

ATTENUATION OF THE EFFECT OF LINDANE ON IMMUNE RESPONSES AND OXIDATIVE STRESS BY *OCIMUM SANCTUM* SEED OIL (OSSO) IN RATS

PRAMOD K. MEDIRATTA*, KRISHNA TANWAR, REETA KH***, RAJNI MATHUR, BASU D. BENERJEE**, SURENDER SINGH***, AND KRISHNA K. SHARMA

*Departments of *Pharmacology and **Biochemistry,
University College of Medical Sciences,
Shahadara, Delhi – 110 095*

and

****Department of Pharmacology,
All India Institute of Medical Sciences,
New Delhi – 110 029*

(Received on February 2, 2008)

Abstract : Present study was done to evaluate the effect of *Ocimum sanctum* seed oil (OSSO) on the immunotoxicity and oxidative activity of lindane in rats. Rats were divided into four groups (n=8) and were treated with lindane (10 mg/kg, po) and/or OSSO (1 mg/kg, po) during the study period. Humoral immunity was assessed by measuring haemagglutination titre to sheep red blood cells (SRBC) and delayed type hypersensitivity (DTH) was assessed by measuring foot pad thickness. Lindane showed significant decrease in anti-SRBC antibody titre and also decreased percentage change in foot pad thickness in DTH response as compared to control group. OSSO *per se* produced significant increase in anti-SRBC antibody titre, but did not produce significant change in the foot pad thickness as compared to control group. However, it significantly antagonized the effect of lindane on the anti-SRBC antibody titre and foot pad thickness parameters. Lindane produced oxidative stress as indicated by increase in the levels of MDA and decrease in GSH levels. Treatment with OSSO *per se* showed antioxidant activity and also reversed the oxidative stress produced by lindane. The results suggest that OSSO can attenuate the immunotoxicity and oxidative stress produced by lindane.

Key words : ocimum sanctum seed oil
immunotoxicity

lindane
oxidative stress

INTRODUCTION

Pesticide is any substance or mixture of

substances intended to be used for preventing, repelling, destroying or mitigating any pest. Presence of pesticides

*Corresponding Author : Dr. Pramod K. Mediratta, Professor, Department of Pharmacology, University College of Medical Sciences, University of Delhi, Shahdara, Delhi – 110 095; Email ID : drpramod_k@yahoo.com; Tel.: +91-9110524879.

is more or less ubiquitous in the environment surrounding us and exposure to the same is more or less inevitable. On one hand they are necessary for mankind, on the other hand their exposure has been shown to produce number of adverse effects including immunotoxicity (1–7). One of the important mechanisms suggested for their deleterious effect is by way of generation of free radicals and derangement of antioxidant mechanisms (8–10).

Lindane is an organochlorine pesticide and gamma isomer of hexachlorocyclohexane. Organohalogen compounds are stable, lipophilic and therefore may biomagnify. Like organophosphate pesticides, lindane has been shown to produce immunosuppression in various species of animals and in humans (2, 3, 11–13). It has also been shown to cause both apoptotic and neurotic cell death in thymocytes (5) and reduction in cytokine production by peripheral blood leukocytes (4). It has been reported to produce neurotoxicity after both acute and chronic exposure (6). It has also been shown to be a strong oxidant causing free radical generation in various tissues including brain, heart, thymocytes, ascites tumour cells, etc. (10, 14–16). Certain plant products like amaranth leaves are reported to ameliorate hexachlorocyclohexane-induced oxidative stress in rats (17).

Ocimum sanctum Linn (OS) commonly known as Tulsi in Hindi and holy basil in English has been extensively used in traditional system of medicine. Different parts of the plant are claimed to be effective in ameliorating different disease states. The fixed oil obtained from seeds of OS is rich in unsaturated fatty acids and has been reported to exhibit a number of activities

like antistress, immunomodulatory, antipyretic, anti-inflammatory and analgesic among others (18–20). It has been shown to reduce lipid peroxidation and to increase the levels of reduced glutathione content in blood of diabetic rabbits (21).

Thus, lindane causes immunosuppression and oxidative stress and OSSO exhibits immune enhancing and antioxidant activities. However, not much work has been done in this direction. Hence, it was thought worth while to study the effect of OSSO on lindane-induced immunotoxicity and oxidative stress in experimental animals.

