Ameliorative effect of vitamin C on cypermethrin-induced hepatotoxicity and renal malfunction of adult male rats.

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Abstract

Cypermethrin is a widely used insecticide belongs to type II pyrethroids. It is highly used in developing countries to control many species of insects as it plays an important role in pest control. Vitamin C is an important intracellular antioxidant against insecticides. The aim of the present study is to analyze the toxic effects of cypermethrin (CYP) and the positive effect of vitamin C (VC) with graded doses (0 CYP, 200 VC, 12 CYP and 12 CYP+200 VC mg/kg body weight of male rats/day) for 30 days on liver and kidney functions. Moreover, antioxidant enzymes, oxidative stress markers, glycogen and glucose levels and also serum lipid profile were studied. Also, this study aimed to evaluate the possible protective role of vitamin C against cypermethrin toxicity.

Keywords: cypermethrin, vitamin C, antioxidant parameters, liver enzymes, kidney function

1.   Introduction

Pyrethroid is a group of highly potent lipophilic insecticides and is broadly divided into two types: Type 1 pyrethroids affect the sodium channels in nerve membranes, opening them for relatively short periods. However, Type II pyrethroids produce a longer delay in sodium channels inactivation, leading to persistent depolarization of the nerve membrane without repetitive discharge (Lamberth et al., 2013).

Studies on pyrethroid I and II and first generation synthetic pyrethroids showed low mammalian toxicity. A large amount of the administered dose was excreted in an unmetabolized form, but the bioavailable dose is metabolized easily in mammals through cleavage by esterases and cytochrome P450 mixed function oxidases in liver or plasma (Manna et al., 2004 and Anand et al., 2006). Previous studies have reported data indicating that these pyrethroids have induced oxidative stress via the generation of free oxygen radicals. Huang et al., (2007) has indicated that abnormal production of free radicals leads to damage of some macromolecules, including lipid, proteins, and nucleic acid, this is believed to be included in the etiology of many chemicals and diseases.

Cypermethrin belongs to type II pyrethroids. These groups of pyrethroids are potent neuropoisons, endocrine disruptors that can cause paralysis (Saxena and Saxena, 2010). Cypermethrin has important role in agriculture, protection of food stuff, disease vector control and home pest control (Sankar et al., 2011). It is greatly used as insecticide in developing countries to control many species of insects. It is reported that human exposure to cypermethrin mainly occurred during application or from pyrethroids residues such as those detected in fruits, vegetables, caws milk and bread (Sankar et al., 2010). The toxicity of cypermethrin depends on the starting doses, time and routes of exposures. It causes toxicity on different levels starting from the biochemical to anatomical and molecular ones (Singh et al., 2012).

Cypermethrin induces neurotoxicity and motor deficits. The oxidative stress is implicated in the cypermethrin-mediated neurotoxicity. The most contributors of oxidative stress are excessive production of reactive nitrogen species and reactive oxygen species in the cells or tissues exposed to cypermethrin or decreased level of components of the antioxidant machinery (Tiwari et al., 2010; Sharma et al., 2014). In addition, cypermethrin lead to DNA damage and reduces mitotic and nuclear divisions (Kocaman and Topaktas, 2009; Hussien et al., 2013). Cypermethrin is absorbed primarily from gastrointestinal tract and may be also absorbed by inhalation of spray mist and simply through the intact skin. Because its lipophilic nature, cypermethrin accumulates in body fat, skin, kidney, liver, brain, adrenal glands and ovaries (Tao et al., 2008).

Liver is connected with metabolism and elimination of toxic material from body so changes in its biochemical parameters are a clue to observe oxidative stress and its reversal by antioxidant. The rise in levels of AST and ALT give a good overview of hepatic damage (Abdou et al., 2012).

Respecting to the kidney, it is known that the elevation of serum urea and creatinine is considered as a significant marker of renal dysfunction. High levels of urea in the blood is correlated either with an increased protein catabolism in the mammalian body or from a more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production (Matsumoto et al., 2019).

