The Pharma Innovation Journal 2020; 9(2): 260-266

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(2): 260-266

www.thepharmajournal.com
Received: 17-12-2019
Accepted: 21-01-2020

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Oxidative stress and biochemical alterations induced by combined exposure of cypermethrin and deltamethrin and their amelioration by *Withania somnifera* and resveratrol

Robin, Vivek, Vikas and SK Jain

Abstract
Pyrethroid insecticides are used over organochlorine and organophosphates due to their high effectiveness, low toxicity to non-target organisms and low biodegradability. The combined exposure of cypermethrin and deltamethrin was given in adult male Wistar rats. Various biochemical and oxidative stress parameters were analysed up to 14 days and their amelioration by *Withania somnifera* and resveratrol was investigated. Cypermethrin and deltamethrin was given orally at rate of 75mg/kg and 4mg/kg body weight in adult male Wistar rats. The combined exposure of cypermethrin and deltamethrin caused significant increase in ALT, AST, GGT, BUN, creatinine, lipid peroxidation (MDA) while it caused decrease in tissue protein level and antioxidants level *viz.* reduced glutathione in rats. In cypermethrin plus deltamethrin plus *Withania somnifera* group and cypermethrin plus deltamethrin plus resveratrol group, both *Withania somnifera* and resveratrol co-treatment restored the changes to normal observed following combined cypermethrin and deltamethrin exposure in rats. The present study showed that combined cypermethrin and deltamethrin exposure cause biochemical alterations and oxidative stress in rats which is reversed and restored to normal values following co-treatment with *Withania somnifera* and resveratrol. This indicates the ameliorating effect in rats exposed to cypermethrin and deltamethrin combination.

Keywords: Cypermethrin, deltamethrin, ALT, AST, GGT, BUN, creatinine, lipid peroxidation (MDA), resveratrol and *Withania somnifera*

1. Introduction
Pyrethroids are modified derivatives of pyrethrins, natural substance obtained from flowers of *Pyrethrum* species. Pyrethroids are widely used in agriculture and veterinary applications due to their high bio-efficacy, enhanced stability and comparatively low mammalian toxicity.

Cypermethrin is a synthetic pyrethroid insecticide which is used to kill insects especially on cotton. It behaves as a fast-acting neurotoxin in insects. Synthetic pyrethroids affect axons of neurons of peripheral nervous system and central nervous system. It interacts with transportation system of sodium ions through cellular membrane. This results a delay in closing of sodium channel and prolonged sodium tail current after membrane gets repolarized. Thus cypermethrin acts as neurotoxic for both insects and mammals [10].

Deltamethrin is a synthetic pyrethroid insecticide used in agriculture, home pest control and disease vector control. Neurotoxic mechanisms of deltamethrin include prolonging the opening of voltage sensitive sodium channels and inhibition of voltage gated chloride channels and GABA receptors [42]. Resveratrol (trans-3,5,4-trihydroxystilbene), a polyphenolic phytoalexin abundantly found in grapes and red wine is a potent antioxidant and cytoprotective agent. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi [16]. The major dietary sources of stilbenes include grapes, wine, soy, peanuts and peanut products [11].

Withania somnifera is used as a herb in ayurvedic medicine. The main chemical constituents are alkaloids and steroidal lactones. *Withania somnifera* possesses anti-inflammatory, antitumor, anti stress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. *Withania somnifera* is a well-known and important medicinal plant widely used in several indigenous system of medicine for treatment of various ailments *viz.* asthma, inflammatory disease, bronchitis, ulcer and stomach problems. Major phytoconstituents of this

species are steroidal lactones. Pharmacological experiments in a number of *in vitro* and *in vivo* models have demonstrated the ability of W*ithania somnifera* to exhibit anti-inflammatory, antiulcer, antidiabetic, central nervous system depressant and hepatoprotective activities leading support to the rationale behind several of its traditional uses [24].

2. Materials and Methods

2.1 Drugs and chemicals

Cypermethrin and deltamethrin formulations were purchased from Bayer Crop Science Ltd., India. Resveratrol was procured from Sigma-Aldrich Company. Methanolic extract of *Withania somnifera* was prepared in the departmental laboratory.

