

BUTYRYLCHOLINESTERASE DISTRIBUTION IN THE LAYERS AND NUCLEI OF CEREBELLUM OF MICE BRAIN FOLLOWING LINDANE TREATMENT; EFFECT OF AN ANTIOXIDANT COMBINATION AS A THERAPY

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ABSTRACT

Present study evaluates the effect of an antioxidants combination on cerebellum of mice, acutely intoxicated by lindane. 40 mg/ kg b.w. dose of lindane was selected for causing lesions in the cerebellum. Resveratrol, ascorbic acid, alpha-lipoic acid and vitamin E were used in a suitable combination for protection of brain against lindane induced toxicity. Butyrylcholinesterase (BChE) activity was used as a histochemical marker for lindane induced toxicity. After lindane treatment, BChE activity decreased in the lobes, layers and nuclei of cerebellum, compared to control. Antioxidants treatment enhanced the enzymatic activity. The group that was treated with both antioxidants and lindane showed enzymatic activity more than lindane treated group where as less as compared to purely antioxidants treated group. This reveals that suggested antioxidants combination is a potent therapy for lindane induced lesions in the cerebellum of mice.

Keywords: Lindane, Resveratrol, Ascorbic acid, Alpha-lipoic acid, Vitamin E, Cerebellum

INTRODUCTION

γ -Hexachlorocyclohexane (γ -HCH) also known as lindane is a white crystalline organic solid that turns into a vapor when released into the air. Once released, it looks colorless but has a musty odor¹. It is extensively used in agriculture worldwide during the 1970s. The United States Environmental Protection Agency (EPA) has declared lindane to be a persistent, bioaccumulative and toxic chemical.

In 1944, the insecticidal properties were found to be due to γ -isomer of lindane². In the environment it is widely spread due to persistent nature³. Lindane was present significantly in cow milk⁴ and breast milk in human⁵. This organochlorine pesticide is used extensively in agriculture and malaria vector control programme. It is used in ointments (lotions, creams and shampoos) for the control of lice and mites (scabies) in humans¹.

Lindane is reported to be a central nervous system stimulant. Symptoms of acute exposure in humans can include mental and motor retardation, central nervous system excitation, clonic (intermittent) and tonic (continuous) convulsions, respiratory failure, pulmonary edema and dermatitis. It enters animal tissues through food chain, respiration or dermal contact and is deposited in the membrane bilayer of all cell types⁶. Lindane and its metabolites persist in the environment, consequently affecting the human being and wildlife.

Resveratrol is the first natural medicine to have solid evidence behind it showing that it blocks or stops many stages of cancer. Resveratrol not only prevents cancer but also proposed as an additional treatment⁷⁻⁹.

In living organisms, ascorbate is an antioxidant, as it protects the body against oxidative stress¹⁰ and is a cofactor in several vital enzymatic reactions.¹¹ Vitamin C (Vit C) may also be able to regenerate other antioxidants such as vitamin E (Vit E).¹²

Alpha-lipoic acid (ALA) also known as thioctic acid is a disulphide compound i.e. a cofactor in vital energy producing reactions in the body. It is a very strong antioxidant that is produced in very small amounts by the body¹³⁻¹⁴ and also found in foods such as potatoes and red meat. It protects against damage by cell ravaging free radicals.

ALA can pass easily into the brain. It has protective effects on brain and nervous tissue and shows promise as a treatment for stroke and other brain disorders involving free radical damage. ALA also works

closely with vitamin C and E and some other antioxidants, recycling them and thus making them much more effective. It neutralizes free radicals in both the fatty and watery regions of cells, in contrast to vitamin C (which is water soluble) and vitamin E (which is fat soluble). ALA has been found to slow the progression of multiple sclerosis.¹⁵⁻¹⁶

