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Original Article

CURCUMIN AMENDS OXIDATIVE STRESS AND ANTIOXIDANTS STATUS IN OLFACTORY LOBES, CEREBRUM, HYPOTHALAMUS-HIPPOCAMPUS, CEREBELLUM AND PONS-MEDULLA OF MICE ACUTELY INTOXICATED WITH LINDANE

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ABSTRACT

Objective: Present study ascertains the neuroprotective potential of curcumin in olfactory lobes, cerebrum, hypothalamus-hippocampus, cerebellum and pons-medulla of mice, intoxicated with lindane.

Methods: For the study, mice were divided into four groups. Olive oil was given as a vehicle to the mice of group I. Mice belonging to groups II and III was administered with lindane and curcumin respectively, for 12 h exposure by intraperitoneal injection. In group IV, curcumin was administered 10-15 min prior to exposure of lindane.

Results: Lindane exposure significantly increased the activities of thiobarbituric acid reactive substance (TBARS) ($p<0.05$) and protein carbonyl content (PCC) ($p<0.05$) whereas decreased the activity of reduced glutathione (GSH) ($p<0.05$) and superoxide dismutase (SOD) ($p<0.05$). Treatment of curcumin alone as well as in combination with lindane significantly declined the level of TBARS and PCC ($p<0.05$) and increased the activity of GSH and SOD ($p<0.05$).

Conclusion: Curcumin has neuroprotective potential. It can be used as a therapeutic agent against lindane induced toxicity.

Keywords: Lindane, Curcumin, Oxidative stress, Endogenous antioxidant

INTRODUCTION

Lindane is an organochlorine pesticide that is persistent in nature and damages liver, kidney, neural and immune system [1]. For rats and mice, lethal dose (LD_{50}) of lindane falls in the range of 100-200 mg/kg body weight. It causes central nervous system (CNS) excitation, clonic and tonic convulsions, increases irritability and impairs spontaneous and conditioned behavior in rats [2].

Lindane has been used as a pesticide on fruits and vegetable crops and found in cow milk in significant concentration [3]. The highest amount of lindane is deposited in humans, as they occupy the top of the food chain. The brain is the most vital organ which controls the functions of other organs in the body. Accumulation of lindane is highest in the brain next to adipose tissue because it is rich in lipid concentration. Order of accumulation of lindane in different body organs is as following-Adipose tissue> Brain> Kidney> Muscle> Lung> Heart> Spleen>Liver>Blood>Breast milk [4]. The brain consumes the highest amount of oxygen in the body but has less amount of antioxidants [5]. Therefore, oxidative stress in the brain induced by lindane is higher, which eventually affects the whole body. Lindane induces oxidative stress in the cells by generating reactive oxygen species (ROS) viz peroxides and super oxides. ROS exert damaging effects on the cells like smash up of DNA, oxidation of polyunsaturated fatty acids (PUFAs), oxidation of amino acids in proteins, inactivation of enzymes by oxidation of co-factors.

Curcumin is an antioxidant that may inhibit the generation of free radicals both *in vitro* and *in vivo* [6, 7]. It is a diarylheptanoid which is obtained from *Curcuma longa L.* Curcumin has anti-inflammatory and antimicrobial properties [8-10]. Various studies report the decreased level of xanthine oxidase, superoxide anions, lipid peroxides and myeloperoxidase while the elevated levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) following curcumin exposure [11, 12].

Curcumin penetrates the blood–brain barrier, modulates the levels of neurotransmitters (norepinephrine, dopamine, and serotonin) and reduces the oxidative stress in the brain [13, 14]. Curcumin is attributed with a good antioxidant potential which may combat the neurotoxicity induced by lindane. Therefore, it is cardinal to understand the mechanism of toxicity scavenging by curcumin in different brain regions, which is attempted in the current investigation.

MATERIALS AND METHODS

Chemicals

Lindane and curcumin were purchased from Sigma Chemical Company (St. Louis MO, USA). 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), ethylenediamine tetraacetic acid (EDTA), tris hydrochloride, trichloroacetic acid (TCA) and 2-thiobarbutric acid (TBA) were purchased from SRL (Mumbai, India). All other chemicals used in current investigation were of analytical grade.

Experimental animals and research design

Healthy Swiss mice (*Mus musculus*) of 15-20 gm used in the studies were procured from C. C. S Haryana Agricultural University, Hissar. All the experiments involving animals were conducted in accordance with the guidelines of CPCSEA (Committee for prevention of cruelty on supervision of experimentation on animals) (Ref. No. BU/BT/383/13-14). Mice were fed with a pelleted diet (Hindustan Unilever Limited) and water *ad libitum.*

The dose of lindane was selected after determination of LD_{50}, then in the second step, minimum tolerance dose (MTD) was determined, thereafter seeking the results 25 mg/kg b.w. the dose of lindane was selected. The dose selection of curcumin was based on a review of the literature. For determination of acute toxicity of dichlorvos and its mitigation by the curcumin, their exposure was made once for 12 h.

