*Journal of Applied and Natural Science* 8 (1): 346 –349 (2015)

JANS

# Role of vitamin C against bifenthrin induced oxidative damage in lungs of Wistar rats

**Muneer Ahmad Dar*, Rajinder Raina, Arshad Hussain Mir, Pawan Kumar Verma and Mahrukh Ahmad**

Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology-Jammu-181102 (J & K), INDIA
*Corresponding author. E-mail: darmuneer79@gmail.com

*Received: July 2, 2015; Revised received: November 4, 2015; Accepted: March 5, 2016*

**Abstract:** The aim of present study was to unravel the protective role of vitamin C on oxidative stress parameters in lung homogenates of bifenthrin intoxicated rats. Rats were divided into four groups. Group I served as control while group II animals were 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, lung samples were taken and analysed for oxidative stress parameters. Significant ($P<0.05$) increase in lipid peroxidation was observed from control value of 4.80±0.39 to 7.90±0.50 in bifenthrin treated animals. Mean control values of SOD, GSH-Px and CAT were 0.55±0.05, 0.98±0.03 and 138.70±6.01 which were significantly ($P<0.05$) decreased to values of 0.27±0.0, 0.53±0.05 and 91.10±9.70 respectively in bifenthrin treated animals. The value of GST increased significantly ($p<0.05$) to 1.05±0.06 in bifenthrin administered animals from control value of 0.70±0.08. Pre-treatment with vitamin C in ameliorative group IV significantly restored the normal values of lipid peroxidation, SOD, GST and CAT but could not reverse the decreased values of GSH-Px. The present research is first of its type where in free radical generation due to bifenthrin –a commonly used insecticide was evaluated in lung homogenates when given orally which might be due to residues present in the lung. Besides it will be helpful in better understanding of toxicological profile of pyrethoids, the most commonly used insecticides.

**Keywords:** Bifenthrin, Oxidative stress, Rats, Vitamin C

## 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 (Walker and Keith, 1992; 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 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*., 2013; 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 lung tissue of wistar rats and also to evaluate the protective role of vitamin C in controlling such damage.

## MATERIALS AND METHODS

**Chemicals:** Biflex$^{R}$, 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 was 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. The experiment was approved by Animal Ethics Committee of the institute. 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 @ 5.8mg/Kg/day ($1/10^{th}$ $LD_{50}$) 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

ISSN : 0974-9411 (Print), 2231-5209 (Online) All Rights Reserved © Applied and Natural Science Foundation www.ansfoundation.org

30 days.

The rats were anaesthetized with diethyl ether after 30th day of oral treatment. From freshly collected lung 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 are presented in Table 1. The main toxic symptoms like incoordination, muscle weakness and irritability recorded in bifenthrin treated animals (Group I) were not observed in animals given vitamin C and bifenthrin together. 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 and glutathione peroxidise 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. However, decreased value of glutathione peroxidise in bifenthrin treated group II was not significantly restored in ameliorative group IV. The concentration of glutathione-S-transferase increased significantly in bifenthrin intoxicated animals and was reduced 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 there was significant restoration of this enzyme in group IV (Bifenthrin+L-Vitamin C) near to control value (Group I).

## 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., 2001) 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, 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 (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

**Table 1.** Effect of repeated oral administration of bifenthrin alone and in combination with vitamin C on lipid peroxidation and other antioxidant enzymes in lung 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) | 4.80±0.39$^{a}$ | 7.90±0.50$^{b}$ | 3.80±0.32$^{a}$ | 5.43±0.47$^{a}$ |
| SOD (Units/mg protein) | 0.55±0.05$^{a}$ | 0.27±0.03$^{b}$ | 0.49±0.04$^{ac}$ | 0.37±0.05$^{bc}$ |
| GSH-Px (Units/mg protein) | 0.98±0.03$^{a}$ | 0.53±0.05$^{b}$ | 0.82±0.04$^{c}$ | 0.79±0.08$^{d}$ |
| GST (µmol of conjugate GSH-CDNB/min/mg protein) | 0.70±0.08$^{a}$ | 1.05±0.06$^{b}$ | 0.89±0.08$^{ab}$ | 0.91±0.07$^{ab}$ |
| CAT ( µmol of $H_2O_2$ decomposition/min/mg protein | 138.70±6.01$^{a}$ | 91.10±9.70$^{b}$ | 127.56±10.79$^{a}$ | 129.49±8.66$^{a}$ |

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.

excess formation of free radicals could be possible reasons for the resultant depletion of these antioxidant enzymes (Eraslan *et al*., 2008). 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 increased in bifenthrin treated animals as compared to control. Contrarily, a 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 glutathione peroxidase as evidenced from ameliorative group IV. The protective role of L-ascorbic acid against oxidative damage of lings in winstar rats are in concurrence with the studies of other authors (Cadenas *et al.*, 1998; Halliwell *et al.*, 1999; Chisolm and Steinberg, 2000; and 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; Buettner and Moseley, 1993)

## Conclusion

Lung tissue homogenates of rats after intoxication with bifenthrin showed significant increase in lipid peroxidation and GST and a significant decrease of SOD, GSH-Px and CAT as compared to control. Vitamin C (L-ascorbic acid) effectively normalized the changed values of various oxidative stress parameters. The present study will be helpful in better understanding of toxicological profile of bifenthrin in particular and pyrethoids in general which are commonly used and are considered safe for non target species.