Vit E deficiency results mainly in neurological symptoms including impaired balance and coordination (ataxia), injury to the sensory nerves (peripheral neuropathy), muscle weakness (myopathy) and damage to the retina of eye (pigmented retinopathy)¹⁷. Vit E may help protect against Parkinson's disease. In cancer cell lines, vit E ester, alpha-tocopheryl succinate, can inhibit proliferation of cells and induce apoptosis.¹⁸⁻¹⁹

Like acetylcholinesterase (AChE), butyrylcholinesterase (BChE) inactivates the neurotransmitter ACh and is hence a viable therapeutic target in Alzheimer's disease, which is characterized by a cholinergic deficit.²⁰ In the absence of AChE, the levels of extracellular ACh in the brain are controlled by the activity of BChE.²¹

MATERIALS AND METHODS

In the present study the effect of certain antioxidants (resveratrol, vitamin C, alpha-lipoic acid and vitamin E) was evaluated on the toxicity of lindane in the cerebellum of mice brain. Lindane was purchased from Sigma chemicals, St. Louis, Mo, USA (CAS No. 58-89-9 and purity 97%). Ascorbic acid, alpha-lipoic acid, vitamin E and butyrylthiocholine iodide were obtained from Hi Media (India). Resveratrol was purchased from Cayman Chemical Company, USA (CAS No. 501-36-0 and purity 98%). All the chemicals used were of analytical grade.

Experimental dose

(A) Dose selection of lindane: 40 mg/kg b.w. dose of lindane was selected for the present investigation. Dose selection of lindane was on the basis of drug tolerance study conducted in our laboratory.

(B) Dose selection of antioxidants: Selection of antioxidant dose was totally based on review of literature and it was found that combination of antioxidants would be better choice than studying single. The following concentrations of antioxidants were used to assess the neuroprotective action-

Resveratrol-5 mg/kg b.w.

Vitamin C-50 mg/kg b.w.

Alpha-lipoic acid-20 mg/kg b.w.

Vitamin E-50 mg/kg b.w.

Total-125 mg/kg b.w.

(C) Mode of exposure: Lindane was dissolved in olive oil. The antioxidants doses were prepared separately in two vials. In one glass vial, resveratrol and ascorbic acid were dissolved in distilled water whereas alpha-lipoic acid and vitamin E were dissolved in olive oil in other vial. The animals were exposed to lindane and combination of antioxidants subcutaneously.

Animals for experiment

Male Swiss mice (*Mus musculus*) 7-8 weeks old, weighing 22±3 gm were used from an inbred colony. Animals were kept in polypropylene cages and fed on standard pellet diet supplied from Aashirwad Industries, Chandigarh. Water was given ad libitum. The care and maintenance of the animals were as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimentations on Animals (CPCSEA).

Experimental design

The animals were divided into following groups for the conduct of study:

| S. No. | Group | No. of animals | Treatment | Dose | Duration |
|--------|-------|----------------|------------------------|--------------------------------|----------|
| 1. | I | 6 | Olive oil (vehicle) | - | 18 hours |
| 2. | II | 6 | Lindane | 40 mg/kg b.w. | 18 hours |
| 3. | III | 6 | Antioxidants | 125 mg/kg b.w. | 18 hours |
| 4. | IV | 6 | Antioxidants + lindane | 125 mg/kg b.w. + 40 mg/kg b.w. | 18 hours |

Procedure

The animals were sacrificed by cervical dislocation and the brain was immediately dissected out. The cerebellum was sliced and quickly fixed in cold calcium formol for 16-24 hours at 0-4°C. Prior to sectioning the required brain areas were washed briefly in distilled water. Frozen sections, 15 µm thick were cut on cryostat. The sections, after quick washing, were immediately transferred to the incubating medium.

Direct colouring method described by Karnovsky and Roots was adapted for the demonstration of BChE.²²

Data analysis

The assessment of enzyme reactions in histoenzymological studies is based on color intensities expressed as under:

| S. No. | Sign | Activity |
|--------|------|---------------------|
| 1. | - | No activity |
| 2. | + | Negligible activity |
| 3. | ++ | Mild activity |
| 4. | +++ | Moderate activity |
| 5. | ++++ | Strong activity |

RESULTS

The BChE activity has been studied in the various layers and nuclei of cerebellum.