Mice were divided into four groups. Group I was controlling and it received olive oil as a vehicle. Lindane (25 mg/kg b. w) and curcumin (45 mg/kg b. w) were administered to the mice of group II and group III. Group IV was treated with the curcumin and lindane both. Curcumin was injected 10-15 min prior to exposure of lindane in group IV.

Doses of lindane and curcumin were prepared by dissolving them in olive oil and water respectively. Mode of exposure of lindane and curcumin was intraperitoneal.

After 12 h of exposure, mice were cervical dislocated, and olfactory lobes, cerebrum, hypothalamus-hippocampus, cerebellum and pons-medulla regions of the brain were immediately removed. After the collection, all the brain regions were washed with normal saline.

Tissue homogenate preparation

10% homogenates of different parts of the brain in phosphate buffer (0.1 M, pH 7.4) were prepared. Tissue homogenates were stored at-20 °C and used for determination of various biochemical parameters. For affirmation of toxicity in different brain regions due to lindane, a change in oxidative stress and endogenous antioxidants was used as a biomarker.

Oxidative stress

TBARS level and protein carbonyl content (PCC) were used as biomarkers of oxidative stress. TBARS level was measured by the method of Ohkawa *et al.* (1961) whereas Levine *et al.* (1990) method was used for determination of PCC [15, 16].

Assay of endogenous antioxidants of brain

Reduced glutathione (GSH) activity served as an index for determining the extent of LPO. GSH activity was determined by Ellman's method (1959) whereas by Dhindsa *et al.* (1981) method was used for determination of SOD activity [17, 18].

Statistical studies

All the results were analyzed statistically by using two way of analysis of variance (SPSS 16). Intergroup comparison was made by post hoc comparison analysis. Data are expressed as mean±SD the level for concluding a significant change was selected at P<0.05.

RESULTS

TBARS

TBARS level was found to be significantly increased in the lindane-treated group compared to groups I, III and IV in olfactory lobe, cerebrum, hypothalamus-hippocampus, cerebellum and pons-medulla oblongata regions of the brain (p<0.05). TBARS level is decreased significantly in curcumin treated group relative to groups II and IV in the olfactory lobe and cerebrum (p<0.05). In hypothalamus-hippocampus and pons-medulla regions of the group that was treated with curcumin, TBARS level was revealed to decline significantly as compared to group II (p<0.05).

Changes in TBARS level of the cerebellum of group III was found significant compared to all others groups (p<0.05). A significant change in TBARS level in group IV was recorded in olfactory lobe compared to group I, II and III (p<0.05). In cerebrum and cerebellum of group IV, TBARS level increased significantly as compared to purely curcumin treated group (p<0.05). Protective role of curcumin is suggested by a significant decline in TBARS level of the cerebrum, hypothalamus-hippocampus and pons-medulla of group IV mice as compared to lindane-treated mice (p<0.05) (fig. 1).

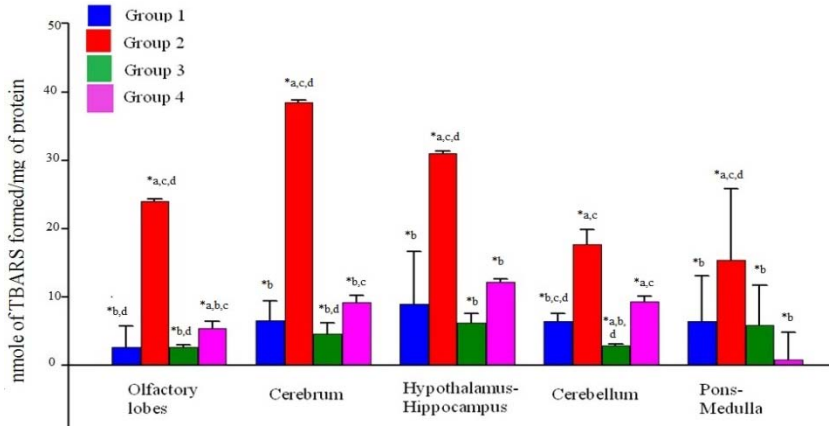

Fig. 1: TBARS level in various brain regions in differently treated groups
*** = p<0.05, a= compared to group I, b= compared to group II, c= compared to group III, d= compared to group IV**

