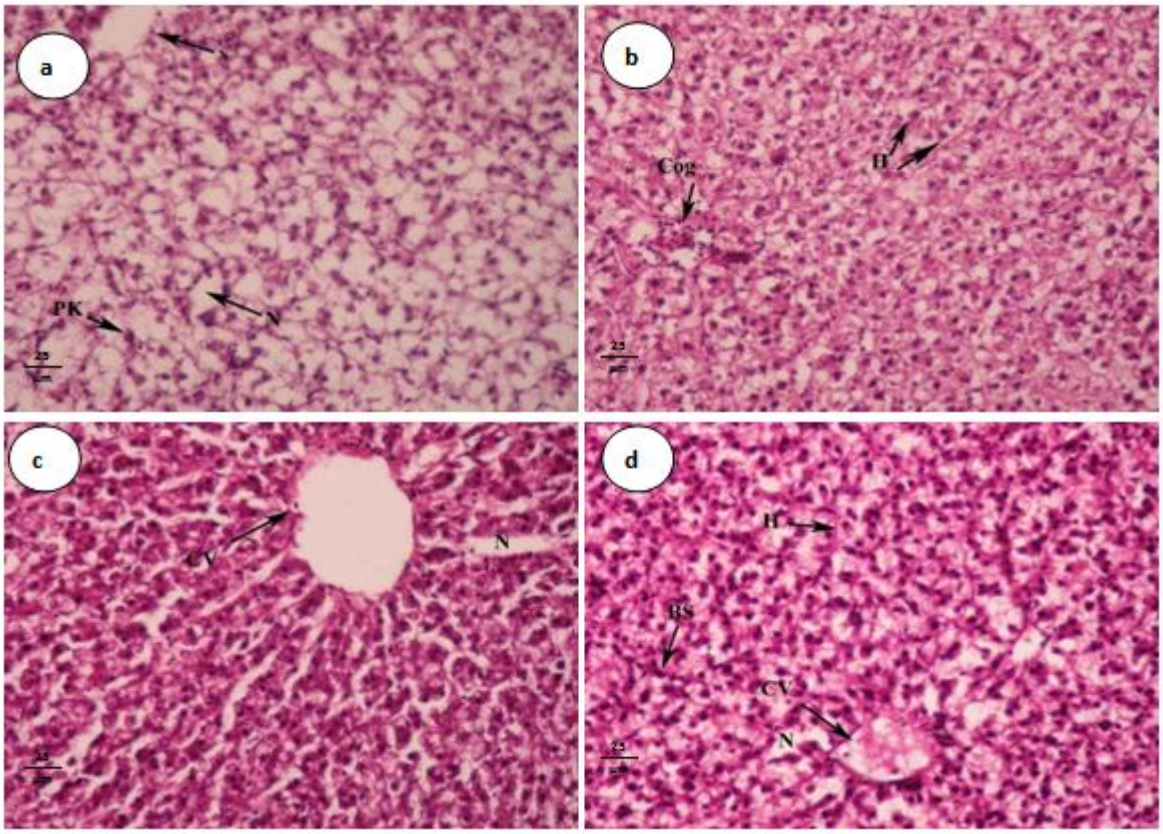

Fig.4. Sections of treated fish liver exposed to (.05 ppm and 0.1 ppm) of cypermethrin plus vitamin C. (a) Exposure to .05 ppm of cypermethrin plus 50 mg/kg vitamin C for 15 days showing improvement with histological changes in the hepatic tissues aspyknosis (PK) and necrosis (N)..
(b) Exposure to .05 ppm of cypermethrin plus 50 mg/kg vitamin C for 30 days showing less normal structure of the liver (CV) central vein with little congestion(Cog) and polygonal hepatocytes (H).
(c) Exposure to 0.1 ppm of cypermethrin plus 50 mg/kg vitamin C for 15 days showing improvement with histological changes in the hepatic tissues. thickened wall (TW) of blood vessel, lymphocytic aggregation (LA) , küpffer cells (K) and necrosis (N).
(d) Exposure to 0.1 ppm of cypermethrin plus 50 mg/kg vitamin C for 30 days showing less normal structure of the liver (CV) central vein , Blood sinusoids (BS) and polygonal hepatocytes (H).

Treatment of fish *Clarias gariepinus* with (0.05 ppm or0.1 ppm) cypermethrin plus 10 ppm propolis and 50 mg/kg vitamin C.