2.2 Animals and treatment

A total of 84 adult male Wistar rats weighing 100-120 g were procured from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, and housed in polyacrylic cages in a group of 7 rats per cage in the departmental animal house. Bedding material (rice husk) was regularly changed on alternate days. The animals were provided with feed and water *ad libitum* and maintained at room temperature with a natural light-dark cycle. Rats were acclimatized to laboratory conditions for 7 days before start of the experiment. Animal house temperature varied between 22 to 27º C throughout the investigation. The prior approval of institutional animal ethics committee was obtained for the use of experimental animals in this study. Forty two rats were used for 14 days study. The rats were randomly divided into six groups, each comprising of seven rats. Group 1 was Naive (control) group which received 3% gum acacia suspension orally. Group 2 was administered cypermethrin (75mg/kg) plus deltamethrin (4mg/kg) as suspension in 3% gum acacia orally. Group 3 animals received cypermethrin (75mg/kg) plus deltamethrin (4mg/kg) as suspension in 3% gum acacia and separately *Withania somnifera* (12.5mg/kg) suspension in 3% gum acacia orally. Group 4 animals were administered cypermethrin (75mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia and separately resveratrol (5 mg/kg) suspension in 3% gum acacia orally. In, Group 5 *Withania somnifera* (12.5 mg/kg) in 3% gum acacia suspension was administered orally, and in Group 6 Resveratrol (5 mg/kg) in 3% gum acacia suspension was administered orally.

2.3 Preparation of tissue homogenates

A 500 mg of tissue (liver, kidney and brain) was weighed and taken in 5 ml of ice-cold PBS (pH 7.4). Another 100 mg of sample (liver, kidney and brain) was weighed separately and taken in 1 ml of 0.02 M ethylene diamine tetra acetic acid (EDTA) solution for reduced glutathione (GSH) estimation. The homogenates (10%) prepared with IKA homogenizer (Germany) under ice-cold condition were centrifuged for 10 min at 3000 rpm. The supernatant was stored at -20°C until assayed for different oxidative stress-related biochemical parameters. A double beam UV-VIS spectrophotometer (UV 5704 SS, ECIL, India) was used for recording the absorbance of the test sample.

2.4 Sampling

Blood samples were taken by sterile hypodermic syringe directly from heart after anaesthetizing rats with ether in heparin coated vials for analysis of blood parameters in sterile tubes.

2.5 Biochemical assay

Different biochemical parameters *viz.* BUN, Creatinine and various blood enzymes like AST, ALT and GGT were determined by autoanalyzer using Erba kits.

2.6 Statistical Analysis

Data were expressed as mean ± SE. Statistical analysis of data was performed using Graph Pad prism 5.03 and Microsoft Excel. Data were analyzed by ANOVA along with Bonferroni multiple comparison post hoc test. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1 Effect of combined treatment of cypermethrin and deltamethrin on ALT, AST, GGT, (IU/L), BUN (mg/dl), creatinine (mg/dl) levels in plasma and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Effect of combined treatment of cypermethrin and deltamethrin on ALT, AST, GGT (IU/L), BUN (mg/dl), creatinine (mg/dl) levels in plasma and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study is presented in Table 1. Combined exposure of cypermethrin and deltamethrin significantly ($p<0.05$) increased the plasma BUN level and creatinine as compared to naive group of animals in 14 days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin and Resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) decreased the elevated BUN value and creatinine as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on plasma BUN level and creatinine as compared to control animals in 14 days study.

Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the plasma ALT, AST and GGT level as compared to naive animals in 14 days study. *Withania somnifera* co-treatment in combined treatment group of cypermethrin and deltamethrin and Resveratrol co-treatment in combined treatment group of cypermethrin and deltamethrin significantly ($p<0.05$) decreased the elevated ALT, AST and GGT level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on plasma ALT, AST and GGT level as compared to naive group in 14 days study.

Table 1: Effect of combined treatment of cypermethrin and deltamethrin on ALT, AST, GGT levels (IU/L), BUN (mg/dl), creatinine (mg/dl) in plasma and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Treatment Groups	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
Naive	35.31 ±3.79	85.59 ±1.23	1.76±0.02	46.56±1.27	1.07±0.01
C + D	47.64^{a} ±2.29	107.27^{a}±1.18	4.84^{a}±0.07	55.17^{a}±1.19	3.52^{a}±0.09
C + D + W	36.77^{b} ±1.78	92.14^{ab} ±0.41	2.65^{ab}±0.13	48.94^{b}±1.04	2.32^{ab}±0.06
C + D + R	36.22^{b} ±2.10	90.53^{ab}±0.50	2.43^{ab}±0.12	47.95^{b}±1.05	2.23^{ab}±0.11
W	34.94^{b}±2.46	84.43^{bcd}±1.08	1.67^{bcd}±0.07	45.99^{b}±1.24	1.23^{bcd}±0.11
R	34.85^{b}±1.88	84.32^{bcd}±0.89	1.64^{bcd}±0.02	46.03^{b}±1.42	1.21^{bcd}±0.09

Values are expressed as Mean ±SEM of seven animals in each group. a, b, c, d, e ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R, respectively. C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.

