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Effects of combined exposure of cypermethrin and deltamethrin on myeloperoxidase level and their amelioration by *Withania somnifera* and resveratrol in male rats

Robin, SK Jain and Pooja Kundu

Abstract
Cypermethrin and deltamethrin are synthetic pyrethroid insecticide used in agriculture, home pest control and disease vector control. Pyrethroids cause oxidative stress and alteration in antioxidant enzymes in erythrocytes of pyrethroid intoxicated rats. *Withania somnifera* and resveratrol possesses anti-inflammatory, antitumor, anti-stress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. Myeloperoxidase (MPO) is an enzyme stored in azurophilic granules of polymorphonuclear cells and macrophages and released into extracellular fluid in the setting of inflammatory process. The assessment of MPO activity is well established for quantifying inflammation. In inflammatory conditions, the neutrophil levels of inflammed tissues, and consequently the MPO enzyme level is increased. The present study was undertaken to investigate effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male Wistar rats in 14 and 28-days experimental study. Combined treatment of cypermethrin and deltamethrin significantly increased levels of MPO in tissues in both 14 and 28 days study as compared to naive which were reduced significantly by resveratrol and *Withania somnifera* co-treatment in cypermethrin and deltamethrin administered groups in both 14 as well as in 28 days. In conclusion, resveratrol co-treatment and *Withania somnifera* co-treatment reduced the MPO activity indicating its protective effect against cellular injury in adult male rats.

Keywords: cypermethrin, deltamethrin, myeloperoxidase, resveratrol and *Withania somnifera*

1. Introduction
Cypermethrin, a synthetic pyrethroid insecticide which is used to kill insects in agriculture field. It is a fast-acting neurotoxin in insects. It interacts with transportation system of sodium ions which presents on cellular membrane. In veterinary science, it is effective in controlling ticks and other ectoparasites on dogs, cattle and sheep. Pyrethroids cause oxidative stress and alteration in antioxidant enzymes in RBC of pyrethroid intoxicated rats [6].
Deltamethrin, a synthetic pyrethroid insecticide which is also mainly used in home pest control, disease vector control and agriculture. Deltamethrin has the ability to pass from a woman's skin into systemic circulation and breast milk [2]. Since deltamethrin is a potent neurotoxin, it attacks the nervous system of any animal and insects with which it comes into contact. Skin contact with pyrethroids can lead to tingling or reddening of the skin. Main mechanism of action of deltamethrin include, prolongs the opening of voltage sensitive sodium channels and inhibition of voltage gated chloride channels and GABA receptors present on cell membrane [13]. Lipid peroxidation is also caused by deltamethrin at high dose when given in combination with chlorpyrifos [12].
Resveratrol, a fat-soluble compound mainly found in *trans* and *cis* configuration. It is a potent antioxidant and cytoprotective agent which is abundantly found in grapes and red wine. Resveratrol was originally isolated from the roots of hellebore by Takaoka. It gains wider attention in 1992, when its presence in wine was suggested as the clarification for cardioprotective effects of wine [1]. It has also been found that Resveratrol also possess a number of potentially cardioprotective effects *in vitro*, including promotion of vasodilation by enhancing the production of nitric oxide (NO) [5] antidiabetic, neuroprotective [1] and anti-inflammatory properties.
Withania somnifera, a plant in the Solanaceae family, mainly used as an herb in ayurvedic medicine. Its main chemical constituents of *Withania somnifera* are alkaloids and

steroidallactones, which include tropine and cuscohygrine. The leaves contain the steroidal lactones mainly withaferin A. Bioactive constituent withaferin A has shown good potential in therapy for glioblastomas, although this is not a traditional use of the plant. Pharmacological experiments in a number of *in-vitro* and *in-vivo* models have demonstrated that *Withania somnifera* also possess anti-inflammatory, anti-tumor, antistress, hemopoetic, antiulcer, hepatoprotective, immunomodulatory, antioxidant and rejuvenating properties [9] leading support to the rationale behind several of its traditional uses [8].

The present study was undertaken to investigate the protective effects of resveratrol and *Withania somnifera* on combined cypermethrin and deltamethrin exposure induced myeloperoxidase level changes in adult male rats.

2. Materials and Methods

2.1 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. All animals were housed in polyacrylic cages in a group of 7 rats per cage in the departmental animal house. Animals were provided with feed and water *ad libitum*. Animals were 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 ºC throughout the study. 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 42 rats were used for 28 days study. Cypermethrin and Deltamethrin formulations were purchased from Bayer Crop Science Ltd., India. Resveratrol was procured from Sigma-Aldrich Company. Methanolic extract of *Withania somnifera* roots were prepared in the departmental laboratory.