MATERIAL AND METHODS

The study was carried out in male Wistar rats weighing between 150–180 g. The animals were divided into 4 groups each comprising 8 rats. The first group was administered the vehicle (1 ml/kg/day), the second group was administered lindane, the third group OSSO and the fourth group was administered a combination of OSSO and lindane. The animals were procured from the Central Animal House, University College of Medical Sciences, Delhi. Animals were housed in standard laboratory conditions. Care of animals was as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and was approved by Institutional Animal Ethical Committee, University College of Medical Sciences, Delhi.

The dried seeds of OS (Plant family: Labiatae) were collected from Maidan Garhi, New Delhi-110068, India and authenticated in the Department of Genetics, Indian

Council of Agricultural Research, Govt. of India, Pusa, New Delhi, India and specimen boucher were deposited in the laboratory. The seeds were crushed and cold macerated in petroleum ether (40–60°C) (SD fine Chemical Ltd., India) for three days. The extract was taken out, petroleum ether evaporated and the oil was filtered. Yield was 17.50% v/w with reference to dried seeds. Oil thus obtained was used in a dose of 1 ml/kg/day orally (po) for various studies. Lindane was procured from Sigma, USA and was dissolved in groundnut oil. It was administered in a dose of 10 mg/kg/day po. OSSO was administered at least 1 h before the administration of lindane.

Immunological parameters

Haemagglutination titers to sheep red blood cells (SRBC)

Rats were immunized with sheep red blood cells (SRBC; 0.5×10^9 cells/ml/100 g, ip) on day 0. On day 14th, they received the same dose of antigen again (booster dose). During the 21 day period, the animals either received vehicle (control group), lindane, OSSO or OSSO + lindane from day 1. On day 21, under mild ether anesthesia blood was collected from retroorbital plexuses, serum separated and haemagglutination titer was estimated as follows: Two fold dilutions (0.025 ml) of sera were made in the microtitre plates with saline. To each well, 0.025 ml of 1% (v/v) SRBC was added. The plates were incubated at 37°C for 1 h and then observed for haemagglutination. The highest dilution giving haemagglutination was taken as antibody titre and expressed in graded manner, the minimum dilution ($\frac{1}{2}$) being ranked as 1 and mean ranks of different

groups were compared for statistical analysis (22).

Delayed type hypersensitivity (DTH)

DTH response was estimated by the method as described by Institoris (23). The animals were sensitized by Keyhole Limpet Hemocyanin (KLH). Each rat was immunized subcutaneously at the base of the tail by 0.3 ml of KLH containing 0.5 mg KLH in 0.15 ml normal saline (NS) and mixed with 0.15 ml Freund's complete adjuvant on the 0 day of treatment. The animals were administered vehicle (control group), lindane, OSSO or OSSO + lindane (test groups) on days 1–14. On day 14, the reaction was challenged by injecting 20 µg KLH in 50 µl NS in left hind foot pad as test group and NS in right hind limb foot pad as control. Foot pad thickness was measured before and 24 h after challenge by antigen using dial calipers. Results were expressed as percentage change in foot pad thickness over vehicle treated control values.

Oxidative stress parameters

Estimation of serum malondialdehyde (MDA)

Thiobarbituric acid (TBA) method as described by Satoh (24) was used for the estimation of serum lipid peroxides. Peroxidation of lipids generates MDA which reacts with TBA to give red species (thiobarbituric acid reactive substances, TBARS).

Estimation of reduced glutathione (GSH) content in blood

Reduced glutathione was estimated by the method of Beutler et al (25). Blood (0.2 ml)

was collected in EDTA vial and was lysed by addition of 1.8 ml of 1 g/l EDTA. To the hemolysate 3 ml of precipitating reagent [metaphosphoric acid (1.67 g) + disodium EDTA (0.2 g) + NaCl (30 g) added to distilled water upto a volume of 100 ml] was added. The mixture was allowed to stand for 5 min and then filtered. To 2 ml of filtrate 4 ml of disodium hydrogen phosphate and 1 ml of DTNB reagent were added. The absorbance was measured at 412 nm and results were expressed as mg/g of hemoglobin (Hb).

Statistical analysis

One way analysis of variance (ANOVA) followed by post hoc Tukey's test was used for analysis. A P value <0.05 was taken as significant.

RESULTS

Immunological parameters

Lindane (10 mg/kg, po) produced significant ($P<0.01$) decrease in anti-SRBC antibody titer compared to control. OSSO (1 ml/kg, po) caused a significant ($P<0.01$) increase in anti-SRBC antibody titre. When OSSO was administered along with lindane, it significantly ($P<0.01$) reversed the lindane-induced decrease in anti-SRBC antibody titer (Table I).