Manna et al., (2006) observed significant elevation in the activities of ALT, AST, ALP, LDH and glucose level in Wister rats given consecutive daily oral dose of 14.5 mg kg^{-1} body weight cypermethrin for 60 days. In addition, Eraslan et al., (2008) demonstrated significant elevation in the levels of serum glucose and uric acid as well as significant increases in the activity of ALP in Wister rats treated with a single dose of 125 mg kg^{-1} body weight cypermethrin. Moreover, treatment with cypermethrin caused a significant increase in urea, and uric acid. Serum creatinine decreased after acute treatment but increased after 14 and 21 days treatment (Saxena and Saxena, 2010).

Vitamin C (VC) is a water-soluble vitamin that scavenges free radicals (Gaziano et al., 2010). It is an antioxidant and consequently potentially involved in protecting cells against oxidative stress (Annae and Creppy, 2001). It is involved in synthesis of collagen, carnitine, and epinephrine, absorption of dietary iron and mobilization of storage iron for erythropoiesis. Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions. The principal pathway of ascorbic acid metabolism involves the loss of two electrons (Arrigoni and De Tulio 2002). It is also widely used as a food additive to prevent oxidation (Padayatty et al., 2003). VC is a cofactor in at least eight enzymatic reactions, including several collagen synthesis reactions that -when dysfunctional- cause the most severe symptoms of scurvy. In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries.

Thus, the aim of the present work is to study the possible effect of various doses of cypermethrin on liver enzymes, kidney functions, lipid profile, glucose and glycogen. Also to investigate the effect of cypermethrin on specific biochemical parameters including lipid peroxidation products, thiobarbituric acid, nitric oxide and activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in liver and kidney. Also to determine the histopathological alterations in liver of male rats and the protective role of VC against such pesticide.

2. Materials and methods

2.1.   Experimental animals

Forty adult male rats (200-250 gm) were obtained from the Medical Technology Center, Medical Research Institute, Alexandria University, Egypt. Animals were housed in plastic cages at an environmentally controlled room (temperature 25-27 ^{0}C, 12h light/dark cycle) for two weeks prior to starting the experiment for adaptation with laboratory conditions and they were provided with tap water and standard rat diet (protein 24%, fat 5%, fiber 4%, carbohydrates 55%, calcium 0.6%, moisture 10% and ash 9%).

2.2.   Chemicals

Cypermethrin was purchased from Faculty of Agriculture, Cairo University. Egypt. VC was purchased from Elhekma Pharma in the city of 6th of October, Egypt.

2.3.   Experimental design

Rats were randomly divided into 4 equal groups as follows: the first group was used as a control and was administrated with distilled water orally. In the second group, VC solution was dissolved in distilled water and administrated at a dose of 200 mg/kg b.w (Aksoy et al., 2005). In the third group, cypermethrin was administrated at a dose of 12mg/kg b.w (Abdou et al., 2012).The fourth group was used to study the effect of the combination of VC with cypermethrin. Animals were treated every day for 30 days. The proper doses of cypermethrin and VC for each animal were applied using a syringe that was inserted orally through stomach tube.

At the end of the experiment. The animals were sacrificed by cervical decapitation and dissected. Liver and kidney were sampled, cleaned from adhering matters, washed with saline solution, blotted and weighted. A portion of liver and kidney from each rat were processed immediately and homogenized (Goldberg and Spooner, 1983) for the biochemical analysis and determination of enzymatic parameters.

2.4.   Blood collection

The blood was collected in heparinized tubes. The heparinized blood samples were used to determine the count of erythrocytes, leukocytes and platelets and also the hematocrit and hemoglobin content.

The remaining non-heparinized blood was allowed to clot in a centrifuge tube and the serum was separated from blood cells by centrifugation at 8000rpm for 5 min. The serum was separated and stored at -20 ^{0}C until biochemical parameters assay.