3.2 Effect of combined treatment of cypermethrin and deltamethrin on reduced glutathione (GSH) (mmol/ml) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Effect of combined treatment of cypermethrin and deltamethrin on reduced glutathione (GSH) (mmol/ml) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study is presented in Table 2 respectively. Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) decreased the GSH level in liver, kidney and brain as compared to naive group in 14 days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the GSH level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. Resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the GSH level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. *Withania somnifera* treatment alone and resveratrol treatment alone significantly ($p<0.05$) increased the GSH level as compared to naive group in 14 days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) have less effect in amelioration as compared to resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin in 14 days study.

3.3 Effect of combined treatment of cypermethrin and deltamethrin on tissue protein (mg protein/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Effect of combined treatment of cypermethrin and deltamethrin on tissue protein (mg protein/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study is presented in Table 3 respectively. Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) decreased the tissue protein level in liver, kidney and brain as compared to naive group in 14 days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the tissue protein level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. Resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the tissue protein level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on tissue protein level as compared to naive group in 14 days study.

3.4 Effect of combined treatment of cypermethrin and deltamethrin on MDA levels (nmol/g tissue) in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Effect of combined treatment of cypermethrin and deltamethrin on MDA levels (nmol/g tissue) in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study is presented in Table 4 respectively. Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the MDA level in liver, kidney and brain as compared to naive group in 14 days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) reduced the MDA level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. Resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) reduced the MDA level as compared to combined treatment of cypermethrin and deltamethrin in 14 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on MDA level as compared to naive group in 14 days study.

Table 2: Effect of combined treatment of cypermethrin and deltamethrin on reduced glutathione (GSH) (mmol/ml) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Treatment Groups	GSH (mmol/ml)		
	Liver	Kidney	Brain
Naive	4.93 ± 0.02	4.38 ± 0.01	3.63 ± 0.02
C + D	2.50^{a} ± 0.02	1.57^{a} ± 0.02	1.69^{a} ± 0.03
C + D + W	3.93^{ab} ± 0.02	3.60^{ab} ± 0.02	2.90^{ab} ± 0.02
C + D + R	4.14^{abc} ± 0.01	3.82^{abc} ± 0.01	3.09^{abc} ± 0.02
W	5.14^{abcd} ± 0.01	4.55^{abcd} ± 0.01	3.76^{abcd} ± 0.01
R	5.20^{abcd} ± 0.01	4.60^{abcd} ± 0.02	3.85^{abcd} ± 0.01

Values are expressed as Mean ±SEM of seven animals in each group. a, b, c, d, e ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R, respectively. C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.

Table 3: Effect of combined treatment of cypermethrin and deltamethrin on tissue protein (mg protein/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Treatment Groups	Tissue protein (mg protein/g tissue)		
	Liver	Kidney	Brain
Naïve	81.12 ± 0.51	85.80 ± 0.75	78.40 ± 0.67
C + D	26.79^{a} ± 0.75	40.60^{a} ± 0.68	32.14^{a} ± 0.65
C + D + W	74.78^{ab} ± 0.74	76.67^{ab} ± 0.48	71.39^{ab} ± 0.80
C + D + R	76.74^{ab} ± 0.27	78.81^{ab} ± 0.41	74.33^{ab} ± 1.09
W	81.35^{bcd} ± 0.77	87.01^{bcd} ± 1.16	79.69^{bcd} ± 0.64
R	81.95^{bcd} ± 1.34	86.93^{bcd} ± 0.65	81.02^{bcd} ± 0.43

Values are expressed as Mean ±SEM of seven animals in each group. a, b, c, d, e ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R, respectively. C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.

