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Effects on Oxidative Balance and Acetylcholinesterase in Liver and Brain of Rats Dermally Exposed to Bifenthrin

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Abstract

The present study was aimed to appraise the oxidative stress potential of bifenthrin and its effects on acetylcholinesterase in liver and brain of wistar rats after its repeated dermal application for a period of 30 days. The rats were divided into four groups with six rats in each group. Group I and III served as control and were applied with distilled water @ 1.8ml/Kg/day where as the animals of group II and IV were dermally applied with bifenthrin @ 45mg/Kg/day for 20 and 30 days respectively. Significant increase in MDA levels was observed in liver and brain homogenates after 20th and 30th day of dermal treatment. Significant decrease in SOD was observed both in liver and brain after 20th and 30th day. GPx decreased significantly after 30th day in liver and after 20th and 30th day in brain. The activities of GST and CAT decreased significantly in liver and brain both after 20th and 30th days of dermal application. Non- significant decrease in AchE was observed in liver and brain homogenates.

Keywords: Bifenthrin, Liver, Brain, Oxidative Stress, Acetyl-Cholinesterase, Rats

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Introduction

Pyrethoid exposure is of considerable magnitude in India and other countries including the United States due their indiscriminate and injudicious use in agriculture and domestic habitations.[1] Humans and animals are constantly exposed to these chemicals from environmental contamination or due to their high concentration in different food products or from inadvertent therapeutic applications or accidental and occupational exposure.

Bifenthrin is a newly introduced type-I pyrethroid with eight stereoisomers of which cisisomer is active 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 foliar pests.[2]It is one of the most potent commercialized pyrethoids to date with dermal lethal dose-50 (LD_{50}) as 4500 mg/Kg in rats. Hyrophobic nature of bifenthrin in commercial preparations ensures its entry via skin and hence its toxic residues have been found in liver and brain of animals.[3]Liver is responsible for metabolism and detoxification of these components while as brain acts as site for pyrethoids to produce untoward effects. [4]The lack of studies demonstrating the toxic effect of bifenthrin formulation on liver and brain was the main reason behind conduction of this experiment. Rats were dermally exposed to bifenthrin for a period of 30 days and an attempt was made to assess the impact on oxidative parameters and acetylcholinesterase levels in liver and brain.

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Material and Methods

Reagents and chemicals

Bifenthrin (Biflex^{R}2.5% EC) obtained from FMC India Pvt. Limited, Tamil Nadu (India) was applied @ 45 mg/Kg (1/100th LD_{50}) on intrascapular region as per method described by Punareewattana *et al.*,[5]

Animals

Wistar rats (150-220 g b.wt) of both sexes were procured from Indian Institute of Integrative Medicine (Council of Scientific and Industrial Research, Jammu, India) and maintained on *adlibitum* feed (pellets) and water. The experimental design with animals was approved by the University Animal Ethical Committee with registration number: 862/ac/04/CPCSEA, dated 16-12-2004. The rats were randomly divided into four groups of six rats each. The animals of groups I and III served as control and were applied with distilled water @ 1.8 ml/Kg/day whereas animals of group II and IV were applied with bifenthrin @ 45mg/Kg/day (1.8 ml undiluted Biflex^{R}) (1/100th LD_{50}).

Sample collection and processing

The rats were anaesthetized with diethyl ether after 20th and 30th day of dermal treatment. From freshly collected liver and brain, one gram of sample was weighed and taken in 10 ml of ice cold 0.1 mol/L phosphate buffer solution (pH 7.4). The homogenates were prepared under cold conditions by using a tissue homogenizer**.** The homogenate was centrifuged at 4000 rpm for 15 minutes to harvest the supernatant which was used for the assay of various oxidative stress parameters.

