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Biochemical alterations following combined exposure of cypermethrin and deltamethrin and their amelioration by *Withania somnifera* and resveratrol in male rats

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Abstract

This experiment was conducted to evaluate the ameliorating effect of *Withania somnifera* and resveratrol against induced biochemical changes by combined exposure of cypermethrin and deltamethrin in rats during month of June, 2017. The combined exposure of cypermethrin and deltamethrin was given in adult male Wistar rats. Various biochemical parameters were analysed up to 28 days and their amelioration by *Withania somnifera* and resveratrol on various biochemical alterations was investigated. Cypermethrin and deltamethrin was given orally at rate of 75mg/kg and 4mg/kg body weight in adult male Wistar rats. Rats did not exhibit any marked changes in their gross behavioural signs and symptoms. The combined exposure of cypermethrin and deltamethrin caused significant increase in ALT (61.28 IU/L), AST(161.38 IU/L), GGT(6.82 IU/L), BUN(64.96mg/dl) and creatinine (4.09 mg/dl) levels 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 near normal observed following combined cypermethrin and deltamethrin exposure in rats. The present study showed that combined cypermethrin and deltamethrin exposure cause biochemical alterations 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, *Withania somnifera* and resveratrol

1. Introduction

Synthetic pyrethroids is an unique group of insecticides having pyrethrin like structure with better performance characterized and account for over 30% of insecticide use globally [5]. 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 [5].

Cypermethrin is a synthetic pyrethroid insecticide which is used to kill insects especially on cotton. It behaves as a fast-acting neurotoxin in insects [5]. Cypermethrin is used in agriculture to control ectoparasites which infest cattle, sheep and poultry [24]. In veterinary practice, it is effective in controlling ticks on dogs. 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 [5].

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 [27].

Resveratrol is a fat soluble compound that occurs in *trans* and *cis* configuration. Resveratrol (trans-3, 5, 4-trihydroxystilbene), a polyphenolic phytoalexin abundantly found in grapes and red wine is a potent antioxidant and cytoprotective agent [6]. 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 [10]. The major dietary sources of stilbenes include grapes, wine, soy, peanuts and peanut products [6].

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Pedimelum cuspidatum root used for long in traditional Asian medicine, as a circulatory tonic, is a source of resveratrol [10].

Withania somnifera is a plant in the Solanaceae or nightshade family. It is used as an herb in ayurvedic medicine. The main chemical constituents are alkaloids and steroidal lactones [19]. *Withania somnifera* possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties [18]. *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 [19]. 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 *Withania 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 [18]. The present study was conducted to investigate the ameliorating effect of *Withania somnifera* and resveratrol against induced biochemical changes by combined exposure of cypermethrin and deltamethrin.

2. Materials and Methods

This experiment was conducted in month of June, 2017.

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 27 °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, while remaining forty two rats were used for 28 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. Experimental groups were same for 14 days as well as for 28 days study.

2.3 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.

2.4 Biochemical assay

Different biochemical parameters viz. BUN, Creatinine and various blood enzymes like AST, ALT and GGT were determined by autoanalyzer using Erba kits [14].

2.5 Statistical Analysis

Data were expressed as mean \pm 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

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 male rats in 14 and 28 days study

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 male rats in 14 and 28 days study is presented in Table 1 and 2 respectively.

Combined treatment of cypermethrin and deltamethrin significantly ($p < 0.05$) increased the plasma creatinine level as compared to control animals in both 14 and 28 days study. *Withania somnifera* co-treatment and Resveratrol co-treatment in combined treatment group of cypermethrin and deltamethrin decreased significantly ($p < 0.05$) the elevated creatinine value in both 14 and 28 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on plasma creatinine level as compared to naive group of animals in both 14 and 28 days study.

Combined exposure of cypermethrin and deltamethrin significantly ($p < 0.05$) increased the plasma BUN level as compared to naive group of animals in both 14 and 28 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 as compared to combined treatment of cypermethrin and deltamethrin in both 14 and 28 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on plasma BUN level as compared to control animals in both 14 and 28 days study.

Combined treatment of cypermethrin and deltamethrin significantly ($p < 0.05$) increased the plasma GGT level as compared to naive animals in both 14 and 28 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 GGT level as compared to combined treatment of cypermethrin and deltamethrin in both 14 and 28 days study.

Withania somnifera treatment alone and resveratrol treatment alone did not have any effect on plasma GGT level as compared to naive group in both 14 and 28 days study.

Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the plasma ALT and AST level as compared to naive animals in both 14 and 28 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 and AST level as compared to combined treatment of cypermethrin and deltamethrin in both 14 and 28 days study. *Withania somnifera* treatment alone and resveratrol treatment alone did not have any effect on plasma ALT and AST level as compared to naive group in both 14 and 28 days study.

