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A Protective Role of Flaxseed Extract against Neuronal Damages Caused by Lead Acetate Toxicity

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***Abstract*: Lead is one of the most common environmental toxicants, exposure to which can cause significant neurotoxicity and an associated decline in brain function. This study was designed to evaluate the neuroprotective and ameliorative effects of flaxseed extract against lead induced neurotoxicity. The mice were distributed into four groups. Mice of the first group were served as control. Mice of the second group were exposed to lead acetate. Mice of the third group were treated with flaxseed extract. Mice of the fourth group were treated with lead acetate and flaxseed extract. Results showed that lead treatment caused a significant decrease in the average body and organ weight as compared to control. Thiobarbituric acid reactive substances (TBARS), an indicator of oxidative stress, and activity of glucose-6- phosphate dehydrogenase (G6PD) significantly increased in lead treated mice. Administration of flaxseed extract prevented and reversed the observed biochemical alterations induced by lead acetate exposure. Hence, the results of this study suggest that flaxseed extract protects against lead acetate induced brain toxicity and the protective influence of flaxseed extract may be attributed to its antioxidant role.**

***Keywords*: Lead acetate, flaxseed, TBARS, oxidative stress, glucose-6-phosphate dehydrogenase.**

1. Introduction

Lead is the most important toxic heavy element in the environment. It's important physico-chemical properties seem to make difficult to give up its use. Due to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards. Lead is a highly poisonous metal affecting almost every organ in the body. Of all the organs, the nervous system is the mostly affected target in lead toxicity, both in children and adults. The direct neurotoxic effects of lead include damage to neuronal mitochondria, leading to programmed cell death and distribution of the release of neurotransmitters. Recent studies have recognized oxidative stress as a major indirect mechanism of lead induced neurotoxicity. Lead causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells. Medicinal plants are now important targets of drug synthesis. Various extracts and drugs of plant origin have shown promise in the treatment of a range of neurological diseases, and many studies have demonstrated how the antioxidant properties of natural products help to mitigate the toxicity of lead acetate. The flaxseed (Linum usitatissimum) is the seed from the flax plant, an annual herb which belongs to Linaceae family. The potential health benefits of flaxseed are anticancer effects, antiviral, bactericidal and anti-inflammatory activities. Most of the reported biological activities and active constituents of this plant may be related to its antioxidant nature. The objective of present investigation was to study the in vitro antioxidant activity of ethanolic extract of Linum usitatissimum for free radical scavenging, superoxide anion radical scavenging and hydrogen peroxide scavenging activities.

2. Review of Literature

Babalola et al. (2005) reported that examples of heavy metals commonly found in the environment include lead, cadmium, mercury, zinc, arsenic, bismuth etc. These metals are particularly dangerous because they tend to bio-accumulate in the body tissues and organs. Markovac and Goldstein (1988) proposed that lead competes with metals like calcium, zinc, iron and that are essential to our body. Lead's ability to substitute for calcium is a factor common to many of its toxic actions. Picomolar concentrations of lead, competes with micromolar concentration of calcium for binding sites on cerebella phosphokinase C, thereby affecting neuronal signalling. Liliana et al. (2010) presented that Lead exposure at low concentrations, causes alterations in the nervous functions, which leads to a reduction of brain ability to use serotonin and dopamine, neurotransmitters directly associated with conduct disorders and aggressiveness in mice. Shawky et al. (2014) reported a correlation between lead and various aspects of human behaviour such as violent and criminal behaviour, homicide, delinquent, antisocial behaviour and aggression. Garcia and Gonzalez (2008) suggested that administration of various antioxidants could prevent and cure the toxic effects of lead that causes the generation of free radicals in the body. They have the ability to scavenge reactive oxygen species at molecular level and chelate lead ions, thereby reversing the toxic effects. Chinthana and Ananthi (2012) reported that the

neurotoxicity induced by lead in albino mice could be significantly reduced by oral administration of extract of Solanum nigrum and Solanum trilobatum leaves for 30 days. Gholaminejhad et al. (2017) proposed that that treatment with flaxseed diminished tissue degeneration and improved locomotor function, which can be explained by a reduction in the oxidative stress level after unilateral spinal cord injury.

3. Materials and methods

Healthy, pathogen free Swiss albino mice (7-8 weeks) were used for the experiment. These animals were maintained in the animal house at a temperature of 24 ± 3°C and normal photoperiod (12 hr light and 12 hr dark). Animals were housed in polypropylene cages and fed standard mice feed (Hindustan Lever Ltd., India). All the experimental procedures were conducted after the approval of Institutional Animal Ethics Committee (IAEC/Bio/8-2009) of Himachal Pradesh University, Shimla.

A. Chemicals

All the chemicals used in the study were of analytical reagent and obtained from SD fine chemicals (Mumbai, India), HIMEDIA (Mumbai, India).