In DTH test, lindane (10 mg/kg, po) caused significant ($P<0.01$) decrease in the percent change in foot pad thickness. OSSO (1 ml/kg po) *per se* did not produce a significant change in the foot pad thickness when compared to control group. However, it significantly ($P<0.01$) antagonized the effect of lindane on foot pad thickness (Table II).

TABLE I: Effect of lindane and its modulation by Ocimum sanctum seed oil (OSSO) on haemagglutination titre to sheep red blood cells (SRBC) in rats.

<i>Treatment</i> (mg-ml/kg/day)	<i>Anti SRBC</i> <i>antibody titer</i> (Mean±SEM)
Vehicle (Control)	9.3±0.52
Lindane (10)	6.3±0.27 ^{*a}
OSSO (1)	11.6±0.42 ^{*a}
OSSO (1)+Lindane (10)	9.8±0.57 ^{*a}
n=8	F=24.55 DF 3, 30

^{*a} $P<0.01$ compared to vehicle (control) - treated group, ^{*b} $P<0.01$ compared to lindane alone-treated group.

TABLE II: Effect of lindane and its modulation by Ocimum sanctum seed oil (OSSO) on Delayed Type Hypersensitivity (DTH) in rats.

<i>Treatment</i> (mg-ml/kg/day)	<i>Percent change in</i> <i>foot pad thickness</i> (Mean±SEM)
Vehicle (Control)	31.47±3.35
Lindane (10)	18.00±1.37 ^{*a}
OSSO (1)	31.65±2.03
OSSO (1)+Lindane (10)	29.35±1.74 ^{*b}
n=8	F=8.933 DF 3, 30

^{*a} $P<0.01$ compared to vehicle (control) - treated group, ^{*b} $P<0.01$ compared to lindane alone-treated group.

Oxidative stress parameters

Lindane (10 mg/kg, po) produced a significant ($P<0.01$) increase in the MDA levels and a significant ($P<0.01$) decrease in reduced glutathione content. OSSO (1 ml/kg, po) administration *per se* caused significant ($P<0.01$) decrease in MDA levels and significant ($P<0.01$) increase in reduced glutathione content. Further, administration of OSSO to lindane treated group significantly ($P<0.01$) antagonized the effect

of lindane on MDA level and reduced glutathione content (Table III).

TABLE III: Effect of lindane and its modulation by *Ocimum sanctum* seed oil (OSSO) on oxidative stress parameters in rats.

Treatment (mg-ml/kg/day)	MDA (nmol/ml) (Mean±SEM)	GSH (mg/g Hb) (Mean±SEM)
Vehicle (Control)	2.89±0.2	4.0±0.30
Lindane (10)	8.55±0.2* ^a	2.7±0.02* ^a
OSSO (1)	1.63±0.15* ^a	5.7±0.11* ^a
OSSO (1) + Lindane (10)	1.68±0.4* ^b	5.6±0.14* ^b
n=8	F=1416.84 DF 3, 30	F=4022.8 DF 3, 30

*P<0.01 compared to vehicle (control) - treated group, *^bP<0.01 compared to lindane alone-treated group.

DISCUSSION

Several reports indicate that pesticides produce immunotoxicity (2, 3, 26). The *in vivo* generation of free radical has been demonstrated to suppress immune responsiveness in experimental animals (27). Exposure to organochlorine and organophosphate has been shown to produce immunotoxicity as well as generation of oxygen free radicals (3, 28).

OSSO has been reported to produce immune enhancing and antioxidant effects (20, 21). Keeping this in mind, the present study was carried out to investigate the effect of OSSO on lindane (an organochlorine pesticide) induced modulation of some immune and oxidative stress parameters.

Rats exposed to lindane in a dose of 10 mg/kg/day for 21 days showed no sign and symptoms of overt toxicity, neurotoxicity or mortality. The effect of lindane on humoral immune response was evaluated via measuring antibody titre to SRBC by

haemagglutination assay. In the present study, exposure of the animals to lindane produced a significant reduction in the secondary antibody response. These results corroborates the finding of other workers who observed reduction in antibody titre on exposure to organochlorine, organophosphate and carbamate pesticides (2, 3, 26). Administration of OSSO to animals showed a significant rise in the anti-SRBC antibody titre, corresponding to our earlier finding (20). Further, co-administration of OSSO along with lindane significantly antagonized immuno-suppressive effect of lindane on anti-SRBC antibody titre. The effect of lindane and OSSO on cell-mediated immune response was evaluated with the help of DTH reaction using KLH as antigen. Rats immunized by KLH and exposed to lindane showed a significant decrease in DTH reaction. Similar suppression of cell-mediated immune response to various pesticides has been reported by other workers (23, 26). Administration of OSSO in a dose of 1 ml/kg/day did not show any effect on DTH response but when given to lindane-treated rats significantly antagonized the effect of lindane on DTH reaction.

