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(RESEARCH ARTICLE)

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Protective effects of selenium against cypermethrin induced hepatorenal damage in Sprague Dawley rats

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Abstract

Introduction: Alpha-Cypermethrin is a synthetic pyrethroid insecticide. Pyrethroids are derived from pyrethrins, which are toxic components found in the *Chrysanthemum cinerariaefolium* flowers. They have been employed in agriculture, home and veterinary formulations to control the insect vectors of disease, agricultural insects and topically applied to eradicate veterinary pests. The extensive use of pyrethroids in agriculture results in severe pyrethroid poisonings in developing countries.

Objectives: This study aimed to evaluate the serum biochemical changes induced by oral exposure of cypermethrin and the protective effects of selenium in female Sprague-Dawley rats.

Methodology: The female rats (n=20) were equally divided into four groups. Group 1, 2, 3 and 4 received 1 ml corn oil, 55 mg/kg/b.w of cypermethrin, 1 ppm of sodium selenite, both cypermethrin and sodium selenite respectively for 21 days. The serum was utilized for estimation of alkaline phosphatase (ALP), alanine transaminase (ALT), urea, creatinine and bilirubin by using Selectra E (Germany). Levels of sodium (Na^+) and potassium (K^+) in serum were measured using Easylyte (Na^+/K^+) analyzer (Japan).

Results: Results obtained showed that activities of ALP and ALT were significantly ($P<0.05$) elevated in cypermethrin treated rats. Total bilirubin, urea and creatinine were insignificantly elevated due to cypermethrin administration. Also, selenium reduced the harmful effects of cypermethrin in the combined treatment group in some parameters.

Conclusion: Oral exposure of cypermethrin induces injuries in liver and kidney of rats as well as alterations in the levels of serum electrolytes which were modulated by selenium co-administration.

Keywords: Cypermethrin; Selenium; Pyrethroid; Electrolytes

1. Introduction

Many plants and insects are increasingly becoming resistant to several types of pesticides. Farmers have to use more toxic pesticides or increase the amount of pesticides they apply, due to these so called “superweeds” and “superbugs”. The use of synthetic pesticides became widespread as the manufacturers began to produce large amounts of them in the 1940s [1].

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The pesticides derived from natural sources are generally considered as organic pesticides. The natural sources usually include minerals, like cryolite, boric acid, or diatomaceous earth, rotenone or ryania (botanical insecticides), or plants, such as pyrethrum (pyrethrins) [1]. Organic pesticides are largely insecticides. Insecticides are classified as either organic or inorganic. Organic insecticides are mainly classified into four groups, carbamates, pyrethroids, organophosphates and organochlorines. All of these possess neurotoxic properties [2-4]. Organochlorines increase the excitability of the central nervous system in several different ways, by increasing the neuronal excitability as a general mechanism [3]. They also affect the reproductive, endocrine and immune system. Organophosphorus compounds and carbamates inhibit acetylcholinesterase [5].

Pyrethroid insecticides are preferable over organochlorines, organophosphates and carbamates [6] and they account for more than 30% of the world-wide pesticide use [7]. Regular farmers greatly depend on synthetic pesticides. These pesticides contain the chemicals which are dangerous not only for the environment but also for our health. They leave chemical residues in our food, destroy wildlife and cause risks to the people applying them as well as bystanders [8].

Among the highly potent insecticides, pyrethroids are considered the safest. Pyrethroids slowly absorb across the skin which normally prevent the systemic poisoning, although epidermis may have a substantial pyrethroid reservoir which remain bound to it. Their toxicity has been reported in humans when applied indoors in poorly ventilated and closed areas, and in farmers who are engaged in spraying insecticides [9]. Allergenic properties of pyrethroids may also cause human toxicity, with the induction of antinuclear antibodies and with the development of some autoimmune disorders [10-12]. According to their chemical structures, pyrethroids are divided into two groups: type I pyrethroids (e.g permethrin) do not have a cyano moiety at the α-position, while type II pyrethroids have an α-cyano moiety (e.g cypermethrin).

Permethrin is generally employed to control various pests, such as moth pests of cotton, vegetable crops and fruit [13]. It is also utilized for spot treatment, crack, and crevice and to control insect pests in warehouses, industrial buildings, stores, apartments and houses, laboratories, railcars, greenhouses, ships, buses, trucks and aircrafts. It may also be utilized in nonfood areas in hospitals, schools, nursing homes, food processing plants, hotels and restaurants. It is being utilized against ectoparasites in veterinary practice [14].

Selenium is found in dietary sources including grain products, cereals, vegetables, meat, seafood, and more abundantly in Brazil nuts [15-17]. It constitutes the active center of about twenty eukaryotic proteins, some of them with redox regulatory properties (primarily antioxidant), hence is a powerful catalytic element [18]. Being a part of these selenoproteins, selenium shows carcinostatic ability by acting as a chemo-protective agent and exerts metabolic function linked with maintaining defenses and integrity of the organism [19].

An inverse relationship has been suggested by an epidemiologic data between cancer mortality rates in humans and selenium levels in the diet [20]. The significance of antioxidants such as selenium in maintaining genomic stability due to cellular injury, such as that inflicted by exposure of ionizing radiation, is already known [21, 22].

2. Material and methods

2.1. Maintenance of animals

In this study, twenty adult female Sprague-Dawley rats (8-10 weeks) were used. The average body weight of rats was approximately 180 g. Standard laboratory conditions were maintained in the Animal House facility of Quaid-i-Azam University, Islamabad where the rats were acclimatized for a week and fed standard rat chow and water *ad libitum* after purchasing from National Institute of Health, Islamabad. The light-dark photoperiod was 12 hrs with air condition system and average temperature of 25°C during the experimental period. The health status of rats was also monitored, and five rats were kept per cage to avoid stress that might have resulted by crowding. The guidelines provided by the ethics committee of the Department of the Zoology, Quaid-i-Azam University, Islamabad was followed to perform all animal handling and subsequent killings.

