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THOMSON REUTERS
International Journal of Medical Research and Pharmaceutical Sciences
Volume 5 (Issue 12): December 2018 ISSN: 2394-9414
DOI- 10.5281/zenodo.2526986 Impact Factor- 4.174

AMELIORATING EFFECTS OF FLAXSEED EXTRACT ON LEAD-INDUCED BIOCHEMICAL CHANGES IN BRAIN OF SWISS ALBINO MICE

Sushma Sharma[1] & Archana Thakur*[2]
[1&*2]Department of Biosciences, Himachal Pradesh University, Summer Hill, Shimla, India-171005

Keywords: *Lead, brain, cytochrome c oxidase, flaxseed.*

Abstract

Lead (Pb) is being currently used in various forms and is getting exposed to living system via different routes. To determine the ameliorative effect of flaxseed extract on biochemical changes due to lead poisoning in Swiss albino mice for thirty days, mice were assigned to four groups i.e. (i) control group (ii) mice treated with 10 mg/kg body weight of lead acetate orally (iii) mice given 100 mg/kg body weight of flaxseed extract orally (iv) flaxseed extract plus lead acetate treated group. Exposure to lead resulted in reduction in brain triglycerides and phospholipids concentrations. A non significant decline in cytochrome c oxidase (COX) activity was observed in lead acetate treated group. However, triglyceride and phospholipid alterations in brain induced by lead acetate were mitigated in mice co treated with flaxseed extract and also exhibited a significant increase in COX activity. These findings indicate that flaxseed has beneficial effects and it mitigates lead acetate induced neurotoxicity.

Introduction

In recent years, lead has become a regulatory concern and subject of much interest among pharmacologist, environmental scientist and clinicians because of its widespread distribution in environment due to its continuous emission from industrial sources, automobile exhaust and its pharmacological behaviour to remain bound to mammalian tissues for a long duration. Next to chlorine, lead is the most common contaminant found in tap water. Most lead in drinking water comes from lead-lined pipes, lead solder and brass plumbing fixtures inside our home. Low levels Exposure of lead has been linked with functional and structural impairments in both human and experimental animals (Reza *et al*., 2008) and this pollutant causes well documented neurological impairment (Soong *et al*., 1999). A number of studies demonstrated that prolonged exposure to lead induces slower nerve conduction and an alteration of calcium homeostasis (Orrenius *et al*., 1992). The hematopoietic, nervous and renal tissues are the main targets of lead. Moreover, it hinders the efficiency of the hepatic, reproductive and immune function (Teijon *et al*., 2006 ; Durgut *et al*., 2008). Recently, there have been many studies on the use of natural products such as vitamins and herbal drugs to expel lead (Xu *et al*., 2005). The major merits of plant based medicine seem to be their perceived efficacy, low incidences of serious adverse effects and low cost (Bhattacharya & Haldar, 2013). Flaxseed (*Linum usitatissimum*) is a seed from flax plant, an annual herb, which is a member of the Linaceae family (Rubilar *et al.*, 2010). It is a rich source of omega-3 fatty acid, lignan phytoestrogen, and soluble fibers (Oomah, 2001). The current study was therefore planned to utilize a mice model to elucidate whether flaxseed extract, when administered with lead acetate can ameliorate lead-induced neurotoxicity.

Material and methods

Male Swiss albino mice weighing between 20-25g were used in this study. These animals were maintained in the animal house of department of Biosciences of Himachal Pradesh University under proper hygienic conditions, room temperature (24 ± 2°C), a 12-h light–dark cycle, and they had free access to food and water. Animal handling was performed following the guidelines of the Institunal Animal Ethics Committee, Himachal Pradesh University, Shimla.

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

Plant material: Seeds of *Linum usitatissimum* were obtained from Kangra district of Himachal Pradesh and identified in Himalayan Forest Research Institute (HFRI), Panthaghatti, Shimla. A flaxseed extract was prepared according to the method of Zhang *et al.* (2007).

Phytochemical screening of flaxseed: Flaxseed extract was screened for the presence of various phytochemical constituents i.e. tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and carbohydrates by using standard methods (Harborne, 2005; Raman, 2006).

Experimental design: Mice were randomly divided into four groups. Each group consists of 6 mice with following description:

- Mice in first group serve as control.
- Mice of second group were given lead acetate (10 mg/kg body wt.) for 30 days.
- Mice of third group were administered flaxseed extract (100 mg/kg body wt.) for 30 days.
- Mice of fourth group were treated with flaxseed extract plus lead acetate.