Table 1: 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 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 ($p<0.05$) 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 2: Effect of combined treatment of cypermethrin and deltamethrin on ALT, AST and GGT levels (IU/L), BUN (mg/dl), creatinine (mg/dl) and its amelioration by *Withania somnifera* and resveratrol in male rats in 28 days study

Treatment Groups	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	BUN (mg/dl)	Creatinine (mg/dl)
Naive	36.92 ±1.27	86.50 ±1.13	1.84 ±0.01	49.31 ±1.02	1.04 ±0.06
C + D	61.28 ^a ±1.50	161.38 ^a ±1.25	6.82 ^a ±0.19	64.96 ^a ±1.67	4.09 ^a ±0.06
C + D + W	39.08 ^b ±1.27	105.95 ^{ab} ±1.38	3.06 ^{ab} ±0.05	52.90 ^b ±0.81	2.27 ^{ab} ±0.05
C + D + R	38.38 ^b ±1.02	102.61 ^{ab} ±0.96	2.98 ^{ab} ±0.04	52.63 ^b ±1.05	2.14 ^{ab} ±0.02
W	37.01 ^b ±1.67	90.04 ^{bcd} ±0.58	1.69 ^{bcd} ±0.04	49.63 ^b ±0.71	1.10 ^{bcd} ±0.03
R	36.91 ^b ±1.42	89.56 ^{bcd} ±0.63	1.61 ^{bcd} ±0.02	49.29 ^b ±0.61	1.04 ^{bcd} ±0.05

Values are expressed as Mean ±SEM of seven animals in each group.

a, b, c, d, e ($p<0.05$) 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 liver biomarker enzyme levels and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days as well as in 28 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 [25, 17, 16, 28, 2].

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 [21]. 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 [21].

GGT catalyzes the transfer of gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water molecule. GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and xenobiotic detoxification [8]. 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 [21].

Biochemical analysis performed in 14 days and 28 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. The hepatic biomarkers value increased due to damage of hepatocytes. Hadi and Yassin [12] also reported a significant increase in AST, ALT and GGT values in plasma of male Wistar rats on administration of cypermethrin orally. Significant increase in hepatic markers enzyme values in plasma of male mice and male rats was also observed in which deltamethrin was administered orally (Abdel-Daim *et al.* [1] and Mongi *et al.* [20]).

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 due to their anti-inflammatory, antistress, antioxidant, and rejuvenating properties. These results are in agreement with findings of Elberry *et al.* [9] 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.

4.2 Effect of combined treatment of cypermethrin and deltamethrin on creatinine and BUN levels and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days as well as in 28 days study

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 ^[23]. Creatinine is useful in early detection of nephrotoxicity induced by exogenous compounds.

The elevation of plasma levels of BUN and creatinine are considered as significant markers of renal dysfunction ^[7] ^[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 ^[13] ^[26].

BUN and creatinine are waste products of protein metabolism that need to be excreted by the kidney. Therefore, marked increase in plasma BUN and creatinine, as observed in this study, confirms an indication of degenerative changes in the kidney. These changes cause disturbance in the transport system of biochemical constituents in the kidney ^[15] ^[11].

In the present investigation, combined treatment of cypermethrin and deltamethrin increased BUN and creatinine levels in 14 days as well as in 28 days. It may be due to the effect of pesticides on liver function, as urea is the end product of protein catabolism ^[23]. 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 ^[22]. A significant increase was found in creatinine and BUN levels, which is a classical sign that kidney was affected by combined treatment of cypermethrin and deltamethrin administration in rats. These results are in agreement with findings of Mongi *et al.* ^[20] and Hadi and Yassin ^[12]. They also reported a significant increase in creatinine and BUN levels in plasma of male Wistar rats following oral administration of deltamethrin and cypermethrin respectively. 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. Conclusion

From the present study it can be concluded that the hepatic biomarkers like plasma ALT, AST and GGT were significantly increased in combined cypermethrin and deltamethrin treated animals as compared to naive indicating its hepatotoxicity which was significantly restored by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin treated rats in both 14 as well as in 28 days study.

Plasma levels of BUN and creatinine were significantly increased in combined cypermethrin and deltamethrin treated animals as compared to naive indicating its nephrotoxicity which were significantly restored by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin treated rats in both 14 as well as in 28 days study.

Future Prospectives

These two insecticides are used indiscriminately in crops by the farmers to combat insects and leads to toxicity in animals. Farmers use these insecticides in fodder crops and are not aware about the toxic effects on their animals. Sometimes these insecticides also lead the animals to death. Resveratrol co-treatment and *Withania somnifera* co-treatment may help to save the lives of these animals. Present findings may also help the field veterinarians to use these treatments to retard toxicity of these insecticides. More avenues are also there to study about the anti-toxic properties of resveratrol and *Withania somnifera* against other groups of insecticides.

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