2.2. Chemicals

Sodium selenite Na_2SeO_3 (Surechem, England) and alpha cypermethrin [(RS)-cyano-(3-phenoxyphenyl) methyl-(IRS)-cis-trans-3-(2,2 dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylate] were employed in this study.

A commercial pyrethroid product "Ripcord" was used in the current study as test compound which was purchased from the local market and contained 10% cypermethrin. 100 g/l of Cypermethrin and a mixture of petrol and xylene (820

g/l) were contained in Ripcord (Basf Aktiengesellschaft, Germany) which was in the form of emulsion. In order to reach test concentration i.e. 55mg/kg, adequate dilutions were made in corn oil. Fresh solutions were prepared before usage. Other reagents (analytical grade) utilized in this study were purchased from Sigma (St. Louise, U.S.A).

2.3. Experimental design

Four equal groups of mature female rats having 5 rats each were used to assess the effects of cypermethrin and selenium on the histopathological and biochemical parameters.

Animals in group 2, 3 and 4 were administered with cypermethrin (55 mg/kg body weight), sodium selenite (1 ppm) or their combination, respectively while group 1 served as control.

Oral LD_{50} was found to be 187 to 326 and 150 to 500 mg/kg in male and female rats [23, 24] respectively. Therefore, for this sub-chronic study of 21 days, a low dose of 55 mg/kg body weight of rat was used.

Oral administration of cypermethrin was done in 1 ml corn oil by gavage for 21 days, and control animals were given an equivalent amount of corn oil in an identical manner. Selenium was daily administered as sodium selenite (Na_2SeO_3) in drinking water.

The body weight was recorded daily during the experimental period and doses were calculated on its basis and given to non-fasted rats in the morning. The behavior of the animals was observed on daily basis and compared with those of control rats.

2.4. Dissections

The animals were sacrificed at the end of experiment. An overdose of chloroform was used to anesthetize the animals in accordance with the guidelines provided by National Institute of Health Islamabad, on human use of animal for basic and medical research. Abdomen was cut opened and sterile syringes (10 ml) were used to aspirate blood through cardiac puncture. Blood was collected in tubes for serum preparation.

Blood was allowed to stand at room temperature for one hour and then centrifuged at 1258g (2500 rpm) for ten minutes to prepare serum (Eppendorf centrifuge 5810R). Serum thus prepared was stored at -20°C until analyzed.

2.5. Biochemical profile

The serum was utilized for estimation of activities of alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, bilirubin, urea by using Selectra E (Germany). Levels of sodium (Na^+) and potassium (K^+) in serum were measured using Easylyte (Na^+/K^+) analyzer (Japan).

2.6. Tissue preparation for Histopathological Examination

Reagent composition

Harris' hematoxylin

1 g of hematoxylin was dissolved in absolute alcohol (10 ml). Alum (20 g) was dissolved in distilled water (warm). In the alum solution, hematoxylin solution was added and rapidly brought to boil, and then mercuric oxide (0.5 g) was added in it. The flask was plunged into cold water to rapidly cool the stain and then was added with glacial acetic acid (8 ml).

Eosin solution

Eosin solution was made by adding 1 g eosin and 0.05 ml acetic acid into 100 ml dH_2O.

2.7. Procedure

Small parts of liver and kidney tissues removed during dissections were kept in ice cold buffered saline and subsequently fixed in 10% formalin for 24 hours, trimmed to appropriate size and processed for dehydration and paraffinization. They were embedded in molten wax pre warmed in a wax dispenser (TBS Triangle biomedical sciences, Durham, NC U.S.A) to 60-70°C. 5µm thick sections were cut on a rotary microtome (Shandon Finesse). Ribbons containing tissue sections were immediately transferred into a water bath (Boekel scientific) at 37°C. Tissue sections were taken on cleaned glass slides and left on a slide warmer at 37°C for 2-3 hrs. Tissues were deparaffinized in xylene

with two changes each for five minutes. They were hydrated in descending series (100%-90%-80%-70%-50%) of alcohols for five minutes in each grade. They were washed in distilled water for a few seconds and then in running tap water for one minute. Tissues were stained with Harris' hematoxylin (1%) for five minutes, washed for 1-3 minutes in running tap water and then differentiated for a few seconds in acid alcohol, washed again for 1 minute in running tap water and stained with eosin (1%) for two minutes. They were washed again for one minute in running tap water and dehydrated in ascending series of alcohols (50%-70%-80%-90%-100% each for 5 minutes), cleared in xylene with two changes each for 5 minutes and finally mounted in DPX mountant medium. Nikon Optiphot BH-2 research microscope (Japan) was used for photographing and observation of tissue sections.

2.8. Statistical analysis

Data are expressed as mean ± SEM (Standard error of mean). GraphPad Prism 5 was used for statistical analysis. Single factor analysis of variance (ANOVA) was used to assess the statistical significance of differences between the means. Dunnett test was used to compare the parameters of treatment groups with those of control. Tukey's test was applied to carry out the comparison among different treatment groups. A difference at $P < 0.05$ was considered significant.

3. Results

3.1. Behavioral Observations

Rats treated daily for 21 days with cypermethrin exhibited considerable physical and behavioral abnormalities throughout the experiment. These changes became more pronounced as the experiment progresses. Food and water consumption was reduced. The skin/fur color changed from white to pale and light brown at some parts of the body. Nervous signs consisted of incoordination, staggering gait, ataxia, dizziness, muscular tremors, jerky movements, restlessness, skin irritation, muscle twitching, and whole-body tremor, gasping and labored breathing.

The animals became very weak and showed less resistance during handling for dose administration. Two animals out of nine treated with cypermethrin were died during the experiment. The above-mentioned symptoms in cypermethrin treated animals were less pronounced in cypermethrin + sodium selenite administered rats. The animals of the control and sodium selenite alone treated groups showed no abnormalities.

3.2. Body Weight

The initial mean body weight of all the groups was similar to each other and not significantly different. Mean final body weight of control, cypermethrin, sodium selenite and cypermethrin + sodium selenite treated rats before and after treatment is given in table 1 and fig 1.