The results of present study thus show that lindane can suppress humoral as well as cell-mediated immune response and these effects are significantly attenuated by co-administration of OSSO with lindane.

The effect of lindane and OSSO could be because of their effect on antioxidant system in the body. In the present study, lindane caused an increase in MDA level, an indicator of lipid peroxidation. Lipid peroxidation products are measured as index of oxygen free radical. MDA is generated in

tissue by free radical injury and appears in serum. Hence, measurement of MDA in serum has been considered a sensitive index of free radical generation. The present result of increased MDA level in serum following sub-chronic lindane exposure indicates an increased oxygen free radical generation by pesticides. This result is in accordance with previous studies using lindane and other pesticides (27, 28). Induction of cytochrome P450 and other microsomal enzyme by various pesticides, e.g. carbamate, have been reported and it is possible that lindane mediated free radical generation may be through induction of this enzyme (29, 30). OSSO *per se* produced a decrease in MDA level. Further, it attenuated the increase in MDA level produced by lindane. These results are similar to the observation of another study where OSSO was shown to decrease MDA level seen in hypercholesterolemic rabbits (21).

GSH is an important molecule in the cellular defense against oxidative stress. In its reduced form, it is necessary for the detoxification of xenobiotics. In the present study, exposure of animals to lindane for 21

days caused a significant decrease in serum GSH levels. Various pesticides including lindane have been shown to decrease GSH level (31). This reduction in GSH level could be due to direct conjugation of GSH with electrophiles whose increased production may result from pesticide exposure or could be due to inhibition of enzymes, like glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase etc which are involved in GSH synthesis and regeneration. OSSO treatment *per se* produced an increase in the level of GSH in blood and also attenuated the reduction in GSH induced by lindane. This effect of OSSO could be due to its direct effect because of free radical scavenging properties which may spare GSH. This could also be due to an indirect stimulant effect on various enzymes involved in GSH synthesis and regeneration.

Results of present study show that OSSO is able to ameliorate lindane-induced immunotoxicity and oxidative stress in sub-acute exposure. This study suggests the potential of OSSO therapy in reducing immunotoxicity and oxidative stress in persons exposed to lindane.

REFERENCES

1. Gleichmann G, Kimbler I, Purchase IF. Immunotoxicology: suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. A discussion. *Arch Toxicol* 1989; 63: 257–273.
2. Banerjee BD, Koner BC, Ray A, Pasha ST. Influence of subchronic exposure to lindane on humoral immunity in mice. *Indian J Exp Biol* 1996; 34: 1109–1113.
3. Banerjee BD, Pash ST, Hussain QZ, Koner BC, Ray A. A comparative evaluation of immunotoxicity of malathion after subchronic exposure in experimental animals. *Indian J Exp Biol* 1998; 36: 273–282.
4. Devos S, Van Den Heuvel R, Hooghe R, Hooghe-Peters EL. Limited effect of selected organic pollutants on cytokine production by peripheral blood leucocytes. *Eur Cytokines Netw* 2004; 15: 145–151.
5. Olgun S, Gogal RM Jr, Adestina F, Choudhary M, Misra HP. Pesticide mixtures potentiate the toxicity in murine thymocytes. *Toxicology* 2004; 196: 181–195.
6. Mariussen E, Fonnum F. Neurochemical targets and behavioral effects of organohalogen compounds: an update. *Cri Rev Toxicol* 2006; 36: 253–289.
7. Wang F, Xu ZR, Su JH. Effect of HCH contamination of diet on the growth performance and immune and antioxidant ability in growing/