(A) BChE activity in the layers of cerebellum

The cerebellum of mice brain is distinguished into different layers such as molecular layer (ML), Purkinje layer (PL), granular layer (GL) and nerve fiber layer (NFL). The description of BChE activity in various layers relates to the vermician lobe of cerebellum.

ML (Plate 1: figs. 1-4; Table 1): ML was completely devoid of BChE activity in control group. Moreover, the enzymatic activity was

present only in blood capillaries. Enzymatic activity remained unaffected by any of the treatments.

PL (Plate 1: figs. 1-4; Table 1): PL was completely devoid of BChE activity in control group. Moreover, the enzymatic activity was present only in blood capillaries. Enzymatic activity remained unaffected by any of the treatments.

GL (Plate 1: figs. 1-4; Table 1): GL was completely devoid of BChE activity in control group. Moreover, the enzymatic activity was present only in blood capillaries. Enzymatic activity remained unaffected by any of the treatments.

NFL (Plate 1: figs. 1-4; Table 1): Moderate BChE staining revealed in group I (fig.1). After treatment with lindane, NFL revealed mild reaction (fig.2). Strong and moderate activities of BChE were observed in NFL for group III (fig.3) and IV (fig.4) respectively.

(B) BChE activity in the deep cerebellar nuclei

Nucleus dentatus (D) (Plate 2: figs. 1-4; Table 1): The BChE preparations revealed strong activity in D in control (fig.1) and purely antioxidant treated group (fig.3). The BChE activity was drastically declined after the lindane exposure (fig.2). Antioxidant treatment prior to lindane exposure in group IV prevented the loss of BChE activity compared to group II (fig.4).

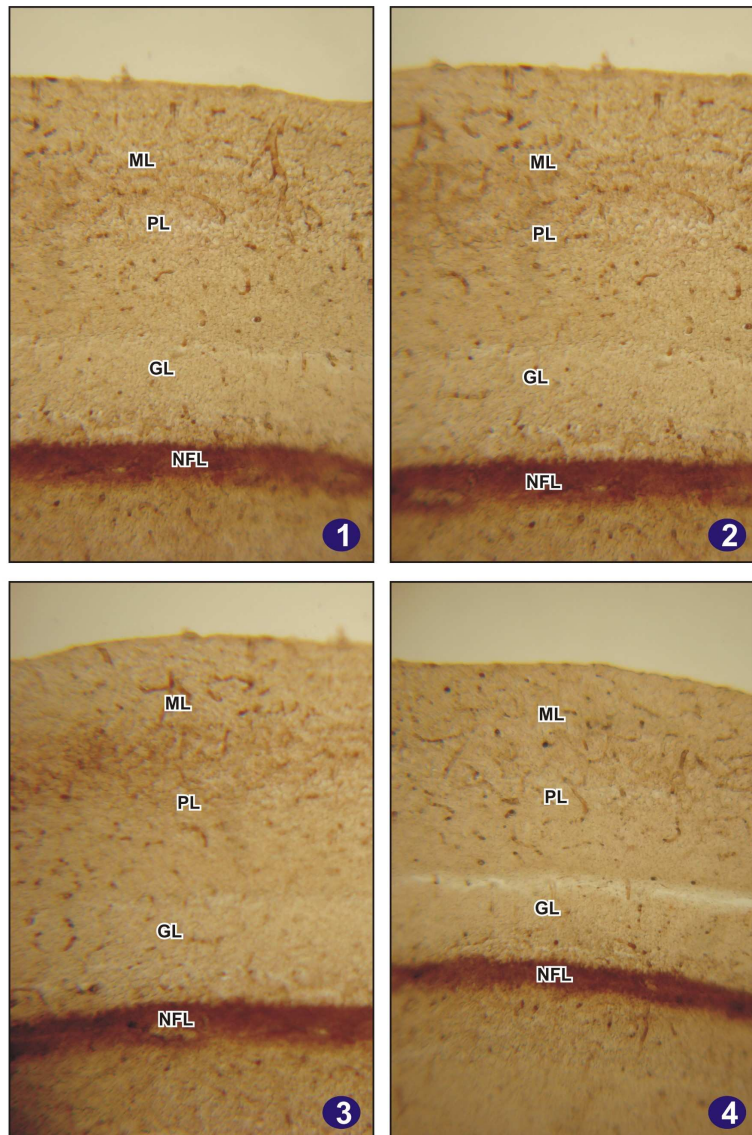
Nucleus fastigii (F) (Plate 2: figs. 1-4; Table 1): In control group, moderate BChE activity was observed in F (fig.1), which completely lacked in group II (fig.2). Antioxidants exposure in group III increased the enzymatic activity to strong (fig.3). In group IV, BChE activity reduced to moderate level (fig.4).