A significant change was observed in body weight gains of cypermethrin administered rats in comparison to control rats ($P < 0.05$) (Table 1). There was a reduction in the weight of animals of treated group after week 1 followed by slight increases at the end of week 2 and 3. But this increase was less as compared to control (Fig 2). The weight gain of selenium group was even more than control. In case of combined group, the weight gain was much better than cypermethrin treated group (Table 2).

3.3. Biochemical profile

Alpha-cypermethrin and sodium selenite treatment caused alterations in the ALP, ALT, creatinine, urea, bilirubin, sodium, and potassium levels in the rat serum in comparison to control rats (Table 3; Fig 3-9).

3.4. Cypermethrin treatment

Cypermethrin significantly raised the levels of serum ALT and ALP ($P < 0.05$). Creatinine, bilirubin and urea increased non-significantly. The serum level of Na^+ decreased significantly ($P < 0.05$) while K^+ decreased non-significantly.

3.5. Sodium Selenite treatment

In rats treated with sodium selenite alone, there was non-significant rise in the activities of ALP, ALT and creatinine. Whereas the levels of total bilirubin, urea and serum electrolytes (Na^+/K^+) were decreased non-significantly.

3.6. Cypermethrin + Sodium Selenite treatment

Treatment of cypermethrin along with sodium selenite resulted in increased levels of ALT, ALP, and creatinine as compared to control. Bilirubin and urea showed values close to control. The level of potassium decreased non-significantly while that of sodium decreased significantly ($P < 0.05$).

3.7. Histopathological Observations

Normal structure of liver was observed in control rats (Fig 10 A and B). It is comprised of pentagonadal or hexagonadal lobules with central veins. The connective tissue is embedded with peripheral hepatic triads or tetrads. Hepatocytes are arranged in trabecules which runs radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with peripheral chromatin distribution and a particularly marked nucleolus. Some cells have two nuclei each. Congestion and sinusoidal dilatation were observed in the cypermethrin treated rats. In most of the animals, hydropic degeneration (ballooning) was also observed in perivenular region (zone 3). In some rats, degeneration had proceeded to midzonal region (zone 2). Additionally, haemorrhage and perivenular necrosis were found in these rats (Fig 10 C and D). Mononuclear cell infiltrates were observed locally, most commonly in the hepatocytes of periportal region (zone 1). The cytoplasm of hepatocytes (zone 2 and 3) was enlarged and contained empty vacuole-like spaces. The walls of most sinusoids showed numerous Kupffer cells and some sinusoids were overfilled with erythrocytes. In the animals of the combined group, the damage caused by cypermethrin was less severe (Fig 10 E and F).

Normal structure of the cortex and medulla was shown by the kidney of control group animals (Fig 11 A and B). Tubular necrosis and glomerular widening were evident in the kidneys of cypermethrin-exposed animals (Fig 11 C and D). Degeneration of epithelia of renal tubules and hypertrophy of epithelial cells, dilation of glomeruli, hyperaemia of cortical and medullary parts with mononuclear cell infiltrates were apparent and the inflowing cells blurred the tubular structure at these sites. Dilatation of capillaries filled with erythrocytes both in the medullary and cortical sections of the kidney and enlargement of renal glomeruli were found. Simultaneous administration of selenium with cypermethrin, reduced the toxic signs in the kidney.

Table 1 Mean body weights of control, cypermethrin, sodium selenite and cypermethrin + sodium selenite treated rats for 21 days. Values are given as ± SEM, $p < 0.05$

Group (n=5)	Initial Weight (g)	Final Weight(g)	Weight gain (g)	Relative Weight gain (g)
Control	154 ± 2.449	199.8 ± 6.248	45.8	29.22
Cypermethrin	186 ± 4	189 ± 6.024	3 [a*]	1.61 [a*]
Selenium	180 ± 1.414	226.4 ± 11.2	46.4	25.5
Cypermethrin + Selenium	180 ± 0.632	200 ± 12.55	20	11.1

[a] Significantly different from Control; * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$

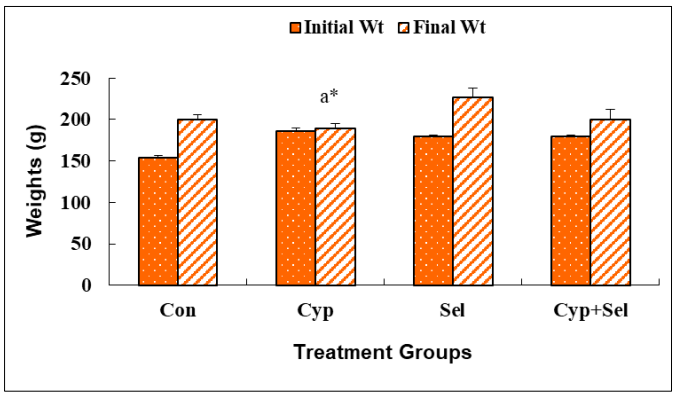


Figure 1 Mean body weights of control, cypermethrin, sodium selenite and cypermethrin + sodium selenite treated rats for 21 days

Table 2 Weekly mean body weights of rats in control, cypermethrin, sodium selenite and cypermethrin + sodium selenite treated groups. Values are given as ± SEM

Group (n=5)	Initial Weight (g)	Week 1	Week 2	Week 3
Control	154 ± 2.449	181 ± 2.449	190 ± 8.367	199.8 ± 6.248
Cypermethrin	186 ± 4	172 ± 13.56	178 ±11.58	189 ± 6.205
Selenium	180 ± 1.414	218 ± 7.348	211.6 ± 7.659	226.4 ± 11.2
Cypermethrin + Selenium	180 ± 0.632	200 ± 6.325	191.8 ± 6.785	200 ± 12.55