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 given cypermethrin (75 mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia orally. Group 3 animals received cypermethrin (75 mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia and separately *Withania somnifera* (12.5 mg/kg) suspension in 3% gum acacia orally. Group 4 animals were administered cypermethrin (75 mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia and separately resveratrol (5mg/kg) as suspension in 3% gum acacia orally. In group 5 *Withania somnifera* (12.5 mg/kg) in 3% gum acacia suspension was administered orally. In group 6 animals, resveratrol (5 mg/kg) in 3% gum acacia suspension was administered orally. Experiment groups were same for 14 days as well as for 28 days study.

2.2 Estimation of myeloperoxidase

MPO activity, a sensitive index of tissue polymorphonuclear leukocytes (PMNL) sequestration in tissue homogenate was measured spectrophotometrically using o-dianisidine and hydrogen peroxide (H_2O_2) was estimated by method of Bradley and co-workers [3]. In the presence of H_2O_2 as oxidizing agent, MPO catalyses the oxidation of o-dianisidine yielding a brown coloured product, oxidized o-dianisidine, with a maximum absorbance at 460 nm, according to the following overall reaction:

$2H_2O_2$ + o-dianisidine = oxidized dianisidine + $4H_2O$

One unit (U) of MPO activity was defined as that degrading 1 µmol of hydrogen peroxide per minute at 25 °C.

Reagents

A. Potassium-Phosphate buffer solution (50 mmol/L, pH-6.0) containing 0.167 mg/ml of dianisidine hydrochloride.
B. Hydrogen peroxide solution: 0.0005%.

Procedure

To 0.1 ml of tissues homogenate in a 3 ml cuvette, 2.9 ml of 50 mmol/L potassium phosphate buffer, pH 6.0, containing 0.167 mg/ml o-dianisidine and 0.0005% hydrogen peroxide was added. The hydrogen peroxide was added just before estimation. The sample absorbance was measured at 460 nm for 2 min against blank. MPO activity per gram tissue (gt) was calculated by the following formula:

$$\text{MPO activity (units/gt)} = (\Delta A460) \times (13.5)/\text{tissue weight (g)}.$$

Where ΔA460 was the changes in absorbance at 460 nm from 30 to 90 seconds (of 2 minutes absorbance measurement) after the initiation of the reaction. The coefficient 13.5 was empirically determined such that 1-unit MPO activity was the amount of enzyme that would reduce 1 µmole peroxide/min.

2.3 Statistical analysis

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

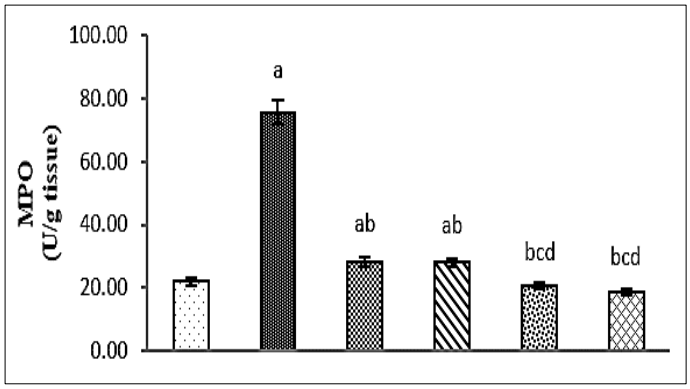
Effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14- and 28-days study is presented in Table 1, 2 and figure 1, 2 respectively.

Combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) increased the MPO level in liver, kidney and brain as compared to naive group in both 14- and 28-days study. *Withania somnifera* co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) reduced the MPO level as compared to combined treatment of cypermethrin and deltamethrin in both 14 and 28-days study. Resveratrol co-treatment along with combined treatment of cypermethrin and deltamethrin significantly ($p<0.05$) reduced the MPO 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 MPO level as compared to naive group in both 14 and 28-days study.

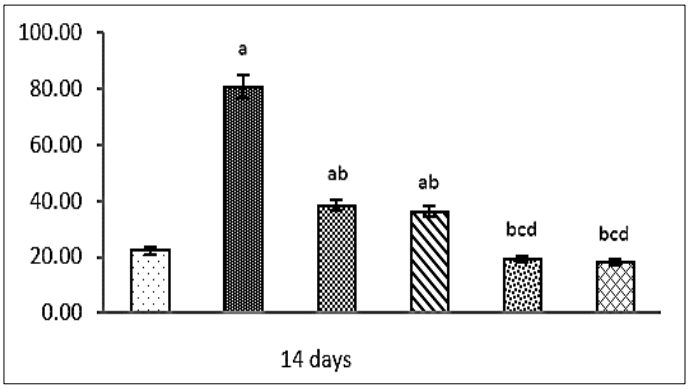
Table 1: Effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study.