Biochemical analysis

Lipid peroxidation (LPO) in terms of MDA levels was determined according to method described by Shafiq-ur-Rehman.[6] The activity of superoxide dismutase (SOD) enzyme in was determined with the help of method adopted by Marklund and Marklund. [7]Catalase (CAT) and glutathione peroxidase (GPx) concentrations were respectively assayed by the methods of Aebi *et al.*,[8]and Hafeman*etal.*[9] The level of glutathione-S-transferas (GST) was measured according to methods described by Habig*et al.*[10] Acetyl-cholinesterase activity in liver and brain was calculated by Voss and Sachsse.[11]

Statistics

All these parameters were assayed in triplicate for each animal. The average value for each triplicate result for an animal represented the individual observation from the animal. Parametric data so obtained was expressed as mean±SE (n=6). The difference between two group means based on individual observations was determined by unpaired Student's t-test. The significance was assayed at$P < 0.05$ and $P < 0.01$ levels.[12] The results in treatment groups were also expressed as percentage of control values (100%).

Results

Dermal application of bifenthrin manifested significant increase in liver malondialdehyde levels (MDA) (63%) after the 20th day of treatment (table 1), which continued to increase by 101% after 30th day of dermal exposure. Superoxide dismutase activity (SOD) decreased significantly (35%) after 20th day of exposure with the values decreasing by 53% after 30th day. Catalase (CAT) activity decreased significantly by 27% and 38% after days 20 and 30 of bifenthrin application while significant decrease (37%) in glutathione peroxidase (GPx) was observed only after 30th day of treatment. The concentration of glutathione-S-transferase (GST) decreased both after 20th and 30th day of dermal exposure respectively by 41% and 45%. No change in liver AchE was observed.

Brain homogenates showed significant increase of MDA by 80% and 73% after 20 and 30 days of treatment (table 2). SOD concentration decreased significantly after 20th day by 34%and after 30th day by 38%. CAT activity decreased significantly by 32% and 60% after 20th and 30th day of dermal treatment. Significant increase in GPx (51%) was observed after 20th day followed by significant decrease by 36% after 30th day. GST levels decreased significantly by 37% both after 20th and 30th day of treatment. Non-significant decrease in AchE was observed in brain.

Discussion

Oxidative stress is associated with toxic reactive oxygen species and mammalian cells are endowed with extensive antioxidant defence mechanisms which counteract the damaging effects of toxic reactive oxygen species.

Table 1: Effect of repeated dermal application of bifenthrinon onacetylcholinesterase and oxidative stress parameters in liver of rats for 30 days

Parameter/Units	Days after dermal application			
	20 days		30 days	
	Group I Control	Group II Bifenthrin	Group III Control	Group IV Bifenthrin
AChE (nmolthiol group formed/min/mg)	989.34±88.56	829.22±79.19	746.45±71.64	676.44±53.84
Lipid peroxidation (n mol MDA formed/g tissue)	5.12±0.37	8.23±0.54[b]	4.59±0.57	9.21±0.25[b]
SOD (Units/mg protein)	0.96±0.08	0.62±0.06[b]	1.04±0.10	0.49±0.05[b]
CAT μ mol H_2O_2 decomposition/min/mg protein	167.52±6.78	122.84±10.03[b]	173.42±10.27	107.32±5.67[b]
GPx Units/mg protein	0.89±0.15	0.95±0.10	1.03±0.06	0.65±0.05[b]
GST μ mol of conjugate GSHCDNB/ min/mg protein	4.35±0.23	2.56±0.32[b]	3.94±0.10	2.15±0.06[b]

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated

[a,b] significantly different as compared to control values at 5% ($P<0.05$) and 1% ($P<0.01$) level of significance respectively.

Pyrethoid insecticides are reputed to have a role in generation of free radicals and oxidative stress mediated injuries in various tissues.[13, 14]SOD is the first and major line of defence against the action of superoxide anion radical (O^{-2}) and CAT is heme-containing enzyme that catalyses the disproportionation of hydrogen peroxide generated by superoxide dismutase.[14]Decreased activity of SOD and CAT has been reported in rats exposed to pyrethoids.[3, 15]

Glutathione peroxidase catalyzes the detoxification of a wide range of peroxides using glutathione as reducing equivalent, reducing the later to oxidized glutathione and water. Significant decrease in GPx activity has been reported in rats with several pyrethroids and bifenthrin intoxication.[16, 17]Glutathione-S-transferase catalyses the conjugation of reduced (GSH) via reaction of the sulphydryl group with electrophilic centres in a wide variety of hydrophilic compounds such as polycyclic aromatic hydrocarbons and other xenobiotics like pyrethroids [18] (Singh et al., 2009). Reduced levels of GST in several tissues have been reported with various pyrethoids[17] (Dubeyet al., 2012 and [19] Singh et al., 2012).The reduction in activity of GPxand GST is often considered to be due to reduced level of GSH, which acts as the substrate for the enzymes. The levels of GSH in liver and brain were not ascertained in the present study although levels of GSH decreased in blood[3] (Dar et al., 2014).