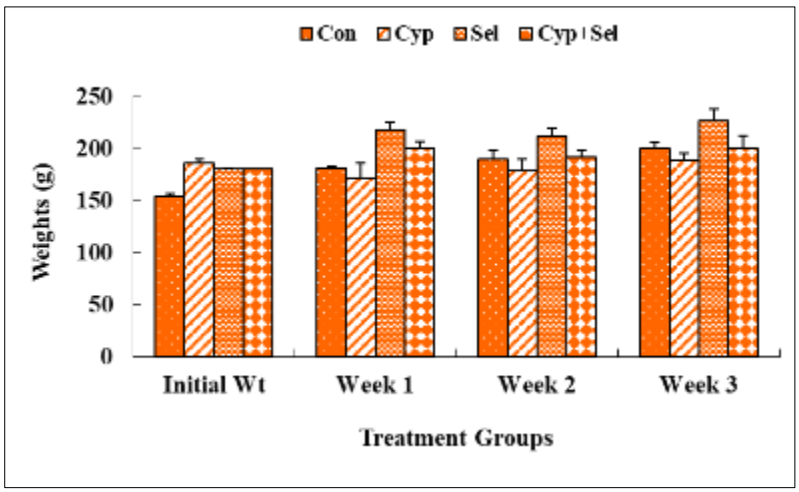
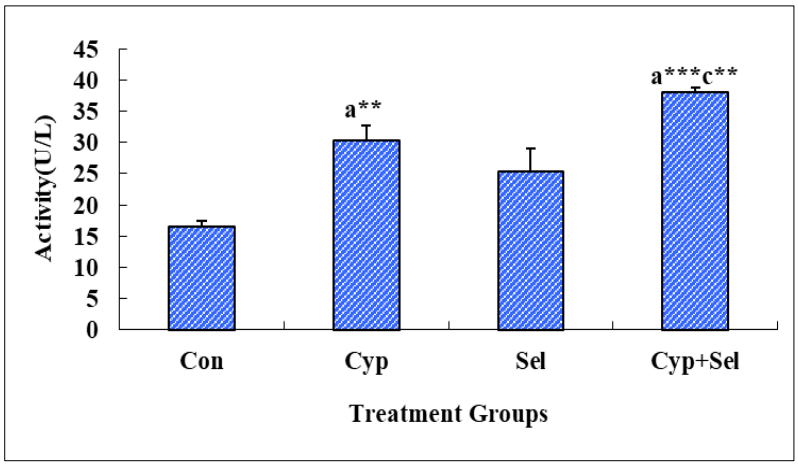


Figure 2 Weekly mean body weights of rats in control, cypermethrin, sodium selenite and cypermethrin + sodium selenite treated groups

Table 3 Biochemical parameters of the female Sprague Dawley rats exposed to sub-chronic dose (21 days) of alpha-cypermethrin. Values are given as ± SEM (n=5), $p < 0.05$

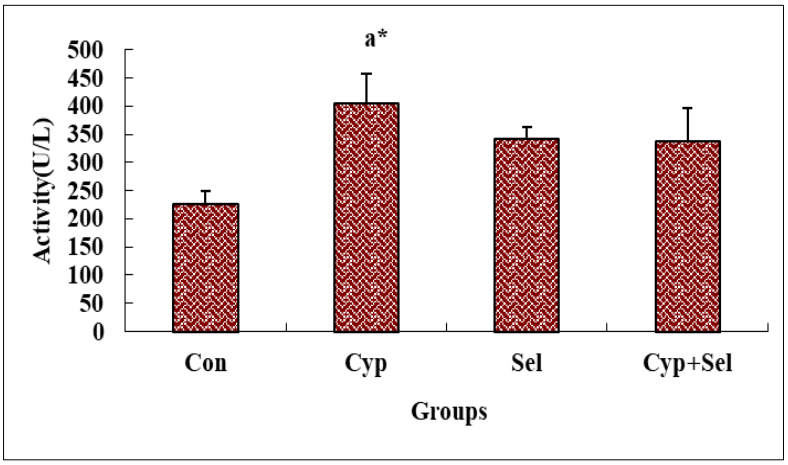
Biochemical Parameters	Control	Cypermethrin	Selenium	Cypermethrin+ Selenium
ALT (U/L)	16.6 ± 0.871	30.4 ± 2.337 [a**]	25.4 ± 3.572	38 ± 0.894 [a***c**]
ALP (U/L)	226.8 ± 23.29	404.8 ± 53.47 [a*]	342.6 ± 19.71	338 ± 57.45
TBIL (mg/dl)	1.4 ± 0.244	2.4 ± 0.509	1.2 ± 0.200	1.4 ± 0.244
Urea (mg/dl)	9.98 ± 0.505	10.06 ± 0.778	8.8 ± 0.363	9.2 ± 0.054
Creatinine(mg/dl)	47.6 ± 3.172	57.6 ± 5.240	60.6 ± 4.632	53.8 ± 2.083
Na^+ (µg/ml)	145.8 ± 1.114	143.2 ± 0.244 [a*]	142 ± 1.049	141.2± 0.734 [a*]
K^+ (µg/ml)	5.84 ± 0.169	5.38 ± 0.217	5.7 ± 0.184	5.6 ± 0.109

[a] Significantly different from Control; [b] Significantly different from cypermethrin; [c] Significantly different from selenium; * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$



[a] Significantly different from Control; [b] Significantly different from Cypermethrin; [c] Significantly different from Selenium; * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$

Figure 3 Changes in ALT (U/L) activities in the serum of female Sprague Dawley rats from control and treated groups



[a] Significantly different from Control; [b] Significantly different from cypermethrin; [c] Significantly different from selenium; * $p < 0.05$; ** $p < 0.01$;*** $p < 0.001$

Figure 4 Changes in ALP (U/L) activities in the serum of female Sprague Dawley rats from control and treated groups

