ORIGINAL RESEARCH PAPER

Zoology

EFFECT OF LEAD ACETATE ON THE LIPID PEROXIDATION IN BRAIN OF CATLA CATLA- A COMPREHENSIVE STUDY OF ANTI-STRESS PROPERTY OF WITHANIA SOMNIFERA

KEY WORDS: *W. Somnifera,* Lead acetate, Lipid peroxidation (LPO), Fluorescence product, Antioxidant

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ABSTRACT

Withania somnifera , belonging to the Solanaceae family. According to Indian Herbal System (Ayurveda), *Withania somnifera* is considered one of the most important herbs for its medicinal properties. In the present investigation, antioxidant activity of *Withania somnifera* administered through feed was tested against lead acetate induced oxidative stress in brain of fresh water fish Catla catla. For the present investigation fresh water fish catla catla were used. They were grouped in to Group I (control), Group II(fishes treated with lead acetate 0.43 ppm), Group III (*W. somnifera* fed at 1%, 2% and 3% of feed) and Group IV (lead acetate +feed with 1%, 2% and 3% *W. somnifera* leaves powder). All the four groups are continued for 48 and 96 hours. Antioxidant effect of plant extract was studied in brain of oxidative stressed fish on Lipid peroxidation (LPO) and fluorescence product.

In the present study, both total as well as mitochondrial lipid peroxidation and fluorescence product in brain was increased in lead acetate exposed fish. After the treatment of 3% ashwagandha feed, there was an significantly reduction in total as well as mitochondrial Lipid Peroxidation (LPO) and fluorescence product in group IV as compared with the oxidative stressed fish Group(II). The result of the present study shows that lead acetate induced oxidative stress and alteration in total and mitochondrial LPO and fluorescence products of the brain region were significantly protected with co-treatment of lead and *W. somnifera*. This could be due to its strong antioxidant potential property.

INTRODUCTION:

The increase in population, increased human activities, indiscriminate use of natural resources and dumping of wastes cause water pollution (Vasantharaja et al;2012). Increasing environmental pollution throughout the world, particularly aquatic pollution, has become one of the global problems of various toxins, such as heavy metals and toxic chemicals, when released into water bodies without proper treatment is most prevalent in developing countries. Such toxic metals and chemicals and their indiscriminate use resulted in problems with contaminants and polluted the aquatic environment. A toxicant is an agent that can produce an adverse response in an organism, seriously damaging its structure or function and resulting in death (Chavan et al;2014). A pollutant or foreign substance may be introduced deliberately or accidently in to the aquatic ecosystem, ruin the quality of the water and making it adverse for aquatic life.

Heavy metals are extremely toxic and ubiquitous in natural environments and they occur in soil, surface water and plants, which readily mobilized by human activities such as mining and dumping of industrial waste in natural habitats such as forests, rivers, lakes and ocean (Larison et al., 2000). As a result, heavy metals pose a potential threat to terrestrial biota. They are known to cause profound reproductive loss in animals (Eeva and Lehikoinen, 1997).

Lead is a ubiquitous heavy metal that exists in the environment due to its natural origin and as a result of industrial uses. Lead toxicity is currently one of the serious problems worldwide, there is still no specific, reliable and safe treatment (Barhoumi et al;2012). Impact of contaminants on aquatic ecosystems can be evaluated by using fishes (Begum G,2004). Fishes are considered as one of the most significant indicators in freshwater systems for the evaluation of environmental pollution (Farombi et al ;2007, Aruldoss Indra K, Sankar Samipillai;2014 , Jiraungkoorskul et al 2002).