2.5.   Determination of serum biochemical parameters

For liver functions, alanine transaminase (ALT) (Tietz, 1976), aspartate transaminase (AST) (Tietz, 1976), alkaline phosphatase (ALP) (Rosalki et al., 1993), acid phosphatase (ALP) (Bessoy et al., 1946), lactate dehydrogenase (LDH) (Henry, 1974), globulin (Wells et al., 1985), total bilirubin (Young, 2001), total protein (Gornall et al., 1949) and glycogen (Nicholas et al., 1956) were determined.
For kidney functions, creatinine (Bartels et al., 1972), urea (Fawcett and Soctt, 1960), uric acid (Fossati et al., 1980), cholesterol (Roeschlau et al., 1974 and Allain et al., 1974), high density lipoprotein (HDL –c) (Burtis et al.,2005) and low-density lipoprotein (LDL – c) (Burtis et al., 2005) were assayed.

−   Determination of glucose, glycogen and lipid profile including total cholesterol, triglyceride (TG), high density lipoprotein (HDL-c)and low density lipoprotein (LDL-c)

Glucose was determined according to the method described by Trinder, 1969), which based on the following reaction

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} H_2O_2 + \text{Gluconic acid}$$
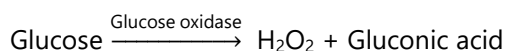
Moreover, glycogen was evaluated using kits as described by **(Nicholas et al., 1956).** Glycogen is broken down into glucose monomers by amyloglucosidase first, glucose is then oxidized by glucose oxidase into D-gluconic acid and hydrogen peroxide.

2.6.   Determination of some enzymatic antioxidant activities
Superoxide dismutase (SOD) **(**Nishikimi et al., 1972), nitric oxide (NO) homogenates (Moshage et al., 1995) and thiobarbituric acid reaction (TBARS) (Okawa et al., 1979) were determined in kidney and liver. Also, protein content (Bishop et al., 2000), calcium (Thomas, 1998), sodium (Kulpmann, 1991), magnesium (Tietz, 1983), potassium (Kulpmann, 1991) and ATPase (Gonzalez-Romo et al., 1992) were assayed.
2.7.   Statistical analysis

The values are expressed as mean ±3a2 SE. the results were computed statistically using statistical package for social sciences (SPSS software package, version 15) using one –way analysis of variance (ANOVA). Post hoc testing was performed for intergroup comparison on using the LSD. $p<0.05$ was considered as significant (Howell, 1995).
3.   Results

3.1. Effect of cypermethrin, VC and their combination on the activity of some liver enzymes, liver function and kidney function.

Table (1) explains that the activities of ALT, AST, ALP, ACP and LDH in all treated groups. The treatment with cypermethrin alone induced a significantly ($p<0.05$) increase in all serum liver enzymes compared to the control group. For VC treated group, there is slightly non-significant difference in ALT, AST, ALP, ACP and LDH compared to the control group. On the other hand, for the group treated with cypermethrin in combination with VC, these is slightly a significant decrease in ALT, AST, ALP and LDH but no significant decrease in ACP was observed compared with cypermethrin -only treated rats.

Table (1): Effect of cypermethrin, vitamin C and their combination on the activity of liver serum enzymes.

Parameters of serum liver function	Experimental groups			
	Control	V C	CYP	CYP+VC
Alanine transaminase (ALT) μ/L	34.60±1.10[b]	34.20±1.06[b]	69.00±3.37[a]	40.86±7.29[b]
Aspartate transaminase(AST) μ/L	70.80±6.99[bc]	65.43±6.13[c]	113.00±8.79[a]	85.57±7.16[b]
Alkaline phosphatase (ALP) μ/L	180.60±40.19[c]	186.71±61.95[c]	325.57±102.09[a]	261.71±34.51[b]
Acid phosphatase(ACP) μ/L	1.71±0.44[b]	2.66±0.19[ab]	3.41±0.58[a]	3.31±0.63[a]
Lactate dehydrogenase LD H) μ/L	584.57±157.84[b]	652.71±106.29[b]	943.20±91.69[a]	833.00±179.3[b]

Values are expressed as mean$\pm$ SE: n = 10

Means followed by different superscript in the same row are significantly different, $p<0.05$.