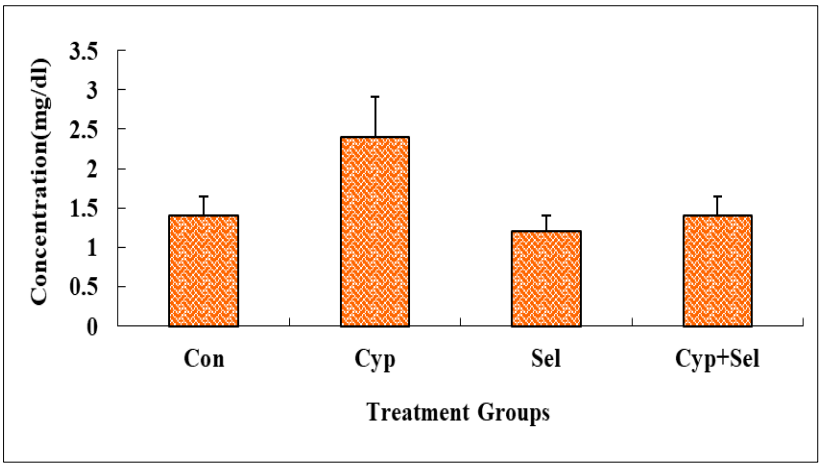


Figure 5 Changes in total bilirubin (TBIL) concentrations (mg/dl) in the serum of female Sprague Dawley rats from control and treated groups

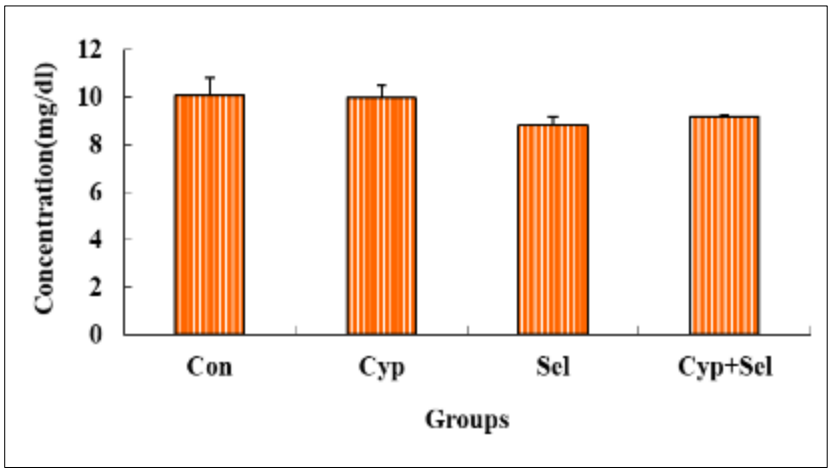


Figure 6 Changes in urea concentrations (mg/dl) in the serum of female Sprague Dawley rats from control and treated groups

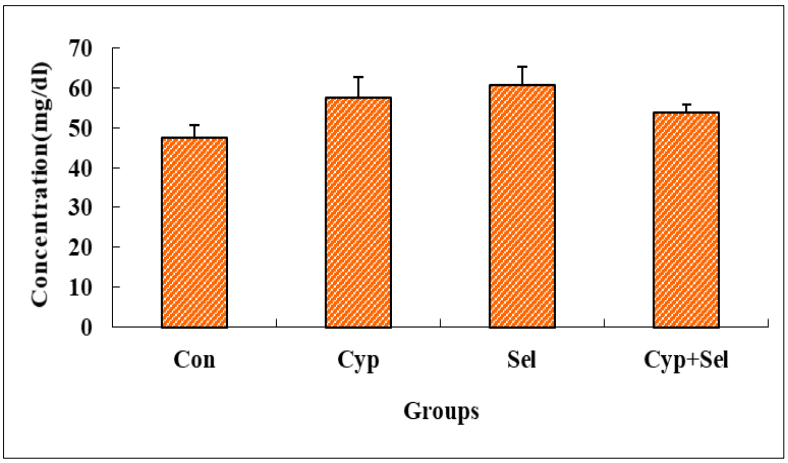
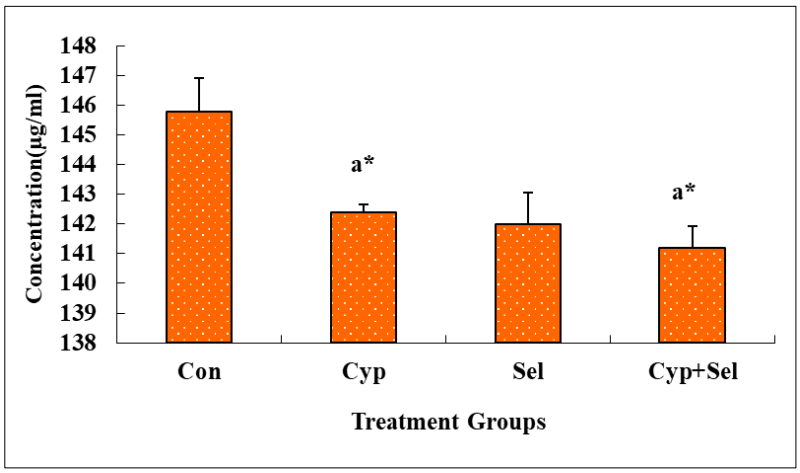


Figure 7 Changes in creatinine (mg/dl) activities in the serum of female Sprague Dawley rats from control and treated groups



[a] Significantly different from Control; * $p < 0.05$

Figure 8 Changes in Sodium (Na^+) (µg/ml) levels in the serum of female Sprague Dawley rats from control and treated groups