- finishing pigs. *Vet Res Commun* 2006; 30: 645–654.
8. el-Shakawy AM, Abdul Rahman SZ, Hassan AA, Gabr MH, el-Zoghby SM, el-Sewedy SM. Biochemical effects of some insecticides on the metabolic enzymes regulating glutathione metabolism. *Bull Environ Contam Toxicol* 1994; 52: 505–510.
 9. Banerjee BD, Seth V, Bhattacharya A, Pasha ST, Chakraborty AK. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicol Letter* 1999; 107: 33–47.
 10. Olgun S, Misra HP. Pesticides induced oxidative stress in thymocytes. *Mol Cell Biochem* 2006; 290: 137–144.
 11. Raszyk J, Toman M, Gajduskova V et al. Effect of environmental pollutants on the porcine and bovine immune systems. *Vet Med* 1997; 42: 313–317.
 12. Daniel V, Huber W, Bauer K, Suesal C, Conradt G, Opelz G. Associations of blood levels of PCB, HCHS and HCB with numbers of lymphocyte subpopulations, *in vitro* lymphocyte response, plasma cytokine levels and immunoglobulin auto antibodies. *Environ Health Perspect* 2001; 109: 173–178.
 13. Christin MS, Menard L, Gendron AD et al. Effects of agricultural pesticides on the immune system of *Xenopus laevis* and *Rana pipiens*. *Aquat Toxicol* 2004; 67: 33–43.
 14. Ananya R, Subeena S, Kumar DA, Kumar DT, Kumar MS. Oxidative stress and histopathological changes in the heart following oral lindane (gamma hexachlorohexane) administration in rats. *Med Sci Monit* 2005; 11: BR325–BR329.
 15. Srivastava A, Shivanandappa T. Hexachlorocyclohexane differentially alters the antioxidant status of the brain regions in rat. *Toxicology* 2005; 214: 123–130.
 16. Srivastava A, Shivanandappa T. Causal relationship between hexachlorocyclohexane cytotoxicity, oxidative stress and Na⁺ K⁺-ATPase in Ehrlich Ascites tumor cells. *Mol Cell Biochem* 2006; 286: 87–93.
 17. Anilakumar KR, Khanum F, Santhanum K. Amelioration of hexachlorocyclohexane-induced oxidative stress by amaranth leaves in rats. *Plant Food Hum Nutr* 2006; 61: 169–173.
 18. Singh S, Majumdar DK. Evaluation of anti-inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J Exp Biol* 1997; 35: 380–383.
 19. Gupta SK, Prakash J, Srivastava S. Validation of traditional claims of Tulsi, *Ocimum sanctum* Linn as a medicinal plant. *Indian J Exp Biol* 2002; 40: 765–773.
 20. Mediratta PK, Sharma KK, Singh S. Evaluation of immunomodulatory potential of *ocimum sanctum* seed oil and its possible mechanism of action. *J Ethanopharmacol* 2002; 80: 15–20.
 21. Gupta S, Mediratta PK, Singh S, Sharma KK, Shukla R. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. *Indian J Exp Biol* 2006; 44: 300–304.
 22. Sharma KK, Mediratta PK, Reeta KH, Mahajan P. Effect of L-arginine on restraint stress induced modulation of immune responses in rats and mice. *Pharmacol Res* 2004; 49: 455–460.
 23. Institoris L, Siroki O, Undeger U, Basaran N, Desi I. Immunotoxicological investigations on rats treated subcutely with dimethoate, As³⁺ and Mg²⁺ in combination. *Hum Exp Toxicol* 2001; 20: 329–336.
 24. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chemica Acta* 1978; 90: 37–48.
 25. Beutler E, Duron O, Kelley BM. Improved method for determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882–885.
 26. Seth V, Banerjee BD, Chakraborty AK, Institoris L, Desi I. Effect of propoxur on humoral and cell-mediated immune responses in albino rats. *Bull Environ Contam Toxicol* 2002; 68: 369–376.
 27. Koner BC, Banerjee BD, Ray A. Organochlorine pesticide induced oxidative stress and immune suppression in rats. *Indian J Exp Biol* 1998; 36: 395–398.
 28. Banerjee BD, Seth V, Ahmed RS. Pesticides induced oxidative stress: perspectiveness and trends. *Rev Environ Health* 2001; 16: 1–40.
 29. Hayes JH. Carbamate pesticides. In: Pesticides stress induced in Man. Baltimore, Waverly Press 1982, 436–462.
 30. Puatanochokchai R, Morimura K, Wanibuchi H et al. Alpha-benzene hexachloride exert hormesis in preneoplastic lesion formation of rat hepatocarcinogenesis with the possible role for hepatic detoxifying enzymes. *Cancer Lett* 2006; 240: 102–113.
 31. Reed DJ. Glutathione: toxicological implications. *Annu Rev Pharmacol Toxicol* 1990; 30: 603–631.