



## Protective Role of L-ascorbic Acid in Oxidative Stress Induced by Repeated Oral Administration of Bifenthrin in Wistar Rats

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### ABSTRACT

The present study was aimed to evaluate the modulatory role of L-ascorbic acid against oxidative stress in bifenthrin intoxicated rats. Rats were divided into four groups with six rats in each group. Group I animals received corn oil and served as control while as group II animals were orally treated with bifenthrin @ 5.8mg/Kg/day. In group III, vitamin C was orally administered @ 60mg/Kg/day where as group IV received both vitamin C and bifenthrin @ 60mg/Kg/day and 5.8mg/Kg/day respectively. After 30<sup>th</sup> day of treatment, blood samples were taken and analysed for oxidative stress parameters. Significant ( $P<0.05$ ) increase in MDA levels was observed in bifenthrin treated animals as compared to control and vitamin C treated animals. Blood glutathione level decreased significantly ( $P<0.05$ ) in bifenthrin intoxicated animals as compared to control. Similarly, the activities of antioxidant enzymes viz., SOD, GSH-Px, GST and CAT decreased significantly ( $P<0.05$ ) in bifenthrin treated rats as compared control. Ameliorative group receiving both bifenthrin and L-ascorbic acid significantly restored the normal values of various oxidative stress parameters except GST.

**Keywords:** Bifenthrin, L-ascorbic acid, oxidative stress, rats

Bifenthrin is a newly introduced type-1 pyrethroid which was first approved for use in UK in 1988. Its properties like low water solubility and photo-stability makes it an effective insecticide and acaricide against a broad range of foliar pests in agriculture and animal husbandry habitations (Walker *et al.* 1992). Human and animal exposure to bifenthrin can occur through oral, pulmonary and dermal routes (Llewellyn *et al.* 1996).

Compared to other pyrethroids, toxic effects of bifenthrin are more and the studies describing the alteration in biochemistry, haematology and histopathology due to its toxicity are limited only to insects (Shakoori *et al.* 1994; Ahmed *et al.* 2004). Very few studies are available which have reported the oxidative stress potential of this insecticide in particular and pyrethroids in general in higher animals (Kale *et al.* 1999; Giray *et al.* 2001; Prasanthi *et al.* 2005; Raina *et al.* 2009). Therefore, present study was envisaged to evaluate the oxidative stress potential of bifenthrin in

rats following its repeated oral administration for a period of 30 days and also to evaluate role of L-ascorbic acid in controlling such oxidative damage.

### MATERIALS AND METHODS

#### Chemicals

Biflex<sup>R</sup>, containing 2.5% bifenthrin was purchased from FMC India Pvt. Limited, Tamil Nadu, while as analytical reagent of L-ascorbic acid procured from High Media Laboratories Pvt. Ltd, Mumbai were used in the study.

#### Animals and Experimental Design

Twenty four adult wistar rats (200-250gm) of either sex procured from Indian Institute of Integrative Medicine, Jammu were used in the present study. All the animals were acclimatized in the laboratory conditions for 2

weeks under standard condition with food and water *ad libitum* following duly approved IAEC protocol. Rats were randomly divided into four groups of six rats each and were orally administered with corn oil in group I as control, bifenthrin @ 5.8mg/Kg/day (1/10<sup>th</sup> LD<sub>50</sub>) in group II, vitamin C @60mg/Kg/day in group III and Vitamin C (@60mg/Kg/day) and bifenthrin @ 5.8mg/Kg/day in group IV for a period of 30 days.

The rats were anaesthetized with diethyl ether and blood samples were collected from retro-orbital fossa using capillary tubes in aliquots containing heparin @ 10 IU/ml of blood. Prior to centrifugation, 200µl whole blood was used for estimation of blood glutathione (GSH) (Beutler 1975). Then 1% haemolysate was used for estimation of superoxide dismutase (SOD) (Marklund and Marklund 1974), catalase (CAT) (Aebi, 1983), glutathione-S-transferase (GST) (Habig *et al.* 1974) and glutathione peroxidase (GSH-Px) (Hafeman *et al.* 1974). Lipid peroxidation in terms of malondialdehyde (MDA) level was estimated in 33% haemolysate (Shafiq-Ur-Rehman, 1984).

## RESULTS AND DISCUSSION

The results of the effect of bifenthrin alone and in combination with L-ascorbic acid on different oxidative stress parameters are presented in table 1. The main toxic symptoms like in-coordination, muscle weakness and irritability recorded in bifenthrin treated animals (Group

I) were not observed in animals given L-ascorbic acid and bifenthrin together. Bifenthrin treated rat manifested significant increase in MDA level as compared to control (Group I) and L-ascorbic treated group III. Significant decrease of MDA level was observed in group IV as compared to bifenthrin treated group II. Blood glutathione level in bifenthrin treated group II decreased significantly as compared to control (Group I) and L-ascorbic treated group III. Ameliorative group IV showed significantly increased blood glutathione as compared to bifenthrin treated animals (Group II). Significant decrease in superoxide dismutase and glutathione peroxidase was observed in group II (Bifenthrin) as compared to group I (Control) and group III (L-ascorbic acid). Co-administration of bifenthrin and L-ascorbic acid (Group IV) manifested significant increase of these enzymes as compared to bifenthrin treated rats (Group II). The concentration of glutathione-S-transferase decreased significantly as compared to control (Group I). However, no significant elevation of this enzyme was observed in ameliorative group IV as compared to group II (Bifenthrin). Catalase activity decreased significantly in group II as compared to group I (Control) and group III (L-ascorbic acid) and there was significant restoration of this enzyme in group IV (Bifenthrin + L-ascorbic acid) near to control value (Group I).