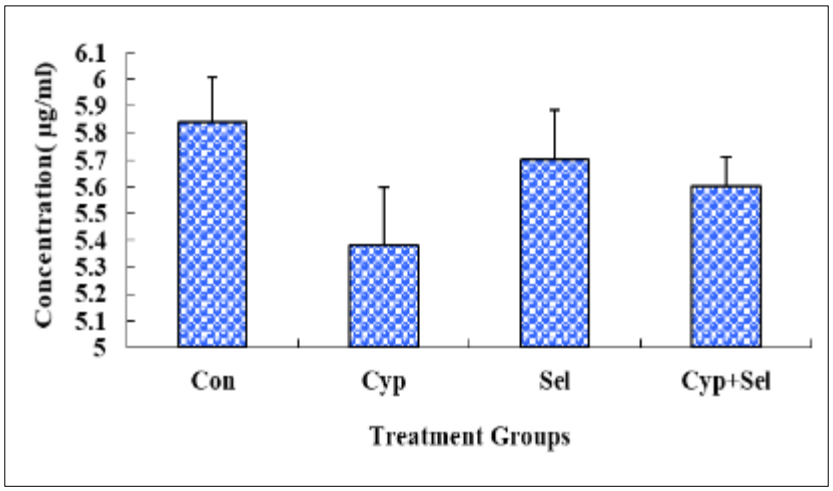


Figure 9 Changes in potassium (K^+) (µg/ml) levels in the serum of female Sprague-Dawley rats from control and treated groups

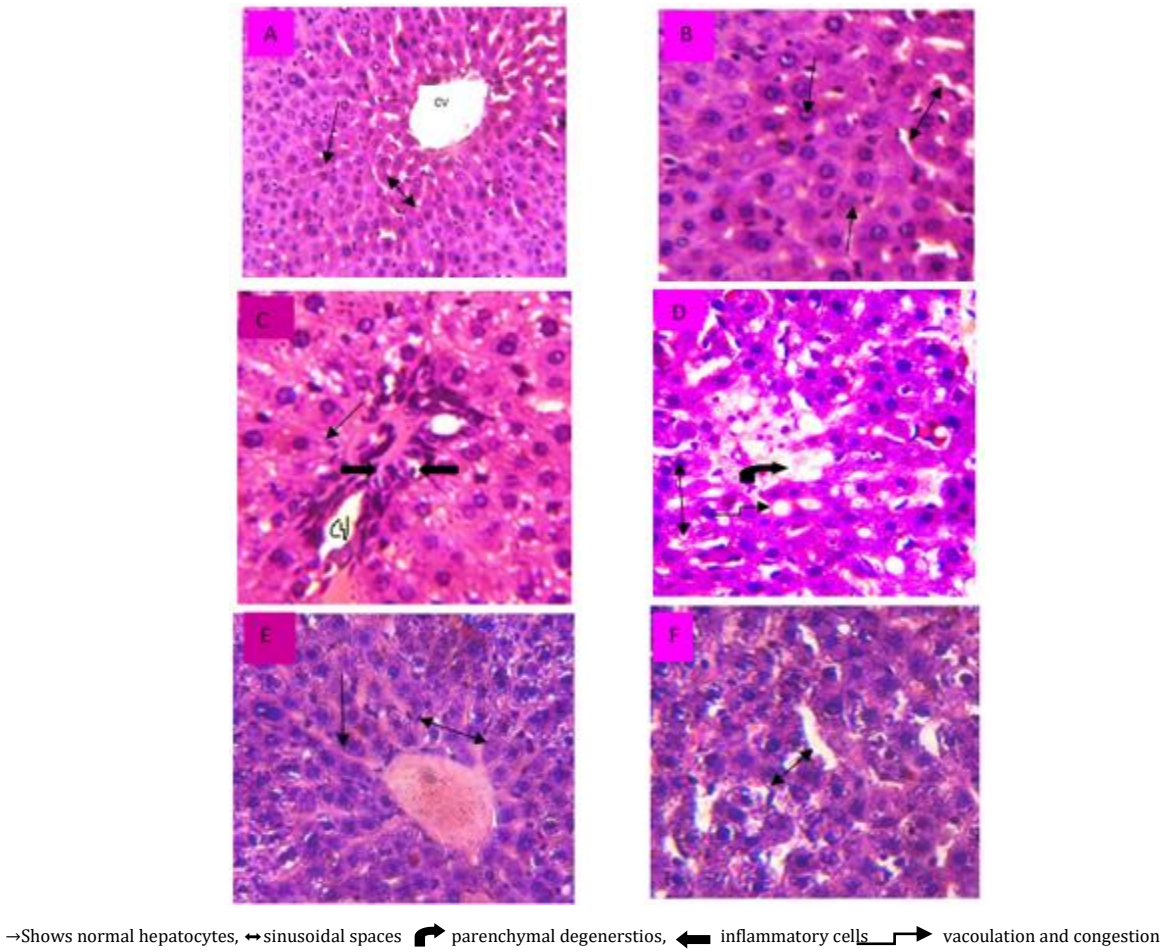


Figure 10 Section through the liver of control rats showing normal cellular architecture with distinct hepatocytes, sinusoidal spaces, and a central vein (A&B). While, cypermethrin intoxicated treatment exhibited severe histopathological changes, such as hepatic degeneration and marked leukocytic infiltration and sinusoidal dilatations (C&D). sections through the liver of rats treated with selenium and cypermethrin to help prevent these histopathological changes, induced by cypermethrin (E&F) (H&E × 400) (CV, central vein)