Withania somnifera also called as 'Ashwagandha' belonging to Solanaceae family. It is one of the important herbs used in Ayurvedic medicine. It is used as general tonic to increase energy, improve all over health and longevity and prevent the diseases in athletes, the elderly, and during pregnancy. It may prevent tumour growth patient with cancer (Chatterjee and Pakrashi ,1995; . Jayaprakasam;2003). Glycowithanolides (Withaferin A) chemically characterized as 4b, 27-dihydroxy 5b-6b-epoxy-1 oxawitha-2, 24-dienolide, is one of the main withanolides active principles isolated from plant. *Withania somnifera* showed chemogenetic variation and so for three chemotype I, II, III had been reported (Abrahamet al;1968). Keeping this perspective in view, present in study was intended with the objective to elucidate the underlying deleterious mechanism of oxidative stress resulted due to lead intoxication through alterations in lipid peroxidation in fish brain. Therefore the aim of the present investigation was to investigate the possible ameliorative potential of *W. Somnifera* as a novel neuroprotective agent against lead toxicity induced oxidative stress in fish brain.

MATERIALS AND METHODS:

Plant:

Plant material in this experimental study, fresh leaves of *Withania somnifera* were collected from Town Hall Garden Kolhapur. The plant was identified by Taxonomist from Botany Department, Shivaji University Kolhapur. Plant extraction were extracted from of Withania somnifera plant as described by Bhattacharya et al (35).

Animal:

Experimental animal Healthy fingerlings (size of 4 to 6 cm in length and 6 ± 0.5 g in weight) of *Catla catla* were obtained from a Government fish seed production center, Dhom, Tal-Wai, Dist- Satara, Maharashtra, India. They were supplied in an aerated polythene bag to the laboratory.

The fish tanks were kept free from fungal infections by washing with potassium permanganate solution. The fishes were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. They were acclimatization before commencement of the experiment in laboratory condition for a week and fed daily on a commercially formulated feed.

Fishes were randomly divided into four groups and simultaneous treatment was given to all 10 animals in each group as follows:

Group I: Fishes were treated with distilled water (vehicle), which serves as control for 48 and 96 hours.

Group II: Fishes were treated with lead acetate 0.43 ppm for 48 and 96 hours (Shaikh, 2020) .

Group III: Fishes were feed with 1%, 2% and 3% W. Somnifera leaves powder for 48 and 96 hours . (Sharma et al. 2010)

Group IV: Fishes were treated with lead acetate +feed with

1%, 2% and 3% W. Somnifera leaves powder for 48 and 96 hours.

After Completion of each exposure period animals were sacrificed for brain i.e. cerebellum were dissected out for total and mitochondrial lipid peroxidation and fluorescence measurement of *W. Somnifera* against lead intoxication.

Determination of total lipid peroxidation (LPO) :
After the completion of doses the animals were sacrificed brain i.e. cerebellum were dissected out, blotted and weighed. The tissues were homogenized in reaction mixture (2 mg/ml) containing 75mM phosphate buffer (pH= 7.04), 1 mM ascorbic acids and 1mM ferric chloride with 20% Trichloroacetic acid (TCA) and 0.67% Thiobarbituric acid (TBA). The mixture were heated in boiling water bath. The Thiobarbituric acid reacting substance TBARS in the form MDA was measured on spectrophotometer (Miltons Roy company) at 532 nm.

Determination of mitochondrial lipid peroxidation :
For the mitochondrial fraction tissue was homogenized in 0.25 M sucrose and 1mM EDTA (2 mg/ml) and centrifugation was carried out at 3000 rpm for 10 min at 4o C (Cooling microfuge, Remi). The supernatant were again centrifuged at 10,000 rpm for 10 min at 4o C. The supernatant thus obtained were discarded, the pellete were resuspended in 0.2 ml 20% Triton X-100 and 0.8 ml distilled water and centrifuged at high speed 10.000 rpm for 10 min 4o C. The pellete obtained after high centrifugation were suspended in reaction mixture and used as sample for estimation of MDA in mitochondrial fraction. The total and mitochondrial lipid peroxidation was studied by Wills methods (Wills;1966), in which thiobarbituric acid reactive substance (TBARS) i.e. Malondialdehyde (MDA) was measured in to form of red coloured malondialdehyde- TBA spectrophotometer (Miltons Roy Co.) at 532 nm against blank (36). Lipid peroxidation was measured in the form of n mole MDA/mg wet tissue.