Treatment groups	MPO (U/g tissue)		
	Liver	Kidney	Brain
Naive	21.98 ± 0.91	22.17 ± 1.21	20.05 ± 0.99
C + D	75.60^{a} ± 1.38	80.61^{a} ± 0.86	96.23^{a} ± 0.86
C + D + W	28.15^{ab} ± 1.29	38.37^{ab} ± 0.71	46.09^{ab} ± 1.45
C + D + R	27.96^{ab} ± 0.70	36.25^{ab} ± 1.72	45.12^{ab} ± 0.77
W	20.63^{bcd} ± 1.20	19.09^{bcd} ± 1.23	19.86^{bcd} ± 0.86
R	18.51^{bcd} ± 1.27	18.12^{bcd} ± 0.57	19.28^{bcd} ± 0.56

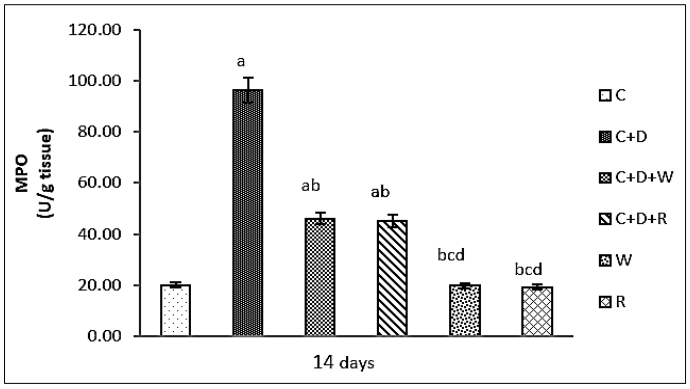
Values are expressed as Mean ±SEM of seven animals in each group.
a, b, c, d, e ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R, respectively.
C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.



Liver



Kidney



Brain

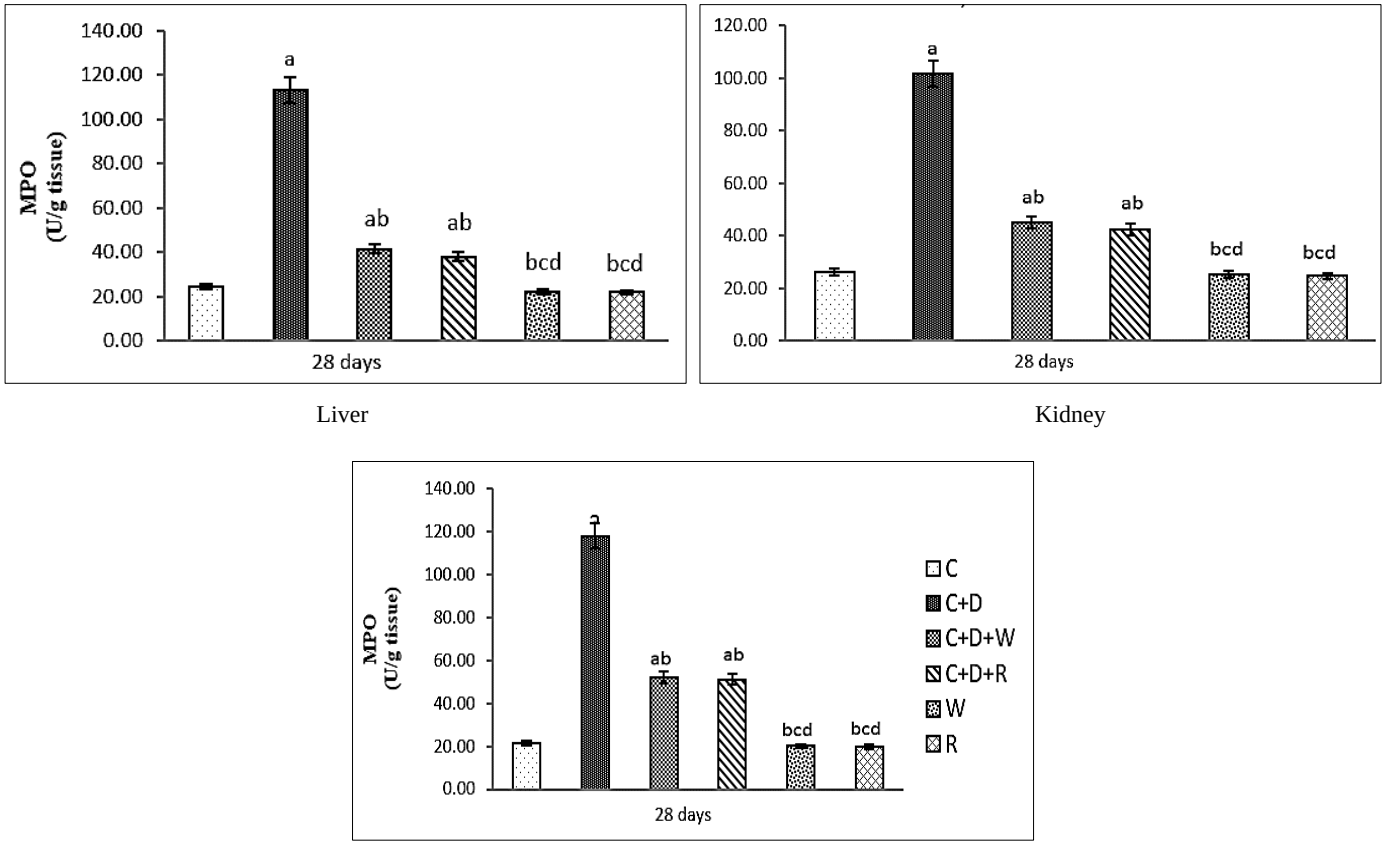
Values are expressed as Mean ±SEM of seven animals in each group. a, b, c, d, e ($p \leq 0.05$) vs. control, C + D, C + D + W, C + D + R, W and R respectively. C + D mean 10% of MTD of cypermethrin and deltamethrin individually used in combination.

