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RESEARCH ARTICLE

MODULATORY ROLE OF L-ASCORBIC ACID IN OXIDATIVE STRESS INDUCED BY REPEATED ORAL ADMINISTRATION OF BIFENTHRIN IN HEART OF 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 30th day of treatment, heart 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. The activities of antioxidant enzymes viz., SOD, GST and CAT decreased significantly ($P<0.05$) in bifenthrin treated rats as compared control. No significant change was observed in the concentration GSH-Px in bifenthrin intoxicated animals. Ameliorative group receiving both bifenthrin and L-ascorbic acid significantly restored the normal values of various altered oxidative stress parameters except catalase activity (CAT).

Key Words: Bifenthrin, Oxidative Stress, Heart, L-ascorbic acid, Rats

INTRODUCTION

Bifenthrin is a newly introduced type-1 pyrethoid which was first approved for use in UK in 1988. Its properties like low water solubility and photostability makes it an effective insecticide and acaricide against a broad range of pests in agriculture and animal husbandry habitations (Pesticide Manual, 1997). Human and animal exposure to bifenthrin as pyrethoid insecticide can occur through oral, pulmonary and dermal routes (US DHHS, 1993; Llewellyn *et al*., 1996). Compared to other pyrethoids, 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). Bifenthrin residues have been found in vital organs like liver, kidney, heart and lung after its oral and dermal exposure (Walker and Keith, 1992). Few studies are available which suggest free radical generation due to intoxication of bifenthrin in blood of animals (Khan *et al*., 2009; Dar *et al*., 2014). However, there is dearth of studies describing its oxidative stress potential in vital organs like liver, kidney and lung. Therefore, present study was an attempt to study oxidative damage due to bifenthrin intoxication in heart tissue of wistar rats and also to evaluate the protective role of vitamin C in controlling such damage.

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MATERIAL AND METHODS

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 libitium following duly approved IAEC protocol. Rats were randomly divided into four groups of six rats each and were orally administered corn oil in group I as control, bifenthrin (Biflex^R 2.5% EC: FMC India Pvt. Limited, Tamil Nadu) @ 5.8mg/Kg/day ($1/10^{th}$ LD50) in group II, vitamin C (L-ascorbic acid, High Media Laboratories Pvt. Ltd, Mumbai) @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. Administration of vitamin C in ameliorative group was done 20 minutes before administration of bifenthrin.

The rats were anaesthetized with diethyl ether after 30th day of oral treatment. From freshly collected heart kept on ice, one gram of sample was weighed and taken in 10 ml of ice cold phosphate buffer solution (PBS, 7.4). The lung homogenates were prepared under cold conditions by using tissue homogenizer. The homogenate was centrifuged at 4000 rpm for 15 minutes to harvest the supernatant which was used for assay of various oxidative stress parameters. The activity of lipid peroxidation (LPO) was determined according to method

described by Shafiq-Ur-Rehman, 1984. The concentration of superoxide dismutase (SOD) in lung homogenate was determined with the help of method adopted by Marklund and Marklund, 1974. Glutathione peroxidise (GSH-Px) activity was assayed by the method of Hafeman *et al*., 1974. Glutathione-S-transferase (GST) and catalase (CAT) were determined by the methods of Habig *et al*., 1974 and Aebi, 1983 respectively.

Statistical analysis

The data were expressed as mean ±SE and statistically analysed by one-way ANOVA followed by Dunnet's test with $P < 0.05$ as limit of significance for comparison.

RESULTS

The results of the effect of bifenthrin alone and in combination with L-ascorbic acid on different oxidative stress parameters in heart are presented in table 1. Bifenthrin treated rat manifested significant increase in MDA level as compared to control (Group I) and vitamin C treated group III. Significant decrease of MDA level was observed in group IV as compared to bifenthrin treated group II. Significant decrease in superoxide dismutase was observed in group II (Bifenthrin) as compared to group I. Co-administration of bifenthrin and vitamin C (Group IV) manifested significant increase of superoxide dismutase comparable to vitamin C treated group III. Non significant increase in glutathione peroxidise was observed in bifenthrin treated group II as compared. The concentration of glutathione-S-transferase increased significantly in bifenthrin intoxicated animals and was significantly increased in group receiving both vitamin C and bifenthrin (Group IV). Catalase activity decreased significantly in group II (Bifenthrin) as compared to group I (control) and group III (Vitamin C) and ameliorative group (Bifenthrin+L-Vitamin C) was not able to reverse the decreased value of this enzyme as observed in bifenthrin treated animals.

Table 1. Effect of repeated oral administration of bifenthrin alone and in combination with vitamin C on lipid peroxidation and other antioxidant enzymes in heart of rats

Parameters (Units)	Control (Group I)	Bifenthrin (Group II)	Vitamin C (Group III)	Bifenthrin + Vitamin C (Group IV)
Lipid Peroxidation (nmol MDA formed/g tissue)	1.79 ± 0.19^{a}	4.21 ± 0.64^{b}	2.54 ± 0.37^{a}	2.24 ± 0.36^{a}
SOD (Units/mg protein)	0.60 ± 0.04^{a}	0.37 ± 0.04^{b}	0.69 ± 0.04^{a}	0.57 ± 0.05^{a}
GSH-Px (Units/mg protein)	0.67 ± 0.05^{a}	0.79 ± 0.06^{a}	0.85 ± 0.07^{a}	0.66 ± 0.05^{a}
GST (µmol of conjugate GSH-CDNB/min/mg protein)	0.49 ± 0.07^{ab}	0.37 ± 0.04^{a}	0.64 ± 0.04^{b}	0.47 ± 0.03^{ab}
CAT (µmol of H_2O_2 decomposition/min/mg protein	166.29 ± 8.45^{a}	108.95 ± 7.57^{b}	153.06 ± 10.29^{a}	103.68 ± 9.49^{bc}

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated. Means with at least one common superscript do not differ significantly at 5% ($P<0.05$) level of significance.

DISCUSSION

Oxidative stress is associated with generation of toxic reactive oxygen species and mammalian cells are endowed with extensive antioxidant defence mechanisms which counteract the damaging effects of these toxic reactive oxygen species (Halliwell and Gutterridge, 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 invitro study with human erythrocytes (Sadowska-Woda *et al*., 2010). Compared to control group, the activity of SOD and CAT decreased significantly in bifenthrin treated animals. The decrease in SOD 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 (Chelikani *et al*., 2004). Superoxide dismutase and catalase are considered as main antioxidant enzymes in oxidative stress produced by synthetic pyrethoids (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 activity of GST was significantly decreased in bifenthrin treated animals as compared to control. Similarly, significant decrease in GST activity was reported in rats treated with several pyrethoids (Kale *et al*., 1991; Singh *et al*., 2009).

Pretreatment with vitamin C has decreased lipid production and reversed the altered values of various antioxidant enzymes except the concentration of catalase 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 (Haliwell *et al*., 1999; Chisolm and Steinberg, 2000; Raina *et al*., 2009). Vitamin C can act as 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 ameliorating role of this molecule as observed in the present study (Bielski *et al*., 1975; Buetlner and Moseley, 1993)

Conclusion

The present study suggests that bifenthrin has tremendous potential of inducing oxidative stress in heart as evidenced by significant increase in lipid peroxidation and alterations in various antioxidant enzymes. L-ascorbic acid has got beneficial effects in normalising the altered values of various oxidative stress parameters except catalase.

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