Measurement of fluorescence product:
Lipofuscinogenesis was studied Dillard and Tapple method (Dillard and Tapple ;1971). The brain i.e. cerebellum were homogenized by using the mixture prepared earlier for lipid peroxidation. The extraction was carried out by addition of chloroform: methanol (2:1 v/v) to 0.5 ml of homogenized tissue sample. It was mixed well on vortex mixer and then 3ml of double distilled water was added and centrifuged at 300 g for 2 min. To 1ml of upper layer 0.1 ml of methanol was added and the fluorescence was measured on photoflurometer calibrated with Quinine sulphate.

STATISTICAL ANALYSIS :
The statistical analysis was performed using One way Analysis of Variance (ANOVA) followed by instat 3 software.

RESULT:
The lipid peroxidation
Total lipid peroxidation
Total lipid peroxidation was found increased in the group II i.e. the lead acetate induced group and it is found directly proportional to the time of exposure period. Group III i.e. *Withania sominofera* treated animal shows no significant change in total lipid peroxidation as compared to the control group. The detailed data were shown in Table no – 1.

Mitochondrial lipid peroxidation
Mitochondrial lipid peroxidation also found directly proportional to the time of exposure period after exposure to lethal concentration of lead acetate. Group II shows significantly increased the mitochondrial lipid peroxidation after 48 and 96 hr of exposure. The detailed data were shown in Table no – 2.

Fluorescence product
In the cerebellum of fingerlimbs *catla catla*, Fluorescent product found increased after exposed to lethal concentration of lead acetate, as compared to control (group I) and this increase was highly significant. While there was decreased in group (IV) fish as compared to lead acetate treated fish exposed after 48 and 96 hours . In *Withania somnifera* treated groups similar results were observed in groups III as compared to group I. The detailed data were shown in Table no – 3

Table 1. Variation of total lipid peroxidation activity (n moles MDA /mg wet weight of tissue) in the brain i.e. cerebellum of fresh water fish Catla catla exposed to lead acetate and W. Somnifera for 48 and 96 hours.

Sr.No.	Hours of Exposure	Group I	Group II Lead Acetate	Group III W. Somnifera			Group IV Lead Acetate+ W. Somnifera		
				1%	2%	3%	1%	2%	3%
1	48 ours	20.189 ±4.082	72.115 ±4.080 ***	20.1898 ±4.082 NS	17.3036 ±4.354 NS	11.5384 ±1.00 NS	66.345 ±4.080 ***	54.8065 ±4.078 ***	49.038 ±4.080 ***
2	96 Hours	20.181 ±4.082	106.72 ±4.073 ***	19.9477 ±3.977 NS	11.538 ±1.00 NS	5.7692 ±1.00 *	90.1885 ±2.996 ***	83.6336 ±4.079 ***	72.115 ±4.080 ***

Table 2. Variation of mitochondrial lipid peroxidation activity (n moles MDA /mg wet weight of tissue) in brain i.e. cerebellum of the fresh water fish *Catla catla* exposed to lead acetate and W. Somnifera for 48 and 96 hours

Sr.No.	Hours of Exposure	Group I	Group II Lead Acetate	Group III W. Somnifera			Group IV Lead Acetate+ W. Somnifera		
				1%	2%	3%	1%	2%	3%
1	48 ours	46.153 ±8.159	147.125 ±4.094 ***	40.384 ±8.159 NS	37.499 ±4.079 NS	28.9610 ±4.080 NS	135.57 ±4.080 ***	124.35 ±4.080 ***	112.455 ±4.023 ***
2	96 Hours	46.153 ±8.159	222.364 ±11.886 ***	49.038 ±4.080 NS	25.711 ±3.726 NS	17.4228 ±4.080 *	204.80 ±8.163 ***	184.612 ±8.163 ***	152.8845 ±4.079 ***

Table 3. Variation of Fluorescence product(ug/mg wet tissue) in the brain i.e. cerebellum of fresh water fish Catla catla exposed to leadacetate and *W. Somnifera* for 48 and 96 hours