Table 4: Effect of combined treatment of cypermethrin and deltamethrin on MDA levels (nmol/g tissue) in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Treatment Groups	MDA (nmol/g tissue)		
	Liver	Kidney	Brain
Naive	155.22 ± 0.45	153.14 ± 0.32	174.72 ± 0.43
C + D	178.35^{a} ± 0.39	172.91^{a} ± 0.31	198.07^{a} ± 0.48
C + D + W	160.27^{ab} ± 0.41	158.90^{ab} ± 0.13	179.28^{ab} ± 0.21
C + D + R	159.78^{ab} ± 0.59	158.02^{ab} ± 0.52	179.56^{ab} ± 0.30
W	155.66^{bcd} ± 0.40	153.84^{bcd} ± 0.20	174.94^{bcd} ± 0.33
R	154.28^{bcd} ± 0.42	153.73^{bcd} ± 0.28	174.61^{bcd} ± 0.35

Values are expressed as Mean ±SEM of seven animals in each group. a, b, c, d, e, f ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R, respectively. C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.

4. Discussion

4.1 Effect of combined treatment of cypermethrin and deltamethrin on MDA levels (nmol/g tissue) in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Malondialdehyde (MDA) is a lipid peroxidation end product. Many pesticides are hydrophobic molecules that bind extensively to biological membranes, especially to phospholipid bilayers and can damage the membrane by inducing lipid peroxidation [41]. However, many pesticides are also known to be associated with enhanced production of ROS [17, 18]. Interaction of ROS with cellular membrane results in membrane lipid peroxidation which can be assessed by MDA. Lipids containing PUFA have abundant sites for the target of ROS because they have double bonds between carbon atoms. Lipid peroxidation is a self-propagating reaction until it is inhibited by antioxidant enzymes. Increased MDA suggests an increased production of free oxide free radicals in rats [27].

The free radical generation leads to DNA damage, protein degradation, LPO (lipid peroxidation) and finally culminating into damage to various vital tissues like liver, kidney, brain and testes [6, 8, 23, 40, 44]. These elevated free radicals and depressed antioxidant defence may lead to cell disruption, oxidative damage to cell membranes and hence increase susceptibility to LPO [41, 22]. In present study of 14 days increase in the values of MDA was observed in liver, kidney and brain tissues in combined cypermethrin and deltamethrin treated animals and *Withania somnifera* co-treatment and resveratrol co-treatment reversed the increased MDA values in combined cypermethrin and deltamethrin treated rats. The findings of this study are similar to those of various workers [5, 9, 25, 31, 32, 33, 35] who reported a significant increase in values of MDA on individual administration of cypermethrin and deltamethrin. These results are also in accordance with those of Ahmad *et al.* [2] and Chaursia *et al.* [12] who reported *Withania somnifera* co-treatment reduced the MDA level in 6-hydroxydopamine induced parkinsonism in rats and in lead treated groups in mouse.

4.2 Effect of combined treatment of cypermethrin and deltamethrin on tissue protein (mg protein/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Tissue protein level is a significant indicator of health condition, metabolic and production features of the organism because of numerous roles in the patho-physiology. In the present study, there was significant decrease in tissue protein level in animals of combined cypermethrin and deltamethrin administered groups was observed in 14 days as well as in 28 days study. These findings were in agreement with Bhatti *et al.* [9] who also reported significant decrease in tissue protein on oral administration of cypermethrin in rats. Resveratrol co-treatment and *Withania somnifera* co-treatment significantly increased the reduced level of tissue protein.

4.3 Effect of combined treatment of cypermethrin and deltamethrin on reduced glutathione (GSH) (mmol/ml) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

Glutathione and glutathione-related enzymes are involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds as well as ROS. Glutathione is synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine. GSH is cell's natural antioxidant which destroys free radical formed in cells. GSH (tripeptide) is an important non-protein thiol which is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx, GR and GST [28, 25]. When oxidative stress occurs, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation. The sulfhydryl (thiol) group (SH) of cysteine serves as a proton donor and is responsible for the biological activity of glutathione. In addition, GSH participates in detoxification of xenobiotics as substrate for

enzyme GST. GSH level is reduced by imidacloprid at high dose which may be through direct utilization of GSH as an antioxidant in terminating free radical reaction [7].

Depletion of GSH, the most abundant cellular non-protein thiol, is associated with oxidative stress and cytotoxicity of pro-oxidant xenobiotics.

In the present study, combined treatment of cypermethrin and deltamethrin induced decrease in the level of GSH. Findings are also in agreement with Manna *et al.* [26] who also observed a dose dependent decrease in GSH content on exposure of deltamethrin in rats. Similar observation has been reported [9, 32, 35] where a dose dependent decrease in GSH content on exposure of cypermethrin in rats.