B. Experimental Plant

Seeds of Linum usitatissimum were obtained from Kangra district of Himachal Pradesh and identified in Himalayan Forest Research Institute (HFRI), Panthaghatti, Shimla. Flaxseeds were ground in a mechanical grinder to obtain a fine powder. The fine powder of flaxseeds was defatted by blending the ground material with hexane (1:6, w/v) for 12h at 25oC. The defatted flaxseed powder was air dried for 12h. Two hundred grams of this powder was blended with 1.2 L mixture solvent of ethanol and water (7:3, v/v) for 24 h at room temperature. The extract was filtered, and then concentrated at 50oC by a rotary evaporator. Light yellow syrup was obtained. It was transferred to a small tube and the volume was made up as required for the experiment.

C. Experimental Design

Normal healthy looking mice showing no sign of morbidity were divided into four groups:

Control group served as normal. Second group received oral administration of lead acetate (10 mg/kg body weight) daily. Third group was given flaxseed extract by oral gavage at a selected dose level (100 mg/kg body weight) daily. Fourth group was exposed to lead acetate plus flaxseed extract. Mice were sacrificed at 10, 20 and 30 days period by cervical dislocation.

D. Body weight & brain weight

Body weight and brain weight of the animals were observed in all the groups both at the start of the experiment and at the time of sacrifice.

E. G6PD Activity

The activity of Glucose -6- phosphate dehydrogenase was spectrophotometrically measured by the reduction of NADP at 340 mμ, using glucose-6- phosphate (G6P) as the substrate. Reaction mixture (4.5ml) consisted of 300 μl supernatant, 5 mM glucose -6-phosphate, 50 mM tris buffer, 4.89 mM MgCl2, 0.1 mM NADP. The reaction was started with the addition of NADP. The reaction mixture was then incubated at 37o C for 25 minutes. A blank was run simultaneously replacing supernatant with distilled water. Absorbance was measured at 340 mμ until a maximum and constant rate of absorbance was obtained.

F. Lipid peroxidation

Tissues were homogenized in 2 ml of 0.1% TCA in a pestle and mortar. Tissue homogenate was then centrifuged at 6000 rpm for 15 minutes. 1 ml of supernatant was taken and 2 ml of 0.5% TBA prepared in 10% TCA was added. The test tubes were then cooled in ice-cold water bath and then centrifuged again. Absorbance of the supernatant was measured at 532 nm and 600 nm. The difference of the two absorbances was taken as actual value and used for calculating the TBA reactive substances (TBARS)/malondialdehyde (MDA) formed. The TBARS or MDA contents formed were calculated in n moles/ml.

4. Results and discussion

In the current study, mice administered lead acetate had a non- significant decrease in body weight (Table & Fig. 1) and brain weight (Table & Fig. 2) of mice. However, in fourth group animals, there was a gradual increase in body weight and brain weight when compared with second group animals. Glucose-6-phosphate dehydrogenase activity (G6PD) was significantly increased (*P< 0.05; **P< 0.01) in the brain of lead acetate treated mice as compared to control. Treatment of lead acetate treated animals with flaxseed completely inhibited the increase of G6PD activity (Table & Fig. 3). Mice brain treated with lead acetate witnessed significant increase (**P< 0.01) in MDA values in comparison to control at all stages of investigation. However, mice administered lead acetate with flaxseed extract induced a decrease of the formation of thiobarbituric acid reactive substances to a certain extent (Table & Fig. 4). The results indicated a potential role of flaxseed extract in mitigating lead acetate mediated toxic effects. Yiin and Lin (1995) demonstrated marked enhancement in malondialdehyde (MDA) as a result of incubation of linoleic, linolenic and arachidonic acid with lead. This finding is proven by many other studies, which have pointed to either elevated lipid peroxidation or decreased intrinsic antioxidant defense in various tissues of lead-exposed animals (Sandhir and Gill, 1995). Glucose-6-Phosphate dehydrogenase is known as a useful biomarker for antioxidant system and its activity is increased in oxidative stress (Salvemini et al., 1999; Gul et al., 2004). In present study, we investigated the effects of lead

acetate on G6PD activities in brain. A general elevation of G6PD activity have been observed. This observation is in agreement with a report by Sadhu et al. (2008) in which a higher activity of G6PD in erythrocytes of the workers occupationally exposed to lead was found. Our findings are in agreement with the results of Ismail et al. (2016) and our study observed normal level of TBARS after flaxseed treatment. This effect is interrelated to the ability of flaxseed to scavenge the free radicals and enhance the antioxidant enzyme activity. Flaxseed might ameliorate lead toxicity by increasing the reactive oxygen species (ROS) detoxification and decreasing ROS generation.