Following various treatments mice were autopsied by cervical dislocation on day 10, 20 and 30. The brain was quickly excised and washed two times with cooled saline. Lipids were extracted by homogenizing the brain with 2:1 chloroform methanol (v/v) as per method of Folch *et al.* (1957). Lipid extract thus obtained was employed for the estimation of triglycerides and phospholipids concentrations.

Triglycerides: Method of Vanhandal and Zilver Smith (1957) using arsenic trioxide and chromotropic acid was employed for the triglycerides estimation and standard curve was drawn using various concentrations of tripalmitin.

Phospholipids: Ammonium molybdate method of Ames (1966) was undertaken for quantitative estimation of phospholipids and standard calibration curve was drawn using various concentrations of KH_2PO_4.

Cytochrome c oxidase: Cytochrome oxidase (COX) activity was measured by the method of Smith (1955). The reaction mixture comprised of 20 mM Tris pH 8.0, 0.5% Tween 20 and 0.05 mM reduced cytochrome C. Samples were preincubated for 6 min at 15^{o}C in the presence of the complete assay mixture prior to the addition of cytochrome C. The rate of change in absorbance was monitored at 550 nm.

Protein determination: The protein content was measured according to the method of Lowry *et al*. (1951).

Results

Phytochemical analysis

The freshly prepared extract was subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of tannins, alkaloids, saponins, flavonoids and carbohydrates. Terpenoids and steroids were absent.

Biochemical analysis

The present results (Table & fig. 1) showed that treatment with lead acetate decreased the triglycerides level in mice brain as compared to normal group. However co-treatment of mice with flaxseed extract protected the triglycerides level from being decreased in brain tissue (*P< 0.05 vs. lead treated group). Table & fig. 2 illustrated that mice treated with lead acetate showed non significant decrease in phospholipids level. Also Table & fig. 2 revealed that treatment of mice administered lead by flaxseed extract showed significant increase in brain phospholipids level. Treatment of mice with lead acetate for 30 days at a dose of 10 mg/ kg body weight also decreased cytochrome c oxidase activity in brain tissue. The activity of this enzyme was found to be significantly protected from being

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decreased compared to lead acetate treated group when mice were co-treated with flaxseed extract (**P< 0.01,*P< 0.05 vs. Lead acetate treated group). Flaxseed extract alone, also increased the activity of this enzyme significantly (**P< 0.01) in the brain tissue (Table & fig. 3).

Table 1

Groups	Days		
	10	20	30
C	7.19 ± 0.80	6.84 ± 0.72	7.05 ± 0.73
L	7.03 ±0.87	4.43 ±0.85	3.86±0.56
F	7.95 ± 0.76	7.47 ± 0.87*	7.86 ± 0.59**
F+L	7.12 ± 0.86	6.46 ± 0.61	6.97±0.87*

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

Fig. 1

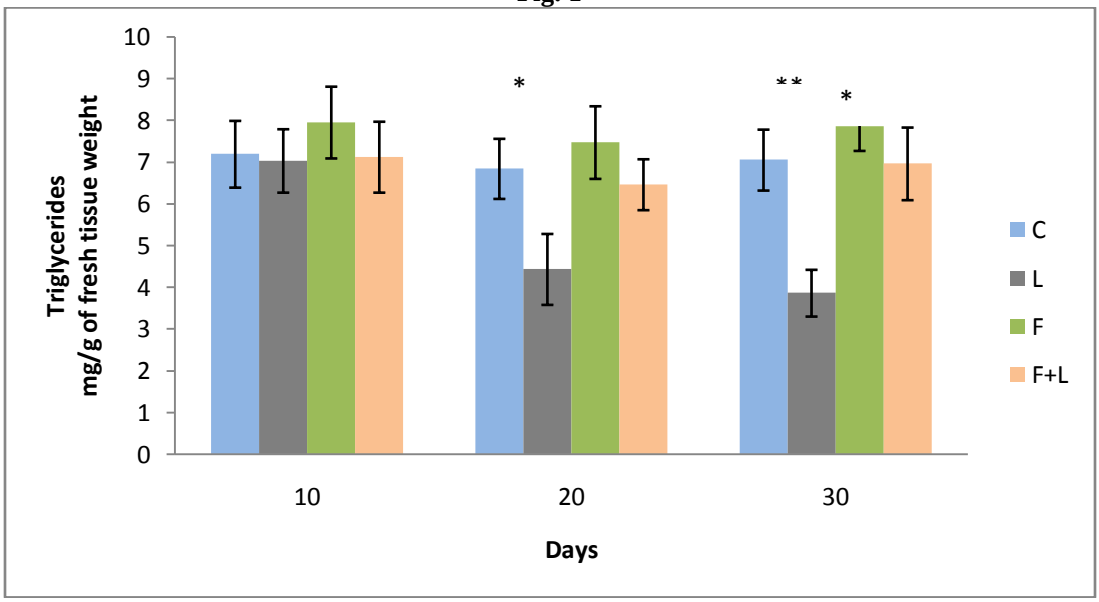

Table & Fig. 1: Triglycerides (mg/g fresh tissue weight) in brain of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Table 2