Table (2) shows significant ($p<0.05$) decrease in tissue liver function (AST, ALT, ALP, ACP and LDH) in cypermethrin treated group compared to the control group. For the group treated with VC alone, no significant difference in ALT, ALP and ACP was observed but there was a significant decrease in AST and LDH compared to the control group. On the other hand, the presence of VC combined with cypermethrin there was slightly significant increase in AST, ALT, ALP, ACP and LDH compared to cypermethrin treated group. Moreover, a significant ($p<0.05$) elevation in urea, uric acid and creatinin level in group treated with cypermethrin alone compared to the control group. The group treated with VC alone showed a significant decrease ($p<0.05$) in urea but significant increase in creatinin while no significant difference in uric acid level compared to the control group. On the other hand, rats treated with vitamin C in combination with cypermethrin preented significant decrease ($p<0.05$) in urea, creatinin and uric acid compared to cypermethrin -treated group

Table (2): Effect of cypermethrin, vitamin C, and their combination on tissue liver and kidney functions.

Parameters Tissue liver function	Experimental groups			
	control	V C	CYP	CYP+VC
ALT μ/mg protein	41.86±5.64 [a]	40.00±2.65 [a]	31.00±1.15[c]	36.00±2.83[b]
AST μ/mg protein	68.71±13.80 [a]	57.14±1.86[b]	40.00±1.41[c]	54.86±13.56[b]
ALP μ/mg protein	352.14±94.13[a]	320.00±118.02[a]	170.00±45.12[c]	283.86±79.17[b]
ACP μ/mg protein	2.71±0.72[a]	2.11±0.90[a]	1.21±0.33[b]	2.41±0.58[a]
LDH μ/mg protein	280.57±74.57[a]	259.14±39.82[b]	220.57±32.57[c]	250.57±48.91[b]
Urea mg/dl	28.60±2.49[c]	22.29±4.07[d]	41.20±8.64[a]	33.71±5.32[b]
Creatinin mg/dl	0.48±0.11[c]	0.54±0.06[b]	0.69±0.08[a]	0.56±0.05[b]
Uric acid mg/dl	5.63±0.68[c]	5.14±1.72[c]	10.33±1.25[a]	6.47±1.21[b]

Values are expressed as mean$\pm$ SE: n = 10

Means followed by different superscript in the same row are significantly different, $p<0.05$.

3.2.   Effect of cypermethrin, VC, and their combination on glycogen and glucose

Table (3) shows significant ($p<0.05$) increase in glucose level but significant ($p<0.05$) decrease in glycogen level in group treated   with cypermethrin compared to the control group. For the VC group a significant increase in glucose but no significant change in glycogen level was observed compared to the control group. On the other hand, the treatment of VC in combination with cypermethrin showed no significant difference in glucose and glycogen levels compared to cypermethrin treated group.

Table (3): Effect of cypermethrin, Vitamin C, and their combination on glycogen and glucose.

Parameters	Experimental groups			
	Control	**V C**	**CYP**	**CYP+VC**
Glycogen mg/dl	5.56±0.78a	5.56±0.68a	3.93±1.42b	4.51±0.74b
Glucose mg/dl	88.60±39.20c	108.43±11.94b	130.00±24.04a	115.00±4.86a

Values are expressed as mean+ SE: n = 10

Means followed by different superscript in the same row are significantly different, p<0.05.

3.3. Effect of cypermethrin, vitamin C and their combination on serum lipid profile.

Lipid profile most frequently includes measurement of total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein- cholesterol (LDL-c). Data listed in table (4) indicated that treatment with cypermethrin alone caused significant (p<0.05) increase in TC, TG, and LDL-c while the HDL-c level was decreased compared to the control group. but in group treated with VC alone observed significant increase in (TC) and (HDL-c)but no significant different in (TG) and (LDL-c) compared to control group. On the other hand, treatment with VC in combination with cypermethrin   had no significant different in (TC) but showed significant decreased in TG and LDL-c, and increased in HDL-c level compared with cypermethrin treated group.