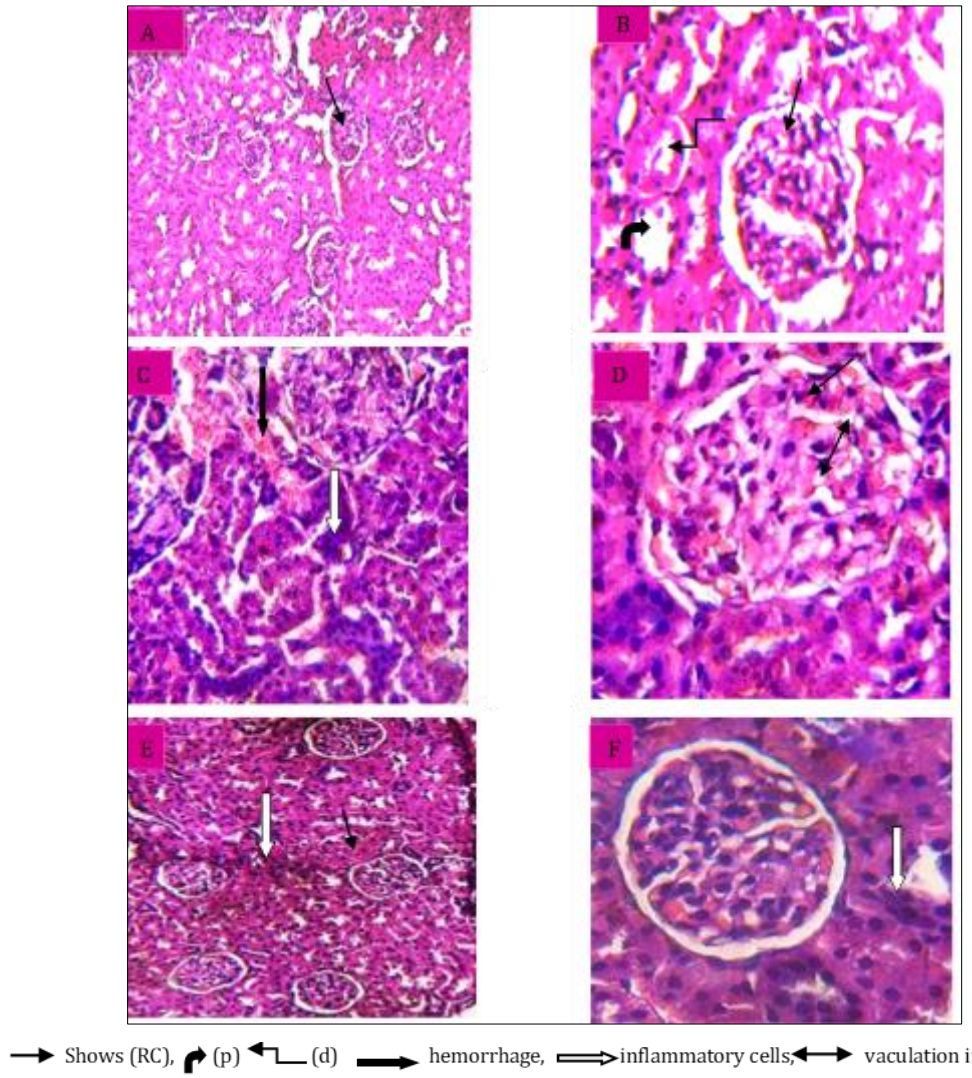


Figure 11 (A) Section through the renal cortex of control rats showing a group of proximal and distal convoluted tubules surrounding renal corpuscles (RC). The proximal convoluted tubules (p) and distal convoluted tubules (d) are seen. (B) Showing lobular organization of the glomerule and a flat epithelium lining the glomerular capsule of control rats. (C) Showing renal cortex of cypermethrin treated group showing tubular epithelial vacuolization and congestion. Some cells of the tubular epithelium show features of oedema. (D) Vascular glomeruli are enlarged, tightly filling the Bowmann's capsule. Capillaries are filled with blood cells. (E & F) showing renal architecture in rats receiving selenium and cypermethrin (H&E stain A,C & E X200, B,D &F X400)

4. Discussion

The effects of commercial pyrethroid product Ripcord on biochemical and histological aspects of female rats and their modulation by co-administration of sodium selenite have been explored in the current study. Clinical signs of cypermethrin toxicity in rats included incoordination, ataxia, staggering gait, dizziness, muscular tremors, jerky movements, restlessness, skin irritation, muscle twitching, and whole-body tremor, gasping and labored breathing. Previously, skin irritation has also been reported in rabbits treated with cypermethrin [25]. Similar nervous signs like mild tremors, hyperexcitability or depression [26], muscle twitching, staggering gait [27] have been reported earlier. Voltage-sensitive sodium channels' gating characteristics are modified by pyrethroids to delay their closure in invertebrate and mammalian neuronal membranes [28] which produces a protracted sodium influx. Cypermethrin produces nervous signs such as restlessness, itching and head shaking by the mechanism of sodium channels' blockage. Similar action has been exerted by pyrethroids and DDT on the neuron system through modulating the voltage-gated sodium channels' function [29] which causes membrane depolarization, leading to discharges from sensory neurons. Indicators of digestive system disturbances have also been reported by other researchers like reduced feed intake as observed in the present study [30, 31]. Both control group animals showed normal behavior. The animals of the combined group showed less severe signs as compared to cypermethrin treated rats.

A significant change was observed in body weight gains of cypermethrin treated group ($P < 0.05$) in comparison to control rats. At the end of week 1 of treatment, there was a decline in the weight of rats followed by slight increases at the end of week 2 and 3. But this increase was less as compared to control. Stress of cypermethrin could be a possible reason for this decrease in weight in treatment groups. Lakkawar *et al.,* [32] have also reported decline in body weight as compared to control group in addition to typical clinical signs of cypermethrin. The weight gain of selenium group was even more than control. In case of combined group, the weight gain was much better than cypermethrin treated group.

The functional status of the liver is represented by serum enzymes like ALT and ALP. When exposed to exogenous modulants like toxins, injury or infection, the overall status of the rats is reflected by content of these enzyme activities in serum.

In the present study, ALP and ALT increases in all the treated groups in comparison to control. In cypermethrin treated group, there was a significant rise in ALP ($p < 0.05$) and ALT ($p < 0.01$). The intensity of cellular damage relates to the increase or decrease of enzyme activity. The alterations in enzyme activities correlates with the pathological changes. Therefore, increase of transaminase activity may be the result of $\alpha-$ cypermethrin induced pathological alterations of the liver. Increased levels of serum ALP and ALT suggest that alpha cypermethrin causes liver injury which may be through free radicals [26].

Total bilirubin, urea and creatinine increases in cypermethrin treated group (non-significantly). The creatinine increases in selenium alone and combined group. While bilirubin and urea decreased in selenium alone and came closer to control values in combined group. Our findings are in accordance with the results of the studies performed employing selenium by [33]. Hoffman and Heinz [34] reported that selenium treatment resulted in decrease of urea concentration.