Groups	Days		
	10	20	30
C	4.31 ± 0.38	4.95 ± 0.49	4.89± 0.43
L	3.02 ± 0.36	2.96 ± 0.38	2.76 ± 0.34
F	4.66 ± 0.58	5.09 ± 0.55*	5.23 ± 0.55*
F+L	3.47 ± 0.52	3.01 ± 0.54*	3.13 ± 0.72

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

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Fig. 2

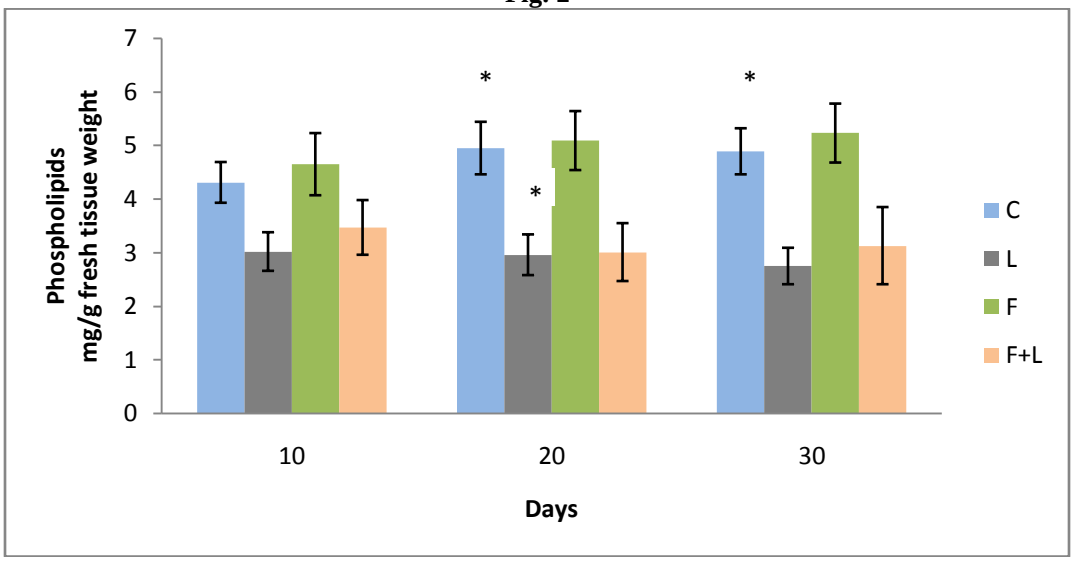

Table & Fig. 2: Phospholipids (mg/g fresh tissue weight) in brain of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Table 3

Groups	Days		
	10	**20**	**30**
C	331.18 ± 5.84	347.84 ± 4.16	374.37 ± 1.05
L	317.02 ± 3.79	314.04 ± 3.49	321.42 ± 1.04
F	359.22 ± 6.83**	367.54 ± 1.41**	383.45 ± 1.11**
F+L	319.39 ± 0.02	331.26 ± 5.58*	347.04 ± 0.84**