Table (4): Effect of cypermethrin, vitamin C and their combination on serum lipid profile.

Parameters	Experimental groups			
	control	**VC**	**CYP**	**CYP+VC**
Cholesterol mg/dl	46.20±5.87[b]	51.20±6.52[a]	59.14±15.66[a]	54.71±8.20[a]
Triglycerides mg/dl	34.60±7.74[b]	31.00±2.31[b]	49.29±15.91[a]	34.43±5.68[b]
HDL mg/dl	25.00+1.91[b]	26.00±0.82[a]	15.14±0.90[c]	23.43±1.13[b]
LDL mg/dl	17.26±5.73[c]	19.16±6.41[c]	32.14±5.73[a]	23.87±8.11[b]

Values are expressed as mean+ SE: n = 10

Means followed by different superscript in the same row are significantly different, p<0.05.

3.4. Effect of cypermethrin, VC, and their combination on activities of antioxidant enzymes (SOD, CAT) and on oxidative stress markers (TBARS, NO).

The activities of superoxide dismutase (SOD) and catalase (CAT) were measured in liver and kidney of male rats treated with cypermethrin, VC and their combination. Table (5) indicated that a significant decrease in the activities of liver and kidney SOD (p<0.001 and (p<0.05) and CAT (p<0.05) levels in rats treated with cypermethrin alone compare with control groups. While, in VC treated group, there was no significant different in SOD in kidney, CAT level in liver and kidney but showed significant decreased in SOD in liver compared to control group. On the other hand VC treatment in combination with cypermethrin improves the reduction in SOD, CAT levels (p<0.05) compared to cypermethrin -only treated group.

Table (5) shows the mean values of the TBARS and NO in liver of male rats treated with cypermethrin, VC and their combination. Treatment with cypermethrin showed significant (p<0.05) increase in the content of NO and TBARS compared to control group. However, treatment with VC alone showed significant decrease in the TBARS, but no significant different in NO level in liver compared to control group. On the other hand, in group treated with VC in combination with cypermethrin observed significant decrease (p<0.05) in NO and TBARS compared to cypermethrin treated group.

Table (5) Effect of Cypermethrin, Vitamin C, and their combination on activities of antioxidant enzymes in tissues of male rats after 30 days.

Parameters	Experimental groups			
	control	VC	CYP	CYP+VC
SOD Liver µ/g. tissue	11.6±0.81[a]	8.8±0.73[b]	4.8±0.71[c]	7.0±0.64[b]
CAT Liver µ/g. tissue	4.8±0.71[a]	4.9±0.77[a]	1.7±0.63[c]	3.6±1.19[b]
TBARS Liver mol/mg. tissue	3.44±0.58[c]	2.11±0.39[d]	5.76±0.39[a]	4.67±0.35[b]
NO Liver µmol/g. tissue	3.70±0.73[c]	3.14±0.68[c]	7.74±0.71[a]	5.64±0.44[b]
SOD Kidney µ/g. tissue	8.8±0.73[a]	8.8±0.78[a]	3.8±2.11[c]	6.1±0.82[b]
CAT Kidney µ/g. tissue	3.1±0.64[ab]	3.8±0.61[a]	1.4±0.52[c]	2.6±0.29[b]

Values are expressed as mean± SE: n = 10

Means followed by different superscript in the same row are significantly different, $p<0.05$.

e bar = 200 & 50)

Discussion

Liver is the center of biotransformation and detoxification of foreign compounds and is the most vulnerable to the chemical assaults such as cypermethrin poisoning. Elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) indicate the utilization of amino acids for the oxidation or for glycogenesis and is used to determine liver damage (Newairy and Abdou, 2013; Essawy et al., 2018). The present study showed a significant increase in activities of several serum enzymes such as ALT, AST, ALP, ACP and LDH. Also, a significant decrease was detected in their activities in liver tissue in cypermethrin – treated group of rats in comparison with the control group. Such serum enzymes reflect the functional state of the liver. The change in the enzyme activity is related to cellular damage. The amplification of activity of the transaminases with the decline of the antioxidant enzymes is the sequence of cypermethrin produced injury of the liver (Manna et al., 2006).

