Original Article

Protective Effects of Flaxseeds (Linum Usitatissimum L.) on Hepatotoxicity Induced by Triazophos on Male Wistar Rats

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Objective: To determine the effects of flaxseeds on triazophos-induced-hepatotoxicity.
Methods: In the current study 24 Wistar albino rats (age: 6-8 weeks, weight 150-200gms) were segregated into four groups comprising six rats each. Group A was kept as control group. Groups B, C and D were labelled as experimentalgroups. Group B was given powdered flaxseeds (800mg/kg b.w.), Group C was administeredtriazophos (8.2mg/kg b.w.) and Group D was giventriazophos (8.2mg/kg b.w.) and flaxseeds (800mg/kg b.w.), each dose was administered twice in a day for 21 consecutive days.On day 22, 24 hours after administration of last dose, all the rats were sacrificed under deep anesthesia. Liver slides were made for H & Estaining. Number of degenerated hepatocytes and nuclear pyknosis were observed in each group.
Results: Number of degenerated hepatocytes and pyknotic nuclei were alleviated in group D with the simultaneous use of flaxseeds. (P value ≤ 0.005).
Conclusions: Flaxseeds in powdered form decreases hepatotoxicity induced by triazophos.
Keywords: pyknosis,degenerated, triazophos, flaxseeds

Introduction

Approximately 2500 years ago, Hippocrates (father of modern medicines) correctly described "Let food be your medicine and medicine be your food".[1] The need for pesticide products and the produced concentration for agricultural efficiency are clear, but the volume of production indicates that the potential for mishandling and accidental exposure is great.[2] Triazophos is a broad spectrum organo- phosphorus pesticide which is abundantly used throughout the world for plant protection, veterinary medicine and public health against different insects, flies and pests that damage variety of crops.[3] Triazophos induces oxidative stress which guides to subsequent histological as well as pathological reorganizations in liver in terms of scattered fatty changes extending from mid-zonal area to the complete liver lobule.[4] Triazophos has also been reported to induce oxidative stress into liver, kidney, brain and fetus of the pregnant rats.[5]

Functional foods are becoming popular alternatives to pharmacological treatments by providing health benefits and decreasing the risk of chronic diseases.[6] Flaxseeds are a dietary botanical supplement with high fiber, minerals, lignans and omega-3 fatty acids having anti-inflammatory and antioxidant properties.[7] Flaxseeds were first grown in the ancient Egypt and China and are full of healthy fat, fiber and antioxidants.[8] Flax plants also contain micronutrients, manganese, vitamin B6 and vitamin B1.[9]

Experimental and clinical research findings showed that due to flaxseed components (omega 3 fatty acids, phytoestrogens, fiber and proteins) powdered flaxseed is a useful strategy to limit several life threatening diseases.[6]

Methods

An experimental animal study was carried out at Animal house and Histology laboratory of Postgraduate Medical Institute (PGMI) Lahore. The study protocol was accepted by Advanced Studies and Research Board of University of Health Sciences, Lahore, and Ethical Committee of PGMI. Healthy adult male Wistar rats of same age group and weighing (170-200mg) were selected for the study. Rats were acclimatized in properly ventilated room at ambient temperature of 25.0 ±2.0ºC under 12 hour light/dark cycles and well administered with standard rat diet and water ad libitum. 24 adult albino Wistar male rats were divided. Four equal groups were made in which rats were divided randomly as group A, group B, group C and group D, so that each group had 6 rats. Details of all the groups are in the following **Table**.

n = Number of rats in each group.

Triazophos is a pesticide. It was weighed on electronic weighing scale and dissolved in distilled water. Flaxseeds were bought from Punjab University Lahore. These are easily available in raw form and then were freshly grinded in grinder machine. The livers of all the groups were dissected out, washed with cold normal saline and fixed in10% neutral

Table-1: Showing detail of the Animal Groups and Experimental Intervention.

Groups	Day of Sacrifice	Specifications for 21 consecutive days	Pintervention and Dosage (orally)
A Control	On day 22	Standard rat feed and distilled water by oral gavage/day	4 ml distilled water (divided in 2 doses)
B Experimental	On day 22	Powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Flaxseeds 800mg/kg b.w in 0.5ml distilled water
C Experimental	On day 22	Triazophos by oral gavage (8.2mg/kg b.w.)/day	Triazophos 8.2mg/kg b.w
D Experimental	On day 22	Triazophos (8.2mg/kg b.w.) and powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Triazophos 8.2mg/kg and high dose of powdered flaxseeds 800 mg/kg b.w. in 0.5ml distilled water

 # Fisher's Exact Test. p-value#< 0.001

Table-2: Comparison of number of degenerated hepatocytes (/mm2) among groups.