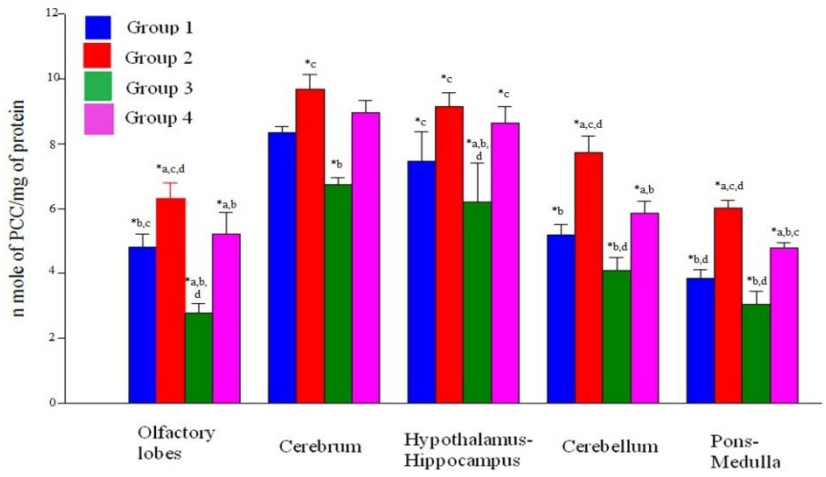

Fig. 2: PCC level in various brain regions in differently treated groups
*** = p<0.05, a= compared to group I, b= compared to group II, c= compared to group III, d= compared to group IV**

PCC

The level of PCC significantly increased in the lindane-treated group compared to group I, III and IV in olfactory lobe, cerebellum and pons-medulla oblongata ($p<0.05$). In cerebrum and hypothalamus-hippocampus of lindane-treated mice, a significant increase in PCC level was observed compared to group III ($p<0.05$). A significant decrease in PCC level was found in curcumin treated group compared to groups II and IV in olfactory lobes, hypothalamus-hippocampus, cerebellum and pons-medulla ($p<0.05$).

PCC level significantly declined in the cerebrum of group III compared to group II ($p<0.05$). The group that was treated with both lindane and curcumin revealed a significantly higher and lower concentration compared to groups I and II respectively, in olfactory lobes, cerebellum and pons-medulla ($p<0.05$). In hypothalamus-hippocampus, a significant elevation in PCC level was found in group IV compared to group III ($p<0.05$) (fig. 2).

GSH

The activity of GSH decreased significantly in the lindane-treated group compared to all groups in cerebrum, hypothalamus-hippocampus, cerebellum and pons-medulla. In olfactory lobes, GSH activity significantly decreased after lindane treatment compared to groups I and III ($p<0.05$). Curcumin treated group showed a significant rise in GSH activity compared to all other groups in studied brain regions. In group IV, GSH activity decreased significantly from control and curcumin-treated group and increased compared to the lindane-treated group in the cerebrum, cerebellum, and pons-medulla ($p<0.05$). In olfactory lobes, a significant decrease in GSH activity was recorded in group IV from groups I and III ($p<0.05$). A significantly increased and decreased GSH activity was found in hypothalamus-hippocampus compared to groups II and III respectively ($p<0.05$). In all brain regions, maximum activity of GSH was recorded for group III whereas the minimum activity of GSH was recorded in the lindane-treated group (fig. 3).

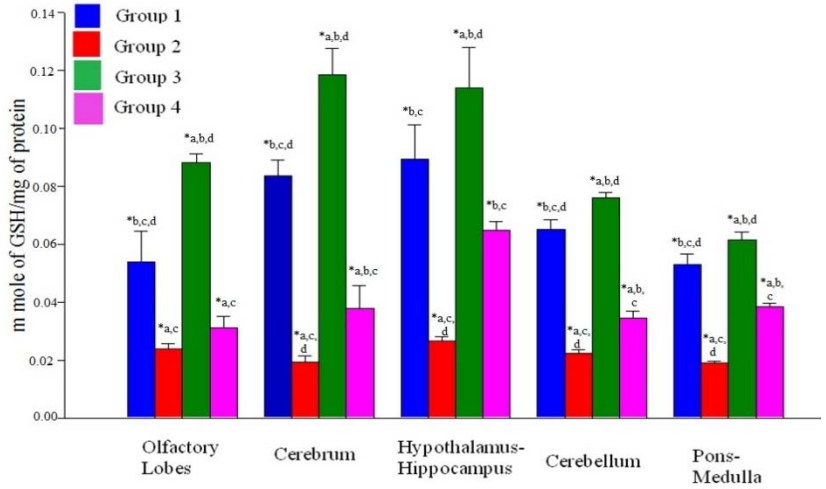

Fig. 3: GSH level in various brain regions in differently treated groups
*** = p<0.05, a= compared to group I, b= compared to group II, c= compared to group III, d= compared to group IV**