The activity of serum alkaline phosphatase (ALP) increased in the current study, this can be attributed initially to some liver patho-physiological changes as a result of pesticide intoxication, as cypermethrin has the capability of changing the normal hepatocellular architecture as reported by Bhushan et al., (2013). Several studies have documented the increase in the acid phosphatase (ACP) level as an indicator of tissue damage. Acid phosphatase is a lysosomal enzyme and stimulated in case of tissue damage (Manna et al., 2004). In agreement with the present results, Abdou et al., (2012), found a marked increase in liver serum enzymes in male rats treated with cypermethrin at doses 12 mg/kg bw for 30 days. Goel et al., (2005) found that the elevation in liver enzymes in cypermethrin treated male rats may be due to liver dysfunction and biosynthesis disturbance of these enzymes. Grewal et al., (2010) showed sharp congestion and degenerative changes of hepatocytes after treatment with 14.5 mg/kg of cypermethrin for 30 consecutive days.

Elevation in serum level of LDH may be as a result of tissue necrosis, particularly of liver. Also, cypermethrin is reported to cause hemolytic anemia resulting in lysis of the red blood cell membrane, which leads to enhance LDH levels in serum of cypermethrin -intoxicated treated rats (Attia and Nasr, 2009; Nair et al., 2010).

These results are in accordance with those reported by Ghassemi et al., (2015) who indicated the damage occurred in the liver tissue due to deltamethrin which induced oxidative stress. Cypermethrin causes membrane lipid peroxidation and disturb the function of cell membrane; it changes membrane permeability and the function of liver antioxidant enzyme. Subsequently, this will lead to the leakage of the above enzymes from liver cytoplasm into blood flow (Bhushan et al., 2013; Soliman et al., 2014).

Cypermethrin caused oxidative stress which increased liver enzymes level in serum and decrease in tissue in cypermethrin treated group compared with the control group. In the group treated with cypermethrin +VC, there was slightly significant decrease in the activity of ALT, AST, ALP, and LDH but non-significant decrease in ACP level in serum compared to cypermethrin treated group.

Moreover, VC had an antioxidant property which scavenged free radicals while cypermethrin had potential to induce free radicals and oxidative stress **(**Hussain et al., 2012; Manal et al., 2012). Several studies showed the hepato-protective property of VC. This is related to its antioxidative property VC was reported to attenuate hepatic damage induced by some chemicals in animals. Ascorbic acid reduced cypermethrin cytotoxicity in rats hepatocytes by recovering 60% of glutathione. Ascorbic acid has also the ability to preserve 100% of cell integrity and modulates ALT and AST activities (Bashandy and Alwasel, 2011).

The influence of cypermethrin on kidney function was assessed through the measurement of urea, creatinine and uric acid. Urea is formed by the liver as an end product of protein breakdown and it is one marker of the kidney function (Tawfik and Al-Badr, 2012). Urea and creatinine concentration was significantly increased in cypermethrin -treated group compared to the control one. Such findings are in agreement with that reported in other studies (Yousef et al. 2003; Saxena and Saxena, 2010y; Sankar et al., 2012 ; Sakr and Albarakai, 2014).

Increase in serum urea detected in the current study may be due to 1) impairment in its synthesis because of impaired hepatic function, 2) disturbance in protein metabolism and 3) decrease in its filtration rate in the kidney. The decline in protein profile recorded in the present study may support this explanation. Moreover, impaired kidney function causes decrease in ability of urea excretion from the blood (Aslam et al., 2010). The increase in serum urea may be due to fast urea production from ammonia and proteins, disserved excretion of urea increased enzymes activities of urea (ornithine carbomoyl transferase, arginase), dehydration and low blood volume (Garba et al., 2007). Decreased serum proteins and the break-down of production of creatine phosphate in muscles is creatinine that is usually produced at a constant rate via the body. Creatinine is chiefly filtered out of the blood by the kidneys and has been found to be indicator of kidney function **(Tawfik and Al-Badr, 2012).**

As the kidneys become impaired in case of cypermethrin poisoning, the creatinine level in the blood will rise due to poor clearance by the kidneys. A rise in blood creatinine level is observed with damage of functioning nephrons and impaired renal function (Ambali et al., 2010). The significant increase in urea and creatinine levels noticed in this study because the kidney was affected by cypermethrin exposure.