After 15 days of exposure to 0.05 ppm cypermethrin plus propolis and vitamin C, liver tissue showed necrosis and congestion in blood vessels with melanomacrophages close to blood vessels (Fig.5a). After 30 days of exposure the same dose of cypermethrin plus propolis and vitamin C, liver tissue showed improvement but little necrosis still present (Fig.5b). After 15 days of exposure to 0.1 ppm cypermethrin plus propolis and vitamin C, liver

tissue showed proliferation and vaculation in hepatocytes with little congestion in blood vessels (Fig.5c). After 30 days of exposure the same dose of cypermethrin plus propolis and vitamin C, liver tissue showed proliferation and pyknosis with congestion of blood vessels (Fig.5d).

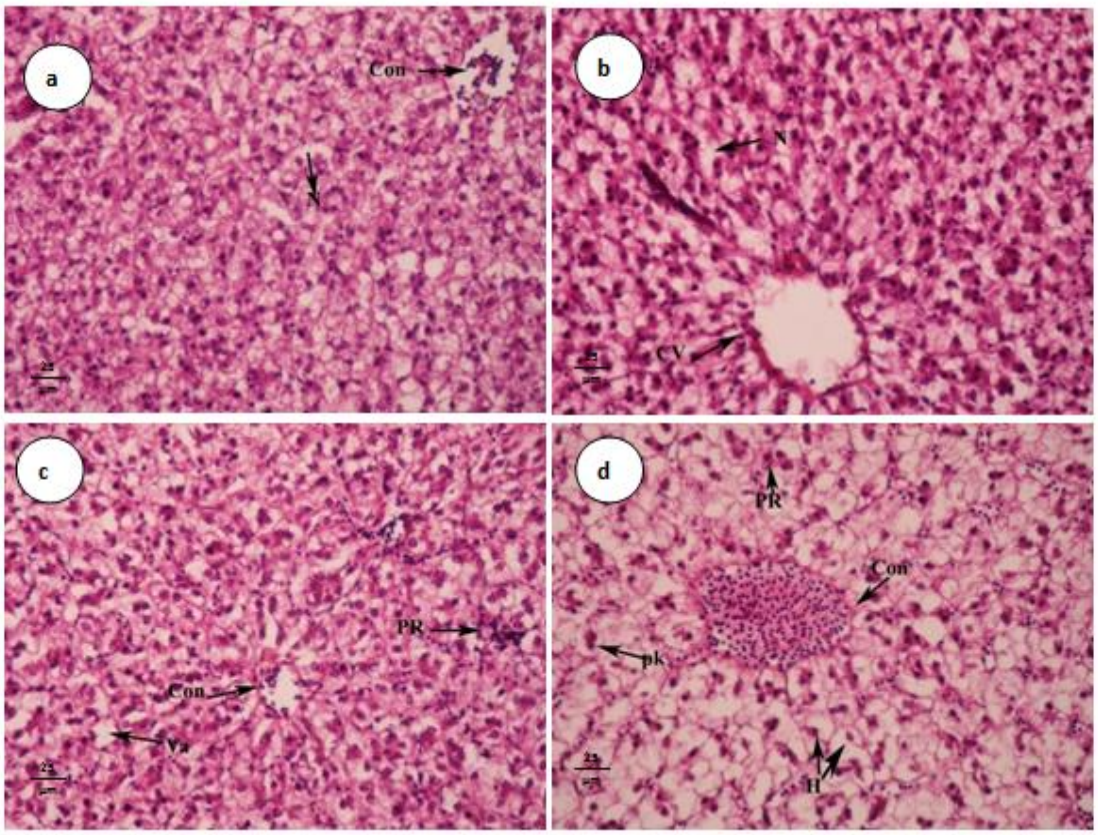

Fig.5. Sections of treated fish liver exposed to (.05 ppm and 0.1 ppm) of cypermethrin plus propolis plus vitamin C. (a) Exposure to .05 ppm of cypermethrin plus10 ppm plus 50 mg/kg vitamin C for 15 days showing improvement with histological changes in the hepatic tissues, congested blood (Con), melanomacrophages (M) and necrosis (N).
(b) Exposure to .05 ppm of cypermethrin plus10 ppm plus 50 mg/kg vitamin C for 30 days showing less normal structure of the liver (CV) central vein with little necrosis (N) of heptocytes .
(c) Exposure to 0.1 ppm of cypermethrin plus10 ppm plus 50 mg/kg vitamin C for 15 days showing congested blood (Con) ,vacuolation (Va) and proliferation (PR).
(d) Exposure to 0.1 ppm of cypermethrin plus10 ppm plus 50 mg/kg vitamin C for 30 days showing congested blood (Con) ,pyknosis (PK) and proliferation (P