Oxidative stress is associated with generation of toxic reactive oxygen species and mammalian cells are endowed with extensive antioxidant defence mechanisms

**Table 1:** Effect of repeated oral administration of bifenthrin alone and in combination with L-ascorbic acid on oxidative stress parameters in rats

Parameters/Units	Control (Group I)	Bifenthrin (Group II)	L-ascorbic acid (Group III)	Bifenthrin + L-ascorbic acid (Group IV)
Lipid Peroxidation (nmol MDA formed/ml erythrocytes)	4.15±0.52 <sup>a</sup>	8.83±0.70 <sup>b</sup>	3.40±0.36 <sup>a</sup>	5.06±0.58 <sup>a</sup>
GSH (nmol/ml)	118.80±7.08 <sup>a</sup>	60.12±8.53 <sup>b</sup>	125.69±6.70 <sup>a</sup>	107.52±5.79 <sup>a</sup>
SOD (Units/mg protein)	0.68±0.05 <sup>a</sup>	0.30±0.03 <sup>b</sup>	0.57±0.04 <sup>ac</sup>	0.54±0.02 <sup>c</sup>
GSH-Px (Units/mg protein)	1.04±0.05 <sup>a</sup>	0.57±0.08 <sup>b</sup>	0.87±0.07 <sup>ac</sup>	0.83±0.06 <sup>c</sup>
GST (µmol of conjugate GSH-CDNB/min/mg protein)	0.046±0.005 <sup>a</sup>	0.024±0.003 <sup>b</sup>	0.040±0.007 <sup>a</sup>	0.032±0.004 <sup>ab</sup>
CAT ( µmol of H <sub>2</sub> O <sub>2</sub> decomposition/min/mg protein)	73.11±6.77 <sup>a</sup>	38.23±4.48 <sup>b</sup>	82.11±6.96 <sup>a</sup>	66.09±5.44 <sup>a</sup>

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated. Means with at least one common superscript do no differ significantly at 5% (P<0.05) level of significance.

which counteract the damaging effects of these toxic reactive oxygen species (Halliwell and Gutteridge, 1989). It is well known that MDA is a terminal product of lipid peroxidation, so the content of MDA can be used to estimate extent of lipid peroxidation. This can indirectly reflect the degree to which the lipid membranes of cells are attacked by free radicals (Raina *et al.* 2009). Increased MDA in present study therefore is indicative of oxidative stress after oral administration of bifenthrin in rats. An increase in LPO has also been observed in rats exposed to cypermethrin (Belma *et al.* 2005) and deltamethrin (Manna *et al.* 2005) and in an *in-vitro* study with human erythrocytes (Sadowska-Woda *et al.* 2010). The level of blood GSH decreased significantly in bifenthrin treated animals as compared to control. Decreased GSH levels were also reported in broiler chicks exposed to deltamethrin (Jayasree *et al.* 2003) and rats exposed to cyfluthrin (Omotuyi *et al.* 2006). GSH is a major non enzymatic endogenous antioxidant which counterbalances free radical mediated damage by eliminating the compounds responsible for LPO or by increasing the efficiency of NADPH that protects detoxifying enzymes (Machlin and Bandlich, 1987). Hence, in the condition of oxidative stress increased utilization of GSH may result in its depletion while its synthesis from reserve may not complete fully to recover the depleted GSH levels as observed in the present study.

Compared to control group, the activity of SOD, GSH-Px and CAT decreased significantly in bifenthrin treated animals. The decrease in SOD, GSH-Px and CAT has also been reported in human erythrocytes exposed to bifenthrin and in rats treated with deltamethrin (Manna *et al.* 2005; Yousef *et al.* 2006). Superoxide dismutase is the first and major line of defence against the action of  $^*O_2^-$  and other ROS (Dubey *et al.* 2012). It converts the superoxide radicals into hydrogen peroxide which is decomposed by catalase to water and oxygen (McCord and Fridovich, 1969; Chelikani *et al.* 2004). Superoxide dismutase and catalase are considered as main antioxidant enzymes in oxidative stress produced by synthetic pyrethroids (Abdollahi *et al.* 2004). The direct inhibition of these enzymes by bifenthrin or increased utilization due to excess formation of free radicals could be possible reasons for the resultant depletion of these antioxidant enzymes (Eraslan *et al.* 2007). The reduction in activity of GSH-Px may be due to reduced level of GSH which acts as

substrate for the enzyme (Raina *et al.* 2009). The activity of GST was significantly decreased in bifenthrin treated animals as compared to control. Similarly, a significant decrease in GST activity was reported in rats treated with several pyrethroids (Kale *et al.* 1991; Singh *et al.* 2009).

Pre-treatment with L-ascorbic acid has decreased lipid production and reversed the altered values of various antioxidant enzymes except the reduced concentration of GST as evidenced from ameliorative group IV. This protective role of L-ascorbic acid against oxidative damage is in concurrence with the studies of other authors (Halliwell *et al.* 1999; Chisolm and Steinberg, 2000; and Raina *et al.* 2009). Ascorbic acid can act an antioxidant by donating two electrons from a double bond between the second and third carbons of the 6-carbon molecule which adequately justifies the ameliorative role of this molecule in the present study (Bielski *et al.* 1975; Buetlner and Moseley, 1993)

It can be concluded that in the present study, oral administration of bifenthrin induced oxidative stress as manifested by increase in LPO and decrease in blood glutathione, SOD, GPx, GST and CAT. L ascorbic acid effectively ameliorates most of the changed oxidative stress parameters.

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