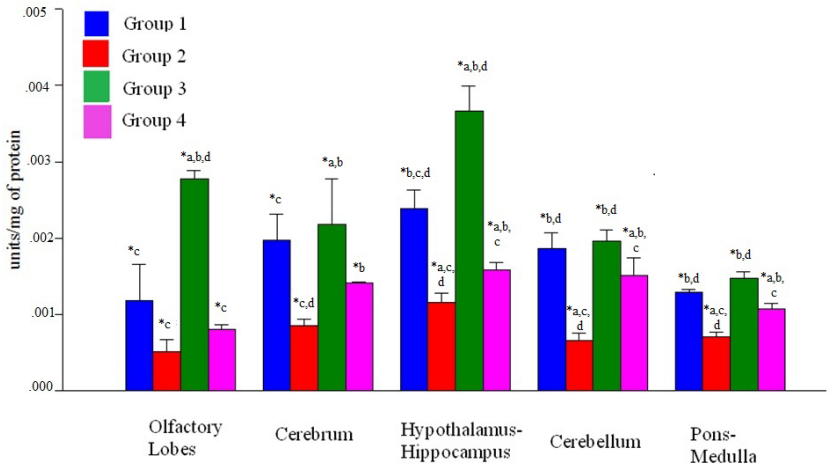

Fig. 4: SOD level in various brain regions in differently treated groups
*** = p<0.05, a= compared to group I, b= compared to group II, c= compared to group III, d= compared to group IV**

SOD

Least level of SOD was found in a lindane-treated group of all studied brain regions. After lindane treatment, SOD level reduced significantly from groups I, III and IV in hypothalamus-hippocampus, cerebellum and pons-medulla ($p<0.05$). In olfactory lobes and cerebrum, a significantly decreased level of SOD was reported in group II as compared to group III ($p<0.05$). In olfactory lobes and hypothalamus-hippocampus of curcumin treated mice, a significantly higher level of SOD was recorded compared to groups I, II and IV. Meanwhile, a significantly increased SOD activity was revealed after curcumin treatment compared to groups II and IV ($p<0.05$). In cerebrum of group III mice, a significantly higher level of SOD was revealed compared to groups I and II ($p<0.05$). In the group that was treated with both curcumin and lindane, a significant alteration in SOD activity was recorded from groups I, II and III in

hypothalamus-hippocampus, cerebellum and pons-medulla ($p<0.05$). In olfactory lobes of group IV, a significant reduction in SOD activity from group III was recorded ($p<0.05$). A significant increase in SOD activity reflects the protective effects of curcumin in the cerebrum of group IV, compared to lindane-treated mice ($p<0.05$) (fig. 4).

DISCUSSION

The brain performs an incredible number of tasks such as regulation of body temperature, blood pressure, heart rate, physical movement, speech, and imagination. All of these tasks are coordinated, controlled and regulated by the brain. If there is an anomaly in the brain that occurred due to a toxicant, it may lead to impairment in coordination and control of above-mentioned tasks. Lindane is a toxicant which damages tissues by generating oxidative stress and reducing the antioxidant status of the brain. The current study reports that curcumin has neuroprotective effects against lindane induced toxicity in different regions of mice brain**.** TBARS is a major end product of lipid peroxidation (LPO) which serves as an indicator of oxidative stress. The present study reports an increase in TBARS and PCC level after lindane administration in different brain regions. Acute administration of lindane increased the level of LPO by an uncompromised generation of free radicals, which disturb the antioxidant defense system leading to oxidative stress. This may be due to the LPO and PCC content induced by free radicals [19]. There are previous studies which also support the enhancement of LPO after lindane exposure [20-27]. Reed (1990) reported that LPO resulted by superoxide radicals leading to oxidation and depletion of GSH [28]. Previous reports establish that GSH level becomes lower in the brain due to lindane toxicity [22, 23, 27, 29, 30]. Administration of lindane decreased SOD activity in different regions of mice brain. It may be due to inhibition of SOD activity which ultimately can increase the concentration of superoxide anions.

Current investigation entails that concomitant exposure of curcumin diminishes the oxidative stress caused by lindane. Curcumin has antioxidant nature that may be reflected by a reduction in LPO and PCC content in the brain of Parkinson's disease [13, 31, 32]. Antioxidant mechanism of curcumin is due to scavenging of super oxides and hydroxyl radicals and ability to inhibit various oxidases [33]. Curcumin increases the level of GSH and SOD. This may be due to the fact that curcumin is able to eliminate the singlet oxygen and nitric oxide [34].

CONCLUSION

Based on results, it is clearly evident that curcumin decreases the TBARS and PCC content, thereby leads to a decline in the oxidative stress in the brain. Meanwhile, curcumin treatment enhances the GSH and SOD activities in different brain regions under investigation. Therefore, it is safely inferred that curcumin have neuroprotective potential, and it can be used as a therapeutic measure against lindane induced toxicity.

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CONFLICT OF INTERESTS

Authors declare that they have no competing interests.

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