The increase in serum creatinine and urea levels is considered as a significant marker of kidney dysfunction in cypermethrin treated animals, and this is encouraged by the result of [35], who found that changes in serum urea may be linked to metabolic disorders (such as cation–anion balance, renal function).

As urea is the end-product of protein catabolism, the rise in serum urea concentrations in cypermethrin administered rats may be because of its effect on liver function, and/or referred to renal dysfunction. The rise in blood creatinine and urea levels in rats treated with cypermethrin agrees with the findings of Al-Qarawi *et al*., [36]. A rise in protein catabolism in mammalian body is correlated with elevated blood urea or from more efficient conversion of ammonia to urea due to increased synthesis of enzyme involved in urea production [37].

The rise in serum total bilirubin may be due to decreased liver uptake, conjugation or increased bilirubin production from hemolysis [38]. The rise in serum total bilirubin concentrations of cypermethrin treated rats are in agreement with the previous work of Kossmann and Mangner-Kreze [39] in pesticides' exposed workers. El-Zahar *et al*., [40] reported malfunction in the liver of rabbits indicated by the plasma bilirubin's induction. Also, Clifford and Rees [41] reported that the onset of periportal necrosis could cause the rise in plasma bilirubin level.

The structure of kidney and liver was evaluated on the basis of histopathological findings. Cypermethrin administration resulted in structural changes to both organs. Our histological results showed that histopathological alterations like mononuclear cell infiltration, degeneration in hepatocytes and hepatic cords and an enlargement of the sinusoids were seen in rats' liver treated with cypermethrin. Similar histopathological alterations such as parenchymatous degenerations of hepatocytes and mononuclear cell infiltration in liver were observed in Wistar rats orally administered with 250 mg/kg α-cypermethrin [42]. However, slight histopathological alterations in liver were reported in rats dermally applied with cypermethrin [43, 44]. Daily oral administration of cyhalothrin in mice for 28 days at levels up to 2000 mg/kg diet showed dose-related histopathological alterations in liver of rats exposed to 100 mg/kg dosages and above [44]. Other studies have also determined histopathological hepatic injuries caused by cypermethrin [45]. *In vitro* and *in vivo* investigations of cypermethrin have indicated that it causes necrosis, inflammation and cytoplasmic hypertrophy in hepatocytes [26].

The kidney is an organ sensitive to external factors, which might induce histopathological change as well as functional deficit. Anatomically, the cortex of the kidney has more functional structures than the medulla and mainly nephrons. The main changes reported in this study were blood congestion in the glomeruli and proximal tubule swollen Bowman capsule lining with minor tubule rupture. However, proximal tubules and glomeruli appeared to be the primary site of action for most toxins because most of the blood flow to the kidney is delivered to the cortex, primarily to the proximal tubules. Previous study demonstrated cypermethrin induced nephrotoxicity in rats [43]. Luty *et al*., [42] have shown few infiltrations of mononuclear cells between the proximal tubules. Renal damage like focal regeneration in tubular

epithelium, focal cortico-medullary mineralization, and mineral/crystal deposition in intertubular region in kidneys was shown in a repeated dose toxicity study [26].

The damage caused by cypermethrin was less pronounced in selenium combined group. These are generally in agreement with the findings of the studies performed using hepatotoxic substances and antioxidants [46]. The mechanism underlying hepatoprotection of selenium might be related to both its indirect effect as a regulator of antioxidative systems and radical scavenging properties. Selenium and cypermethrin coadministration group kidneys showed near to normal architecture of renal cortex. Indeed, there are antioxidant functions of selenium in inhibition of the oxidative processes of lipids and lipoproteins in cell membranes [47-49].

5. Conclusion

Overall, the results of our study demonstrated that cypermethrin administration (oral) induces biochemical and histopathological changes in kidney and liver of rats. Moreover, it also provides information as regards levels of electrolytes and gross physiological abnormalities, in case of cypermethrin toxicity and effect of the selenium co-administration in modulating its toxicity. Co-administration of selenium provided protection against cypermethrin toxicity to some extent. Further studies are required to investigate the mechanism underlying the effects of cypermethrin on electrolytes of the body.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All animal handling and subsequent killings were done according to the guidelines provided by the ethics committee of the Department of Zoology, Quaid-i-Azam University, Islamabad.

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Dr. Umbreen Rashid has completed her MSc, MPhil and PhD from Quaid-i-Azam University, Islamabad, Pakistan.

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She is reviewer and editorial board member (invited) of various International Journals.

She is pioneer in launching a new Department of Environmental Sciences as team member in G.C Women University, Sialkot, Pakistan. Also, established a new lab for the first time in GC Women University, Sialkot to conduct in vivo assays.

Got 1st Position as teacher for the course "Biodiversity and Wildlife" in GC Women University, Sialkot (MS Zoology).

Two MS research students (from Dept. of Zoology and Botany) got first prizes in poster competition held at GC Women University, Sialkot (November 11, 2017).

Her Research interests are Ethnopharmacology, Toxicology, Nanotechnology, Food Biotechnology, Medical Microbiology, Antimicrobial Resistance, Fisheries and Health Care.

Got 378th position in the Directory of Productive Scientists of Pakistan (2017), out of 969 candidates in the field of Biological Sciences.

Achieved the grand score of 3.21 in Research Productivity award list 2017-18.

Acted as Judge for poster session (BS to M.Phil) in 1st National conference on "Bioactivity of Phytochemicals" held on October 04-06, 2017 organized by The University of Lahore.

Currently serving as visiting faculty in Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan and Assistant Professor in the Department of Life Sciences, Abasyn University, Islamabad, Pakistan. Launched MS Biochemistry Program as team member and serving as its coordinator in Abasyn University, Islamabad, Pakistan.

Being one of the lead researchers in the Department of Life Sciences, Abasyn University, she has several publications in HEC recognised journals to her credit.

She has presented number of scientific papers in various international conferences and published about 25 abstracts in the year 2021-22.