Significant inhibition of acetylcholiesterase by pyrethoid intoxication are available from few studies. [19, 14] Few mechanisms have been forwaded for inhibition of acetlycholinesterase activity in pyrethoid

Table 2: Effect of repeated dermal application of bifenthrinonacetylcholinesterase and oxidative stress parameters in brainof rats for 30 days

Parameter/Units	Days after dermal application			
	20 days		30 days	
	Group I Control	Group II Bifenthrin	Group III Control	Group IV Bifenthrin
AChE (nmolthiol group formed/ min/mg)	5527.95±336.11	4986.16±501.56	6072.71±520.33	5692.13±337.77
Lipid peroxidation (n mol MDA formed/g tissue)	5.35±0.47	9.66±0.22[b]	5.69±0.50	9.82±0.46[b]
SOD (Units/mg protein)	0.58±0.04	0.38±0.05[a]	0.50±0.02	0.31±0.02[b]
GSH-Px Units/mg protein	0.53±0.02	0.80±0.10[a]	0.66±0.05	0.42±0.04[b]
GST µ mol of conjugate GSHCD-NB/ min/mg protein	0.51±0.05	0.32±0.03[a]	0.54±0.07	0.34±0.02[a]
CAT µ mol H_2O_2 decomposition/min/mg protein	100.56±5.40	68.52±9.56[a]	89.62±7.48	35.88±5.32[b]

Values given are mean ± SE of the results obtained from 6 animals unless otherwise stated.
[a,b]significantly different as compared to control values at 5% ($P<0.05$) and 1% ($P<0.01$) level of significance respectively

toxicities. Rao and Rao[20] suggested high lipophilicity of pyrethoids as the key mechanism to interact at the aromatic hydrophobic surface of AChE resulting in its inhibited activity.[21]AchEcould be inhibited due to oxidative damage as well the occupation of its active sites by pollutants including insecticides.[22]. However, AchE is also upregulated in response to oxidative stress.[23] The alterations in AchE activities in liver and brain in the present study were non-significant which is contrary to our earlier results on bifenthrin oral toxicity suggesting dose dependent inhibition of AChE of bifenthrintoxicity.[15]

Liver and brain are vital organs where toxic residues of bifenthrin are found after dermal absorption and evaluation of the toxic insults was necessary. In this study the commercial preparation was used so as to imitate natural exposure of this pesticide in environment. However, for better understanding of these toxic effects on vital organs, additional studies should be carried out with standard grade of bifenthrin. To provide a strong insight to the hypothesis that whether AChE is inhibited by oxidative damage due to pyrethoid toxicity, a correlative study between the levels of free radicals and their effect on the AChE in tissues with varying doses of pyrethoids should be undertaken.

Conclusion

The study concludes as commercialized bifenthrin at dermal dose of 1/100th LD_{50}produces toxic effects, evidenced by a significant LPO and alterations in various antioxidant enzymes in the liver and brain tissue. However statistically non-significant change in AChE was observed in liver and brain tissue.