The increased concentration of creatinine in serum might be due to the alteration in removing of creatinine via glomerular filtration through tubular secretion (Ravel, 1995). Khurshid, (2003) reported the rise in uric acid content attributed to sub lethal doses of cypermethrin in the chick. The elevation may be as a result of the increase formation or the decreased excretion. In group treated with cypermethrin +VC, it was observed a decrease in kidney function enzymes compared to cypermethrin treated group. Treatment with VC stabilizes uric acid in plasma and protects it from oxidation as reported by Patterson et al., (2003).

The current study shows a marked increase in glucose but a decrease in glycogen observed in cypermethrin treated group compared to the control group. The increase in glucose reported by Veerappan et al., (2012) is affected by cypermethrin administration. The mechanism by which this pyrethroid induces hyperglycemia may involve one or more mechanisms: reduction in insulin secretion due to the destructive action of cypermethrin on the beta cells of Langerhans islets in the pancreas (Kalender et al., 2005; Eraslan et al., 2008). Impairment in

hepatic function due to oxidative changes, which reduce liver ability to glycogenesis (Abdou et al., 2012; Bhatti et al., 2014). Stimulation of hepatic gluconeogenesis and glycogenolysis (Veerappan et al., 2012; Bhanu and Deepak, 2015). Korkmaz et al. (2009) reported a marked decrease in glycogen level in different organs of Nile tilapia (Oreochromis niloticus) exposed to cypermethrin for 10 days.

The severe hyperglycemia may be resulted from glycogenolysis due to the effect of increase in catecholamine level, and this may be the reason of significant decline in liver glycogen. Reduction in glycogen content might be due to the elevated glycolytic activity in animals during cypermethrin stress. Reduction in glycogen level was found in fish due to pesticide effect (Manna et al., 2004)

In the present study, no significant difference observed in the changes of glycogen and glucose in cypermethrin +VC treated group compared to cypermethrin treated group. Korkmaz et al., (2009) recorded similar results of ascorbic acid against cypermethin-induced toxicity in Nile tilapia, Oreochromis niloticus.

In the current study, total cholesterol, triglycerides, and low density lipoprotein – cholesterol concentration recorded to be significantly increased after exposure to cypermethrin. On the other hand, a significant decrease in serum high density lipoprotein-cholesterol was detected compared to the control group. Similar results were reported by Youssef et al., (2003).

Cypermethrin is a lipophilic molecule that has the capability to pass through the cell lipid bilayer and damage its integrity. The altered lipid profile, demonstrated in this study, may be an indication that cypermethrin exposure may affect lipid metabolism (Manna et al., 2004).

Disturbance of carbohydrate metabolism causes an increase in cholesterol and triglycerides concentrations due to the possible cytotoxic effect of cypermethrin on the pancreatic cells that leading to relative deficiency of insulin (Kalender et al., 2005). Reddy et al., (1991) found that the increase of cholesterol and triglycerides were due to lipogenesis and lipolysis occurring during cypermethrin stress.

The increase in cholesterol level due to liver necrosis by the toxicant which leads to the impairment in its metabolism. Similar result was reported by Yaji et al. (2011). The level of triglycerides is usually used to evaluate lipid metabolism. High concentration may be found due to the nephritic syndrome or glycogen storage diseases as recorded by Bernet et al., (2001).

In the present study, the group treated with cypermethrin +VC showed significant decrease in cholesterol and triglycerides levels. VC reduces CHO, prevents oxidation of LDL, reduces TG, raises HDL level and decreases oxidative damage to oxidized LDL-cholesterol by scavenging free radicals (Kurowska et al., 2000).