Table 1
Groups and Days

Groups	Days		
	10	20	30
C	23.66 ± 0.25	24.33 ± 0.35	25.17 ± 0.31
L	23.16 ± 0.33	23.08 ± 0.30	22.75 ± 0.17
F	23.83 ± 0.31	24.42 ± 0.45	25.25 ± 0.34**
F+L	23.60 ± 0.81	24.08 ± 0.37	24.92 ± 0.27**

C = Control; L = Lead treated; F = Flax seed; F+L= Flaxseed + Lead treated

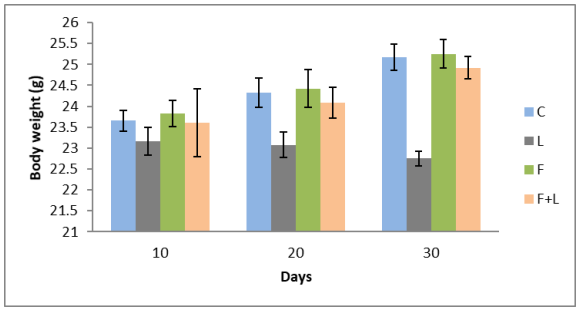

Fig. 1. Changes in body weight (g) of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Table 2
Groups and days

Groups	Days		
	10	20	30
C	496.81 ± 0.72	498.27 ± 0.35	499.07 ± 0.34
L	494.32 ± 0.43	494.27 ± 0.91	493.93 ± 0.86
F	497.78 ± 0.49**	498.23 ± 0.48	500.73 ± 0.84**
F+L	495.39 ± 0.59	498.39 ± 0.51	499.89 ± 0.64**

C = Control; L = Lead treated; F = Flax seed; F+L= Flaxseed + Lead treated

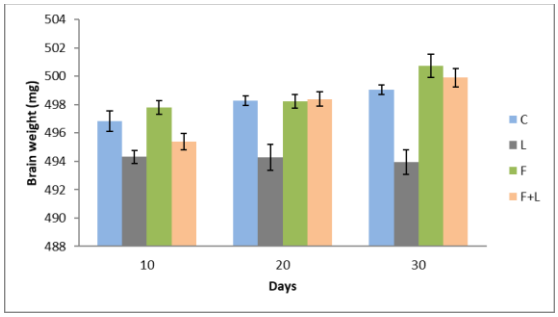

Fig. 2. Changes in brain weight (mg) of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Table 3
Groups and days

Groups	Days		
	10	20	30
C	0.39 ± 0.03	0.39 ± 0.02	0.38 ± 0.01
L	0.48 ± 0.01**	0.49 ± 0.01**	0.52 ± 0.06*
F	0.35 ± 0.01	0.39 ± 0.05	0.34 ± 0.03
F+L	0.43 ± 0.01*	0.41 ± 0.01	0.43 ± 0.04

C = Control; L = Lead treated; F = Flax seed; F+L= Flaxseed + Lead treated

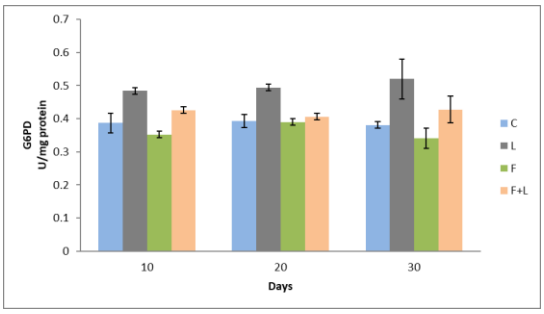

Fig. 3. G6PD specific activity (U/ mg protein) in brain of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Table 4
Groups and Days

Groups	Days		
	10	20	30
C	51.08 ± 1.92	52.25 ± 1.86	55.09 ± 1.54
L	58.89 ± 2.17**	61.85 ± 2.04**	64.02 ± 1.23**
F	49.25 ± 1.28	48.93 ± 1.25	50.53 ± 1.30
F+L	54.19 ± 1.62	55.52 ± 1.98	57.35 ± 1.93*

C = Control; L = Lead treated; F = Flax seed; F+L= Flaxseed + Lead treated

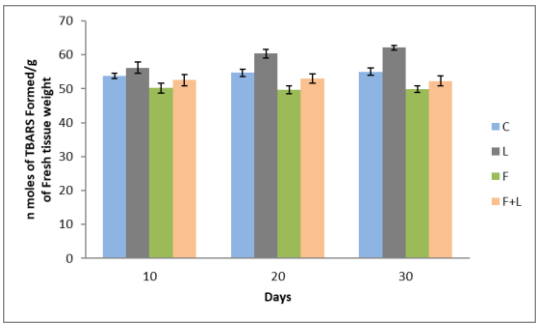

Fig. 4. Lipid peroxides (n moles of TBARS formed/g of fresh tissue weight) in brain of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n=6 (*P< 0.05; **P< 0.01).

5. Conclusion

Collectively, our study proposed that lead acetate caused alterations in all the parameters studied which are clearly confirmed by our results. All these changes were mitigated when the mice were administered with flaxseed extract.

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