Fig 1: Effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 14 days study.

Table 2: Effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 28 days study.

Treatment groups	MPO (U/g tissue)		
	Liver	Kidney	Brain
Naive	24.30 ± 1.41	26.22 ± 0.77	21.40 ± 0.90
C + D	113.20^{a} ± 1.03	101.63^{a} ± 0.70	117.84^{a} ± 1.20
C + D + W	41.46^{ab} ± 0.86	44.93^{ab} ± 1.05	52.07^{ab} ± 1.09
C + D + R	37.99^{ab} ± 0.95	42.42^{ab} ± 0.97	51.10^{ab} ± 0.54
W	22.17^{bcd} ± 1.13	25.26^{bcd} ± 0.76	20.05^{bcd} ± 0.68
R	21.79^{bcd} ± 1.45	24.68^{bcd} ± 0.81	19.86^{bcd} ± 0.63

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.



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.

Fig 2: Effect of combined treatment of cypermethrin and deltamethrin on MPO (U/g tissue) levels in liver, kidney and brain and its amelioration by *Withania somnifera* and resveratrol in adult male rats in 28 days study.

4. Discussion

Myeloperoxidase (MPO) is an enzyme stored in azurophilic granules of polymorphonuclear cells and macrophages and released into extracellular fluid in the setting of inflammatory process. Its activity in the tissue is related linearly to neutrophilic infiltration. The assessment of MPO activity is well established for quantifying inflammation [7]. In inflammatory conditions, the neutrophil levels of inflammed tissues, and consequently the MPO enzyme level is increased. MPO is a major contributing enzyme in the formation of ROS leading to tissue damage and an efficient marker of oxidative stress. In the present study of 14 days and 28 days, combined treatment of cypermethrin and deltamethrin resulted in increase in MPO activity in tissues. Resveratrol co-treatment and *Withania somnifera* co-treatment reduced the MPO activity indicating its protective effect against cellular injury. Chang and co-workers [4] reported that resveratrol influences glial responses to rotenone by regulating MPO and thus protects against rotenone-induced neuronal injury. Likewise, Sener *et al.* also reported similar findings indicating protective effects of resveratrol against acetaminophen-induced toxicity in mice where resveratrol treatment reversed back neutrophilic infiltration to normal [10]. Resveratrol exerts protective effects via its radical scavenging and antioxidant activities, which appear to involve the inhibition of tissue neutrophil infiltration [11].

5. Conclusion

In conclusion, Combined treatment of cypermethrin and deltamethrin significantly increased levels of MPO in tissues in both 14 as well as in 28 days as compared to naive which were reduced significantly by resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin administered groups in both 14 as well as in 28 days.

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