In the present study, resveratrol co-treatment and *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly restored the values towards normal. Our results are in accordance with that of Sharma *et al.* [38] where similar observations were recorded in cypermethrin treated rats. Sener *et al.* [36, 37] also reported protective effect of resveratrol in ischemia induced oxidative renal injury in rats where it restored the GSH levels. Findings of our study are also in agreement with Roy *et al.* [34] where protective effect of resveratrol in carbon tetrachloride induced hepatotoxicity in rats was reported. *Withania somnifera* also restored the value of GSH towards normal in acephate treated rats [21]. *Withania somnifera* alone treatment also increased the GSH level as compared to naive group. These findings are in agreement with Davis and Kuttan [14] who reported an increase in GSH level in *Withania somnifera* treated animals as compared to naive.

4.4 Effect of combined treatment of cypermethrin and deltamethrin on ALT, AST and GGT levels (IU/L), BUN (mg/dl), creatinine (mg/dl) in plasma and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study

The liver function transaminases (ALT and AST) enzyme activity in plasma are most frequently measured for diagnosis of liver diseases particularly infective hepatitis, alcoholic cirrhosis, biliary obstruction, toxic hepatitis and liver cancer [1, 43]. AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidney, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute haemolytic anaemia, severe burns, acute renal disease, musculoskeletal diseases and trauma.

GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and xenobiotic detoxification [13]. GGT is predominantly used as a diagnostic marker for liver disease. Elevated GGT activity can be found in diseases of the liver, biliary system and pancreas.

Biochemical analysis performed in 14 days experiments showed that the oral intake of combined cypermethrin and deltamethrin resulted in rise in liver functional enzymes activities in plasma of treated animals. Hadi and Yassin [19] have also reported a significant increase in AST, ALT and GGT values in plasma of male Wistar rats on administration of cypermethrin orally.

In present study, resveratrol co-treatment and *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly reduced the elevated plasma level of liver biomarker enzymes. Our results are in agreement with findings of Elberry *et al.* [15] and Amandeep and Yadav [4] who reported that *Withania somnifera* co-treatment reduces the elevated levels of AST, ALT in carbon tetrachloride treated rats and in lead treated chickens.

Creatinine is derived mainly from the catabolism of creatinine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Increase in creatinine levels may be the indicator of the degeneration of the kidney, heart muscle and other muscles. The elevation of plasma levels of BUN and creatinine are considered as significant markers of renal dysfunction [3]. Elevation of BUN and creatinine levels in plasma of treated male rats may be attributed to reduction in glomerular filtration in the kidney tubules [20].

Combined treatment of cypermethrin and deltamethrin induced increase in BUN and creatinine levels which were observed in rats in the present investigation in 14 days study, may be due to the effect of pesticides on liver function, as urea is the end product of protein catabolism. Elevated BUN is correlated with an increased protein catabolism in mammalian body or from more efficient conversion to urea as a result of increased synthesis of enzyme involved in urea production. A significant increase was found in creatinine and BUN levels in present study which is a classical sign that kidney was affected by combined treatment of cypermethrin and deltamethrin administration in rats. Mongi *et al.* [30] have also reported an increase in BUN and creatinine in male Wistar rats on oral administration of deltamethrin. Hadi and Yassin [19] have also reported a significant increase in creatinine and BUN levels in plasma of male Wistar rats following oral administration of cypermethrin.

In the present study, resveratrol co-treatment and *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly reduced the elevated levels of BUN and creatinine in rats.

5. Summary and Conclusion

Hepatic biomarkers like plasma ALT, AST, GGT and renal biomarkers like BUN and creatinine were significantly increased in combined cypermethrin and deltamethrin treated animals as compared to naive indicating its hepatotoxicity and nephrotoxicity which was significantly restored by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin treated rats in 14 days study. Combined treatment of cypermethrin and deltamethrin resulted in oxidative stress as compared to naive which was normalized significantly by resveratrol co-treatment and *Withania somnifera* co-treatment. A statistically significant increase in levels of MDA was observed in combined cypermethrin and deltamethrin treated animals as compared to naive in 14 days study indicating increase in lipid peroxidation which was decreased by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin administered animals in 14 days study. Tissue protein levels and reduced glutathione were significantly decreased in combined cypermethrin and deltamethrin treated animals as compared to naive which were significantly restored by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin

and deltamethrin treated rats in 14 days study.

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