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Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Kakko I, Toimela T, Tahti H. The synaptosomal membrane bound ATPase as a target for neurotoxic effects of pyrethroids, permethrin and cypermethrin. *Chemosphere*. 2003; 51: 475-480.
2. Pesticide Manual. Tomlin CDS,ed. The Pesticide Manual. London: The British Crop Protection Council. 1997
3. Dar MA,Raina R., Mir AH, Sultana M, Pankaj NP. Effect of dermal application of bifenthrin on acetlycholinesterase and oxidative stress indices in rat blood, lung and Kidney. *ProcNatlAcadSci India Sect. B Biol Sci.* 2014; DOI 10.1007/s40011-014-0390-z
4. Yang L, Li L. Actions of the Pyrethroid Insecticide Bifenthrin on Sodium Channels Expressed in Rat Cerebral Cortical Neurons. *ToxicolMech Method*. 2015; 25:63-69.
5. Punareewattana K., Smith BJ, Blaylock BL, Longstreth J, Snodgrass HL, Gogal RM, Prater RM, Holladay SD. Topical permethrin exposure inhibits antibody production and macrophage function in C57B1/6N mice. *Food ChemToxicol.* 2001; 39: 133.
6. Shafiq-ur-Rehman. Lead-induced regional lipid peroxidation in brain. *ToxicolLett.* 1984; 21(3): 333-337.
7. Marklund S, Marklund M. Involvement of superoxide anion radical in auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. *European J Biochem*. 1974; 47: 469-474.
8. Aebi HE. *Catalase.* In *Methods Enzymatic analysis* (ed.HU. Bergmeyer, New York: Academic Press).1983.
9. Hafeman DG, Sunde RA, Hoekstra WG. Effect of dietry selenium on erythrocyte and liver glutathione peroxidase in rat. *J Nutr.* 1974;104; 580-587.
10. Habig WH, Pabst MJ, Jakoby WB. Glutathione –S-transferase. The first enzymatic step in mercapturic acid foramtion. *J BiolChem*. 1974; 249:7130-7139.
11. Voss G, Sachsse K.Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylcholine/DTNB procedure. *ToxicolAppl Pharm.* 1970; 16: 764-842.
12. Snedecor GW, Cochran WG. *Statistical Methods*. (6thed. Ames: Iowa State University Press). 1967.
13. Dar MA, Khan AM, Raina R, Verma PK , Sultana M. Effect of repeated oral administration of bifenthrin on lipid peroxidation and anti-oxidant parameters in wistar rats. *Bull Environ ContamToxicol. 2013;90*: *doi.10.1007/s00128-1022-7*.
14. Khan AM, Sultana M, Raina R., Dubey N, Dar SA. Effect of sub-acute toxicity of bifenthrin on antioxidant status and haematology after the oral exposure in goats. *ProcNatlAcadSci* India Sect B Biol Sci. 2013: doi:10.1007/s4001-013-0157-y.
15. Dar MA, Khan AM,Raina R,Beigh, SA, Sultana M. Effect of repeated oral administration of bifenthrin antioxidant status and acetylcholinesterase activity in brain of rats. *Toxicol Environ Chem.* 2015; http//dx.doi.org/10.1080/02772248.1070160.
16. Raina R, Verma PK, Kusum, Vinay Kant. Effect of repeated dermal application of α-cypermethrin on lipid peroxidation and antioxidant system in rats. *Toxicol. Int.* 2009;16: 27-30.
17. Dubey N, Raina R, Khan AM. Toxic effects of deltamethrin and fluoride on antioxidant parameters in rats. *Fluoride.* 2012; 45: 242-246.
18. Singh AK, Saxena PN, Sharma HN. Stress induced by beta-cyfluthrin, a type-2 pyrethoid, on brain biochemistry of Albino rat (*Rattusnorvegicus*). *Biol med***.** 2009;1:74.
19. Singh AK, Tiwari MN, Upadhyay G. Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: Postnatal exposure enhances the susceptibility during adulthood. *Neurobiol Aging*. 2012; 33: 404-415.
20. Rao GV,Rao K S. Modulation in acetylcholinesterase of rat brain by pyrethroids in vivo and an in vitro kinetic Study. *J Neurochem*. 1995; 65: 2259-2266.
21. Shah MAA, Gupta PK P. Influence of permethrina synthetic pyrethoids insecticide on cholinesterase activities in erythrocytes, plasma, brain and liver of rats. *Indian J Toxicol.* 2001; 8: 169-172
22. Piner P, Unerb N. Oxidative and apoptotic effects of lambda-cyhalothrin modulated by piperonylbutoxide in the liver of Oreochromisniloticus. *Environ ToxicolPharmacol*. 2012; 33:414-420.
23. Bond CE and GreenfieldSA. Multiple cascade effects of oxidative stress on Astroglia. *Glia*. 2007; 55: 1348-1361.