Nucleus interpositus (I) (Plate 2: figs. 1-4; Table 1): The strong BChE activity in control and antioxidant treated groups was found in I (figs. 1 and 3). The enzymatic activity became negligible after lindane treatment (fig.2). Moderate BChE activity was observed in the nucleus interpositus in group IV (fig.4).

Table 1: Demonstrating the distribution of BChE in layers and nuclei of cerebellum of variously treated mice

| Layer | Abbreviation | Group I | Group II | Group III | Group IV |
|----------------------|--------------|---------|----------|-----------|----------|
| Molecular layer | ML | - | - | - | - |
| Purkinje layer | PL | - | - | - | - |
| Granular layer | GL | - | - | - | - |
| Nerve fiber layer | NFL | +++ | ++ | ++++ | +++ |
| Nucleus dentatus | D | ++++ | - | ++++ | +++ |
| Nucleus fastigii | F | +++ | - | ++++ | +++ |
| Nucleus interpositus | I | ++++ | + | ++++ | +++ |

Activities are expressed by '-' and '+' and are graded from negative to strong; - =Negative, + =Negligible, ++ =Mild, +++ =Moderate, ++++ =Strong



GL : Granular layer

NFL : Nerve fiber layer

1. Control group

3. Antioxidants treated group

ML : Molecular layer

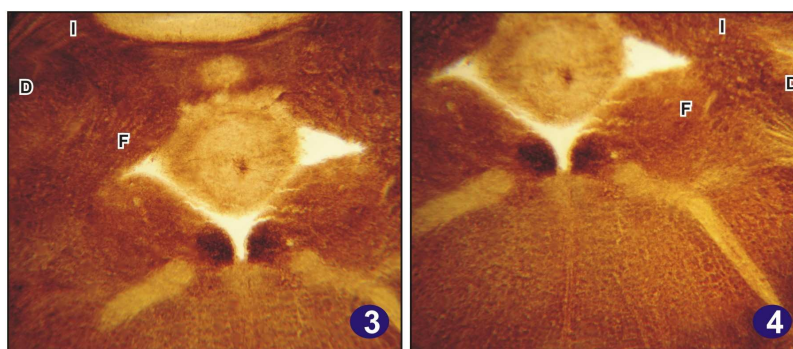
PL : Purkinje layer

2. Lindane treated group

4. Antioxidants + Lindane treated group

Plate 1: Different layers of vermean lobe of cerebellum showing BChE activity. ×100