## ACKNOWLEDGEMENTS

Authors are thankful to the authorities of Faculty of Veterinary Sciences and Animal Husbandry, SKUAST -Jammu for providing facilities and granting permission to take up this work.

## REFERENCES

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S. and Rezaiee, A. (2004). Pesticides and oxidative stress: a review. *Med Sci Monit.* 10: 141-147.

Aebi, H.E. (1983). Catalase. In *Methods Enzymatic analysis*, ed.HU. Bergmeyer, New York: Academic Press.

Ahmed, S., Saleem, M.A. and Khurram, R.K. (2004). Effect of cypermethrin (10 EC) and bifenthin (10 EC) on levels of acid and alkaline phosphatases in a strain of *tribolium castaneum* (Herbst ) (Coleoptera: tenebrionidae). *Pak. Entomol. 26*: 47-51.

Belma, G., Aylin, G. and Filiz, H. (2001). Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. *Toxicol Lett.* 118:139-46.

Bielski, B.H., Richter, H.W. and Chan, P.C. (1975). Some properties of the ascorbate free radical. *Ann N Y Acad Sci.* 258:231–237

Buettner, G.R., Moseley, P.L. (1993). EPR spin trapping of free radicals produced by bleomycin and ascorbate. *Free Radic Res Commun*. 19:89 -93.

Cadenas, S., Rojas, C. and Barja, G. (1998). Endotoxin increases oxidative injury to proteins in guinea pig liver: protection by dietary vitamin C. *Pharmacol Toxicol*. 82:11–18

*Chelikani, P., Fita, I. and Loewen, P.C. (2004). Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 61 (2): 192–208.*

Chisolm, G.M. and Steinberg, D. (2000). The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med.* 28:1815– 1826

Dar, M.A., Raina , R., Mir, A.H., Sultana, M. and Pankaj, N.K. (2014). Effect of dermal application of bifenthrin on acetylcholinesterase and oxidative stress indices in rat blood, lung and kidney. *Proc Natl Acad Sci India Sect B Bio Sci.doi*: 10.1007/s4001-014-0390-z.

Dubey, N., Raina, R. and Khan, A.M. (2012). Toxic effects of deltamethrin and fluoride on anti-oxidant parameters in rats. *Fluoride.* 45: 242-246

Eraslan, G., Kanbur, M. and Silici, S. (2008). Effect of cypermethrin on some biochemical changes in rats: the protective role of prospolis. *Exp.Anim*. 57: 453-60.

Habig, W.H, Pabst, M.J and Jakoby, W.B. (1974) .Glutathione –S-transferase. The first enzymatic step in mercapturic acid foramtion. *J Biol Chem*. 249**:** 7130-7139.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. (1974). Effect of dietry selenium on erythrocyte and liver glutathione peroxidase in rat. *J Nutr.* 104: 580-587.

Halliwell, B. and Gutterridge, J.M.C. (1989). Free radicals in biology and medicine, 2nd edition. Clarendon press, Oxford.pp 188-276.

Halliwell, B., Zhao, K. And Whiteman, M. (1999). Nitric oxide and peroxynitrite. The ugly, the uglier and the not so good: a personal view of recent controversies. Vitamin C: Antioxidant Actions in Humans. *Free Radic Res.* 31:651–669,

Khan, A.M, Sultana, M., Raina, R., Dubey, N. and Dar, S.A. (2013). Effect of sub acute toxicityof bifenthrin on antioxidant status and haematology after the oral exposure in goats. *ProcNatl Acad Sci India Sect B Bio Sci.doi*: 10.1007/s4001-01300157-y

Llewellyn, D.M, Brazier, A., Brown, R., Cocker, J., Evans, M.L. and Hampton, J. (1996) Occupational exposure to permethrin during its use as a public hygiene insecticide. *Ann Occup Hyg*. 40**:** 499-509.

Manna, S., Bhattacharyya, D., Mandal, T.K. and Das, S. (2005). Repeated dose toxicity of deltamethrin in rats. *Indian J Pharmacol.* 37: 160-164.

Marklund, S. and Marklund, M. (1974). Involvement of superoxide anion radical in auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. *European J. Biochem*. 47: 469-474.

Pesticide Manual (1997). Tomlin CDS,ed. The Pesticide Manual. London: The British Crop Protection Council.

Raina, R., Verma, P.K., Kusum. and Vinay Kant. (2009). Effect of repeated dermal application of α-cypermethrin on lipid peroxidation and antioxidant system in rats. *Toxicol. Int.* 16: 27-30.

Sadowska-Woda, I., Popwicz, D. and Karowicz-Billinska, A.

(2010). Bifenthrin induced oxidative stress in human erythrocytes and protective effect of selected flavonols. *Toxicol In Vitro.* 24:460-464

Shafiq-ur-Rehman. (1984). Lead-induced regional lipid peroxidation in brain. *Toxicol Lett.* 21 (3): 333-337.

Shakoori, A.R., Naveeda, T. and Sameem, M.A. (1994). Response of melathion-resistant and susceptible strains of *Tribolium castaneum* (Herbst) to bifenthrin toxicity. *Pakistan J.Zool*. 26 (2): 169-178.

US Department of Health and Human Services (1993). Hazardous Substances Data Base.HHS. Washington, D

Walker, M.H. and Keith, L.H. (1992). EPA's Pesticide Fact Sheet Database.Lewis Publishers, Chelsea, MI : 2-12

Yousef, M.I, Awad, T.I. and Mohamed, E.H. (2006). Deltamethrin induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. *Toxicology*. 227(3): 240-247.