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

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Fig. 3

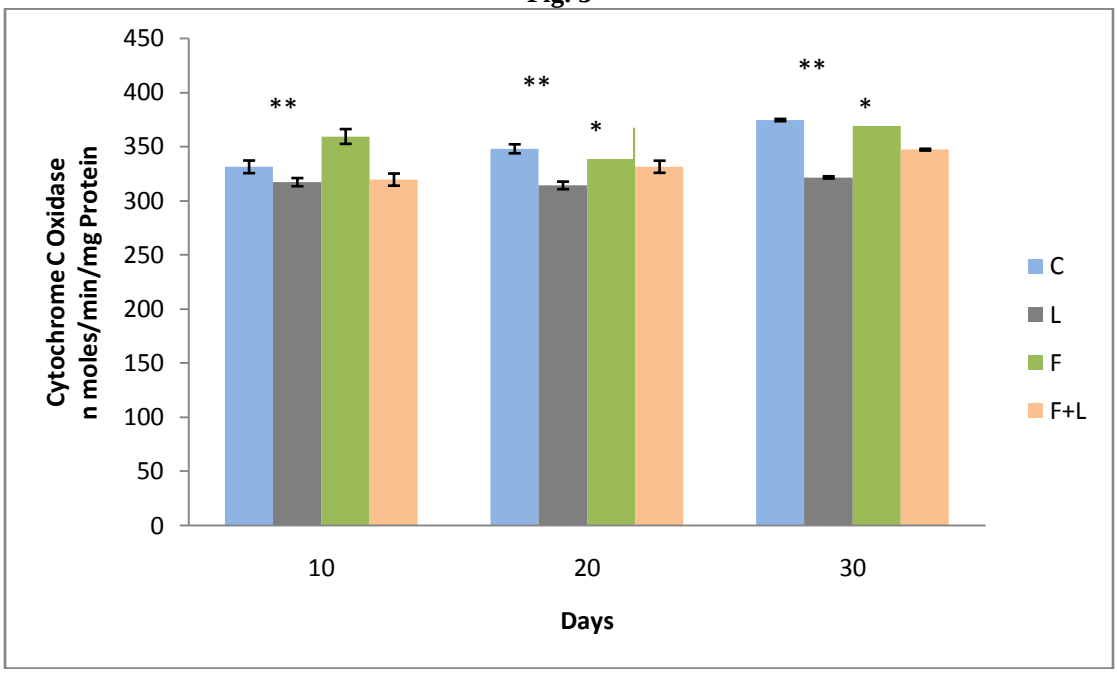

Table & Fig. XVI: Cytochrome oxidase (COX) activity (n moles cytochrome c oxidized/min/mg protein in brain of normal, lead treated, extract treated and lead treated plus extract treated mice from 1-30 days period. Values are mean ± SEM; n =6 (*P< 0.05; **P< 0.01).

Discussion

Triglyceride uptake and/or its availability in any cell at a time are strongly related to lipoprotein lipase (LPL) activity (Belahsen and Deshaies, 1992). It has been suggested that modulation of triglyceride metabolism particularly tissue specific alterations in LPL activity, may be one of the pathways by which heavy metals affect partitioning of lipid substrates (Lucia *et al.*, 2009). In the present work the lipid profile indicated a significant decrease in triglycerides levels in all lead acetate treated groups. These findings are in parallel with those mentioned by El-Moneum Goma and Tohamy, (2016). Ogunrinola (2015) observed reduced brain triacylglycerol after exposure to a heavy metal (Cd). Recovery action of flaxseed has been noticed in present study and small improvement in triglyceride level was also seen. A similar increase in serum triglyceride levels was observed by Stuglin and Prasad (2005) after flaxseed consumption. In the present work, lead acetate treatment has shown a decrease in the phospholipid level in brain. Similar results were obtained by Tulasi *et al.* (1992) in different tissues of *Anabas festudineus.* On contrary, Ogunrinola (2015) showed that phospholipid levels in brain of heavy metal treated groups were higher however the levels of phospholipid contents in plasma, erythrocytes and hepatic tissue were reduced in similar experiment. However recovery action of flaxseed extract was observed. This finding is congruent with those of Dell *et al.* (2001) who indicated a positive effect of flaxssed diet on the concentration of liver phospholipids. Cytochrome oxidase (COX) representative enzyme of electron transport chain is a key to synthesize ATP hence it is involved in the derivation of energy for tissue use. Therefore, this enzyme is vital for optimal cellular functions (Fontanesi *et al*., 2006). Heavy metals are also known to affect respiratory chain complexes and there is substrate specificity (Belyaeva *et al.*, 2011). Findings of present study are in parallel with those of El-Masry *et al.* (2011). All these changes are mitigated when the mice are co treated with flaxseed extract. The results indicate that the flaxseed ameliorates lead-induced toxicity in experimental mice by antioxidants particularly flavonoids present in the extract.

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Conclusion

The present study conclude that the alterations in triglycerides and phospholipids level, changes in cytochrome oxidase activity could play an important role in lead induced neurotoxicity. Ethanolic extract of the flaxseed attenuates lead induced neurotoxicity as it greatly improves triglycerides and phospholipids concentrations and cytochrome c oxidase activity.

Acknowledgment

Thanks are due to UGC-BSR, New Delhi for providing financial support to one of us (AT).

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