D : Nucleus dentatus

F : Nucleus fastigii

I : Nucleus interpositus

1. Control group

2. Lindane treated group

3. Antioxidants treated group

4. Antioxidants + Lindane treated group

Plate 2: BChE activity in the deep cerebellar nuclei of mice brain. x25

DISCUSSION

In the present investigation lindane induced reduction of BChE level in the cerebellum of mice brain. BChE inactivates the neurotransmitter ACh and hence a viable therapeutic target against neurological disorders²⁰. Thus, inhibition of BChE by lindane treatment leads to disturbance in neurotransmission and is also in agreement with earlier investigations²³. However, the BChE is less efficient in ACh hydrolysis at low concentration, but highly efficient at high ones²⁴. Hence, a possible role for brain BChE, particularly when associated with glia, is supportive in hydrolysis of ACh. Under the conditions of high BChE activity in brain, local synaptic ACh can reach at micromolar levels. The spatial relationship of glial BChE would allow BChE mediated synergistic hydrolysis of ACh to permit the maintenance of normal cholinergic function.

The functional importance of BChE was previously underestimated because of its relatively low expression in the normal mammalian brain compared with AChE²⁵. However, research has led to increased interest in defining the role of this "orphan enzyme" within the nervous system and evaluating its importance in coregulating local concentrations of ACh²⁶⁻²⁸.

Biochemical change in BChE level is also supported by histochemical findings. In the control group, all the layers of cerebellum were devoid of BChE reaction except NFL. NFL revealed strong BChE reaction. In ML, PL and GL, BChE activity was observed in blood capillaries (endothelium) which is in agreement with earlier investigation that BChE is associated primarily with glial cells, endothelial cells and neurons²⁷.

The presence of BChE activity in the deep cerebellar nuclei suggests that the enzyme may be related to ionic balances in these regions. There seem to be a strong possibility that the ionic balances may be managed at such sites through the BChE activity present on the glial cells²⁹. BChE reaction declined in the deep cerebellar nuclei after treatment with lindane. It may lead to the disturbance in ionic balance.

In light of the suggested role of BChE in central cholinergic transmission, its altered expression in brain, and probable association with the development of neurochemical changes, it is hypothesized that low BChE activity would be detrimental in neurological disorders and that increase in the activity of this enzyme would be of clinical value.

Our body has several mechanisms to counteract the damage caused by free radicals. The basic and the most prominent defense mechanisms of human body are mediated by antioxidant agents. The

endogenous level of a single antioxidant enzyme is not adequate to detoxify the increased level of ROS that are generated in the body. Therefore a combination of antioxidants was used in the present investigation. Antioxidants treatment significantly increased the activity of BChE in histoenzymological preparations of cerebellum.

Pretreatment of antioxidants in lindane treated group revealed increased BChE activity as compared to lindane treatment group. Mammalian cells are equipped with both enzymatic and nonenzymatic antioxidant defense mechanisms to minimize the cellular damage resulting from interactions between cellular constituents and ROS. Despite the presence of cellular antioxidant systems, an overproduction of ROS in both intra- and extracellular spaces often occurs upon exposure of cells or individuals to radiation, hyperoxia, and certain chemicals. An unbalanced production of ROS in localized compartments has been postulated to play a role in the pathogenesis of a number of clinical disorders such as adult respiratory distress syndrome, ischemia reperfusion injury, atherosclerosis, neurodegenerative diseases, and cancer. This understanding illustrates the importance of the antioxidant defense system in maintaining normal cellular physiology.

ALA and vit E in combination were recorded to decrease lindane induced oxidative stress in brain³⁰. Resveratrol and vit C are water soluble antioxidants, thus protect the intracellular environment from free radicals damage. Both of these antioxidants in combination with ALA and vit E were reported to induce protective effect on brain in a recent study³¹.

However, because of the overlapping activity among some of the antioxidant enzymes, as well as the different intra- and extracellular sites of ROS production and expression of antioxidant enzymes, it is generally difficult to clearly define the physiological role of each of these enzymes. In conclusion, this study provides a suitable antioxidant combination for neuroprotection against lindane induced toxicity and its further elaboration may lead to development of effective therapeutic strategies of individual antioxidant system.

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