	Mumber of degenrated hepatocytes (/mm^2)				
Groups	Absent	Mild	Moderate	Severe	Total
Group A	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (100.0%)
Group B	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (100.0%)
Group C	0 (0.0%)	0 (0.0%)	1 (16.7%)	5 (83.3%)	6 (100.0%)
Group D	0 (0.0%)	2 (33.3%)	4 (66.7%)	0 (0.0%)	6 (100.0%)

 # Fisher's Exact Test. p-value#< 0.001

buffered Formalin solution. Slides were stained with standard procedures of Hematoxylin and Eosin. Nuclear pyknosis was evaluated for any structural change under bright field microscope, while number of degenerated hepatocytes were seen under 20/20 grid microscope. Degenerated hepatocytes on 4 corners of slide and in the center (counted the 5 different places in 1 slide) were counted and then took the mean of all the readings. During counting, margins of lower and left side are left. Area of central vein was avoided as it occupied most of the space and can affect the number of degenerated hepatocytes.

Results

Two parameters were studied under this experiment, number of degenerated hepatocytes and nuclear pyknosis. Out of 24, 12 (50.0%) animal had degenerated hepatocytes. No degenerated hapatocytes were seen in group A and B. In group C, 1 (16.7%) animal had moderate degenerated hepatocytes while 5 (83.3%) animal had severe degenerated hepatocytes. In group D, 2 (33.3%) animal had mild degenerated hepatocytes while 4 (66.7%) animal had moderate degenerated hepatocytes **(Table 2, Fig-1).** Nuclear pyknosis in hepatocytes was noticed and it was found that out of 24, 7 (29.2%) animal had nuclear pyknosis in hepatocytes. No nuclear pyknosis in hepatocytes were seen in both the groups (A and B). In group C all animal had nuclear pyknosis in hepatocytes. In group D, only 1 (16.7%) animal had nuclear pyknosis in hepatocytes **(Table 3, Fig-2)**.

Table-3: Comparison of nuclear pyknosis in hepatocytes among groups.

Groups	Nuclear Pyknosis in Hepatocytes Absent	Present	Total
Group A	6 (100.0%)	0 (0.0%)	6 (100%
Group B	6 (100.0%)	0 (0.0%)	6 (100%)
Group C	0 (0.0%)	6 (100.0%)	6 (100%)
Group D	5 (83.3%)	1 (16.7%)	6 (100%)

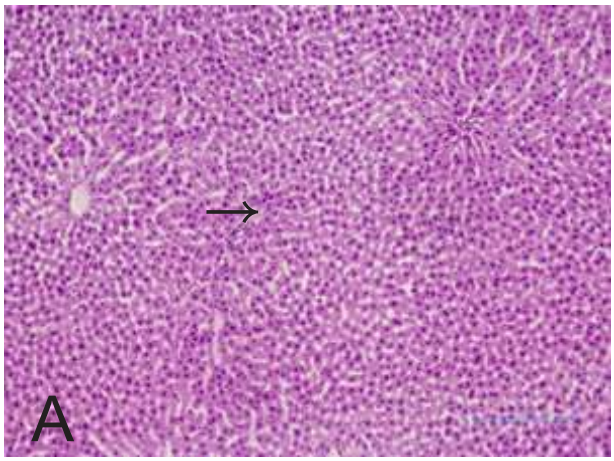

Fig-1: Photomicrograph of the liver from the Group A showing normal hepatocytes (black arrow) and no pyknosis. H & E stain. X 10.

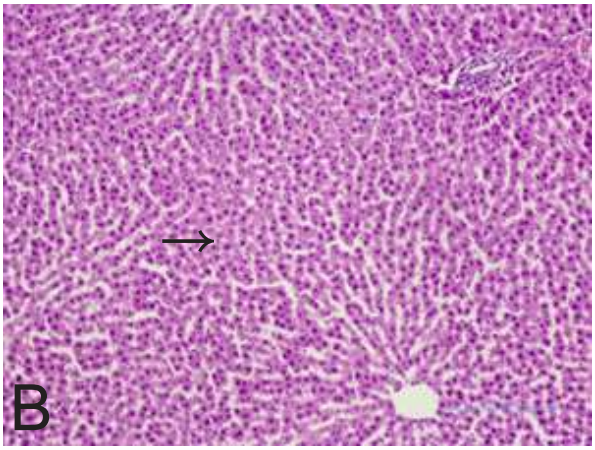

Fig-2: Photomicrograph of myocardium of ventricular wall of group A; showing mature myocardial cells with central oval nuclei (black arrow) and striated cytoplasm (yellow arrow) (Stain H&E. X400).