7-   effect of VC administration on cypermethrin induced changes in antioxidant of SOD and CAT in liver and kidney and oxidative stress of NO and TBARS.

In the present study, it was noted a decrease in the activities of SOD and CAT but an increase in nitric oxide and thiobarbituric acide in group treated with cypermethrin compared to control group. In accordance to the present study, Lidova et al., (2016) showed the ability of cypermethrin to disrupt the antioxidant and are in parallel with those reported in related previous studies.

Thus, the cypermethrin hepatotoxicity presented in the current work may be due to oxidative stress induced by cypermethrin as evidenced by significant increase in TBARS and NO levels. Afolabi et al., (2019) found an increase in level of MDA and lipid hydroperoxid in the kidney and liver.

Cypermethrin induced lipid peroxidation which leads to tissue damage, it is metabolized by cytochrome p450 which associated with generation of oxidative stress as reported by Veerappan et al., (2012). Inhibition of cytochrome p450 by cypermethrin (Manna et al., 2006) may produce oxidative stress resulting in increasing NO and TBARS and decreasing the activities of SOD, CAT. Administration of cypermethrin has been reported to generates reactive oxygen species which produce oxidative stress and reducing the antioxidant defense systems (Atessahin et al., 2005).

Eraslan et al., (2008) reported that cypermethrin accelerate the generation of free radicals which cause an increase in lipid peroxidation of the lipid membrane, and the inhibition of the antioxidant enzymes. SOD is believed to be the first line of defense against harmful effects of ROS in the cell. SOD catalysis and dismutase's

the superoxide radicals to form H_2O_2. Cypermethrin exposure may have depleted cellular SOD level and induced the oxidative stress. Decrease in SOD level have been reported on pesticide exposure in previous studies (Youssef et al., 2003; Vaithinathan et al., 2009).

CAT alleviates oxidative stress by catalyzing the formation of H_2O and O_2 from H_2O_2 (Rajeshkumar and kuttan, 2003). Latchoumycandane and Mathur, (2002) observed a decline in activity of CAT after administration with cypermethrin in rat testis, due to accumulation of hydrogen peroxide in testis.

The increase in H_2O_2 from the activity of SOD on the free radicals could be partly responsible for the inhibition of CAT activity (Badgujar et al., 2015). Uner et al., (2001) observed induction of lipid peroxidation in liver and kidney of the fresh water fish this may due to intoxication with cypermethrin. Lipid peroxidation increases in the kidney of animals, this might be a result of the decrease in antioxidant enzymes.

Oxidative stress has been suggested to play a vital role in damage of kidney in which oxidative stress increases and antioxidant status is reduced. ELGohary et al., (1999) demonstrated that the administration of a single cypermethrin dose of 80 mg/kg was determined to cause oxidative damage of the different tissues and organs. Many studies demonstrated that taking single dose of antioxidants such as vitamin E or VC protect the kidney from injury as well as other oxidative stress related diseases as reported by Shirzad et al., (2011). VC has been recognized as antioxidant and some studies have confirmed that this vitamin prevent free radicals and it can improve the toxic effects of some pesticides (Kalender et al., 2007).

In the current study, it was observed a significant increase in SOD and CAT activities but decreased in NO and TBARS levels in group treated with cypermethrin +VC. Antioxidants such as vitamins E and C have been reported to stop the ROS formation chain reaction (Gordon, 2012). VC scavenges the free radicals formed by the metabolic reaction. VC ameliorated the cypermethrin -induced toxicity by decreasing the cytotoxicity and oxidative stress generated in liver and kidney tissues and increasing the body's ability in removing free radicals (Kashif et al., 2004). Sharma and Bhardwaj, (2018) reported the ameliorative effects of VC in reversing permethrin induced alterations in antioxidant system of goat testis. Siman and Eriksson, (1997) reported that VC intake reduces the elevated concentrations of TBARS in serum of pregnant diabetic rats.

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