IV. Discussion

The present study showed hepatic lesions including vacuolization , nuclear pyknosis,hydropic degenerations, sinusoids enlargement ,hemorrhage ,focal necrosis , increase of kuffer cells ,circulatory disturbance ,narrowing of sinusoids and congestion. Similar results were obtained by Ojutiku *et al* .(2014); Saravanan *et al.*(2016) and Arslan *et al.*(2017) after exposure of *Clarias gariepinus,Esomus danricus* and *Cyprinus carpio* to cypermethrin, respectively. Similar results were obtained by Ezemonye and Ogbomida (2010) after exposure of *Clarias gariepinus* to Gammalin 20.

Pacheco and Santos (2002) described increased vacuolization of the hepatocytes as a signal of degenerative process that suggests metabolic damage ,possibly related to exposure to contaminated water.In the present study the disruption or dissolution of normal arrangement of the hepatocytes was observed .This result was reported by Hacking *et al.*(1978).In the present investigation the liver of *Clarias gariepinus* treated with cypermethrin showed cellular degeneration followed by necrosis .Similar results were observed by Kadry (1989) after exposure of *Clarias lazera* to cypermethrin . In fish liver, the presence of necrosis area is also related with xenobiotic concentration

during the detoxifying process. Ayoola (2009) and Olufayo and Alade (2012) reported that necrosis of some areas in the liver tissue were probably resulted from the excessive work required by the fish to get rid of the toxicant during the process of detoxification by the liver.

The present study showed pyknosis position of nuclei, hypertrophy of hepatocytes,increase of kuffer cells , necrosis in the liver , and lymphocytic aggregation. These results were obtained by Fanta *et al*.(2003)on *Corydoraspaleatus* exposed to organophosphate pesticides,Bernet *et al.(*2004)on *Salmo trutta* exposed to waste water ,Sakr and Al-lail (2005) on *Clarias gariepinus* exposed to fenvalerate ,Cengiz and Unlu (2006)on *Gambusia affinis* exposed to deltamethrin ,Velmurugan *et al .(*2007) on *Cirrhinus mrigala* exposed to lambda-cyhalothrin ,Glover *et al*.(2007)on *Salmo salar* exposed to endosulfan . Increasing in the number and size of küpffer cells which attached to the walls of the sinusoids (phagocytic cells of the liver) and participate (with the spleen) in the removal of spent erythrocytes and other particulate debris from circulation were observed (Hampton *et al*., 1989).Similar findings were observed in the present study and confirmed by Kadry (1989) on *Clarias lazera* exposed to cypermethrin . Mahmoud (1999) attributed the hyperplasia of küpffer cells to the increased phagocytic activities of these cells as a defensive response against any foreign material in the circulating blood while Olojo *et al*. (2005) suggested that the function of the küpffer cells will be impeded due to the clogging up of the sinusoids. This makes old erythrocytes to accumulate in the blood. These erythrocytes are weak and their binding power to oxygen is seriously reduced such that not enough oxygen is taken into the body system.The treated fishes showed migration of macrophages into the liver tissue after exposure to cypermethrin. Similar result was observed by Figueiredo-Fernandes *et al*.(2006) and Ada *et al*.(2012) in Nile tilapia, *Oreochromis niloticus* liver under sub-lethal paraquat exposure and Whabi and El-Greisy (2007) on *Siganus rivulatus* exposed to waste sources.The migration of macrophages and removal of damaged cell components as well as necrotic hepatocytes represented adaptive rather than degenerative features (Oulmi *et al*., 1995).

The present results demonstrated the protective effect of propolis and vitamin C against histolopathological changes in the liver treated with cypermethrin. Similar results were obtained by Fuat Gulhan *et al*.(2014) who reported that the treatment by propolis having antioxidant properties were observed in damaged tissues of fish by different concentrations of cypermethrin. Korkmaz *et al*. (2009) studied 0.22 and 0.44 µg/l cypermethrin + control diet, 0.22 and 0.44 µg/l cypermethrin + ascorbic acid supplement diet for 20 days in Nile tilapia. These results exhibited that ascorbic acid may serve as an antitoxic agent against cypermethrin toxicity in fish.

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