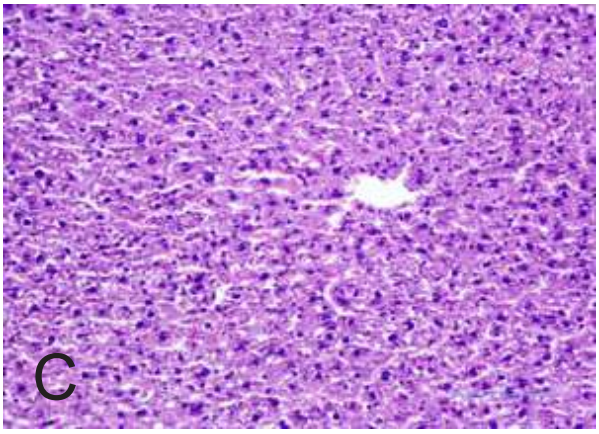

Fig-3: Photom icrograph of the liver from the group C treated with triazophos showing degenerated hepatocytes (black arrow), pyknosis (yellow arrow). H & E stain X 10.

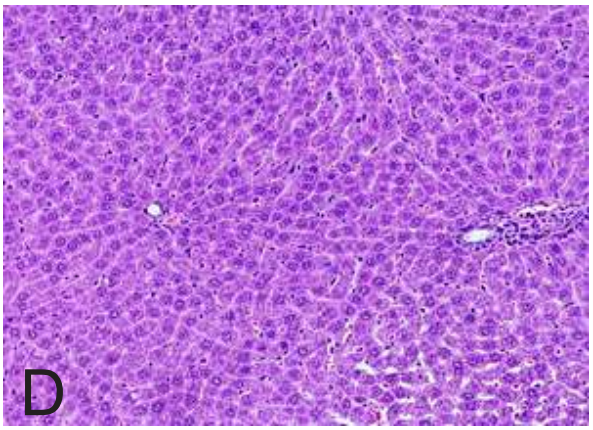

Fig-4: Photomicrogr ph of the liver from group D treated with flaxseeds and triazophos simultaneously showing no pyknosis and decreased degenerated hepatocytes. H & E stain X 10.

Discussion

In present investigation, hepatoprotective potential of flaxseed powder was assessed against pesticide/triazo- phos induced hepatotoxicity in rats. Administration of flaxseed powder had protected hepatic architecture damage with marked improvement in hepatic function and normalization of hepatic enzyme profiles (Hendawiet al10 used 500mg/kg flaxseeds). This study demonstrated that the architecture of hepatocytes remains the same in group A (controlled) and group B (given only flaxseeds 800mg/kg orally for 21 days). While massive destruction of hepatocytes in group C (rats treated with 8.2mg/kg triazophos only) occurred due to oxidative stress. Hepatic degeneration was evaluated under 40X magnification. Findings of Raj et al11 also supported the present study. They used pesticides with the dose of 208mg/kg orally in male Wistar albino rats and showed that malathion and other pesticides induced architectural disarray in hepatocytes in liver of the experimental animals. Jain et al4 also agreed with the results. The group D in which triazophos and flaxseeds both were given, observed the minimized architectural disarray in hepatocytes due to antioxidative property of flaxseeds. The presence or absence of nuclear pyknosis was also found in the cells of hepatocytes of all the animals in this study. The animals of control group had no cells with pyknotic nuclei. It was observed that the hepatocytes in group B showed normal architecture of hepatic cells with no pyknosis. The conclusion of this study was in concordance with those of Hendawiet al10, in this study 500mg/kg b.w flaxseed powder was given to group of rats. In the group C of current study various pyknotic nuclei were observed. Pyknotic nuclei formation in hepatocytes of triazophos treated rats is due to the reactive oxygen species produced as a result of necrosis mostly characterized by pyknosis. Sherma and Sanga12 have also indicated that oxidative stress through reactive oxygen species causes necrosis. Group D demonstrated that the flaxseed powder co-treatment alleviated the lesions caused by triazophos toxicity in rats.

Conclusion

The current study proved that the flaxseeds reduce hepatotoxicity induced by triazophos. It is hoped that this study will produce an awareness of the advantages of supplementation with flaxseed powder in daily life and restriction of unlimited use of triazophos pesticide especially at living places.

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