Sr.No.	Hours of Exposure	Group I	Group II Lead Acetate	Group III W. Somnifera			Group IV Lead Acetate+ W. Somnifera		
				1%	2%	3%	1%	2%	3%
1	48 ours	0.00882 ±0.0013	0.01862 ±0.0013	0.007843 ±0.0027	0.00538 ±0.0027	0.00398 ±0.00	0.01638 ±0.0018	0.0137 ±1.00	0.1176 ±0.0748
2	96 Hours	0.00882 ±0.0013	0.02254 ±0.0013	0.0098 ±0.00	0.00761 ±0.0002	0.00490 ±0.0013	0.2156 ±0.1386	0.01764 ±0.098	0.01470 ±0.0013

DISCUSSION:

In the present study, total and mitochondrial LPO level of the fish organs increased mainly in the brain i.e Cerebral hemisphere of group II(Lead acetate exposed group for 48 and 96 hrs group). Brain was exposed to the toxin, so that brain TBARS increased, lead induced oxidative stress in the brain tissues. Lipid per oxidation has been extensively used as a marker of oxidative stress (Hugett et.al., 1992). .Yousef et al., 2003; Manna et al., 2004 showed that basically, the main mechanism of the toxic effect of pesticides involves the generation of a high level of free radicals, and thereby the damage of tissues and organs .These radicals attack the cell membrane and lead to destabilization and disintegration of cell membranes a result of lipid per oxidation (Stajn et al., 1997). TBARS is a major oxidation product of per oxidized polyunsaturated fatty acids, and increased TBARS content is an important indicator of lipid per oxidation (Celik and Suzek, 2009)

Free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health and disease management.(Aroma) Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids.(M. C. Cord)

Halliwell B reported that one of the major reasons for lead-induced toxicity to mammalian tissues might be attributed to its ability to generate reactive oxygen species (ROS) leading to enhancement of complex mechanism of lipid peroxidation as the outcome of peroxidative chain events. Kang et al and Caylak et al demonstrated that the underlying mechanism of lead toxicity involves stimulation of oxidative stress. Janero and Caylak Et al and research showed that exposure to low level dose of lead acetate doses is extremely dangerous and results in a variety of neurological disturbances. Number of studies revealed that lead acetate responsible for variety of neurological disorders include nerve damage, Alzheimer's disease, schizophrenia and Parkinson's disease , mental retardation, behavioral problems and also result in many biochemical alterations. In the present investigation , lead exposure was associated with a increase in lipid peroxidation and fluorescence product as compared to control group , which was consistent with the previous studies . Pramod et al also demonstrated that increased levels of lead in the brain of rat region, which further provides experimental evidence that lead can cause blood-brain barrier and impart its toxic effects. Soltaninejad et al has been reported that exposure of lead responsible for the generation of free radicals associated with the enhanced oxidative stress. Velaga et al and sharma et al experimental studies have shown an increase in lipid peroxidation and impaired antioxidant defence enzymes in brain following lead exposure, suggesting enhanced oxidative stress.

W. somnifera supplementary alone exposed fish (group III) shows more towards normalcy in lipid peroxidation content and fluorescence product in brain tissues compared to control (group I). *W. Somnifera* is a essential plant in various traditional system of medicine. *W. somnifera* improves energy and also memory by enhancing the brain and nervous functions; shows anxiolytic effects, has hepatoprotective property, raises haemoglobin level and red blood count , improves energy level; has potent antioxidant activity, improve the cell mediated immunity; promotes vigour and vitality along with cheerful sexual life and reproductive equilibrium and acts as powerful adaptogen (Umadevi et al.2012; Ziauddin et all996) Walvekar et al 2015 demonstrated that *Withania somnifera* has a capability of preventing oxidative stress and also combating stress induced infertility. Lipid peroxidation and fluorescence products were significantly decreased with co-treatment of lead and *Withania somnifera* (group IV) as compared to lead acetate (group II). This could be due to its strong antioxidant potential and metal-binding property. In conclusion, our findings clearly indicate that lead induced oxidative stress and increased concentrations of LPO and fluorescence product of fish brain suggesting that it is one of the hazardous heavy metals. This plant extract is a significant source of natural antioxidant which has a capacity to reduces ROS and inhibiting the over production of lipid peroxidation. This indicates that *W. somnifera* offer protection against lead acetate induced oxidative stress in brain.

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