

HEPATOPROTECTIVE EFFECT OF FLAXSEEDS (*LINUM USITATISSIMUM L.*) ON TRIAZOPHOS INDUCED STEATOSIS AND HEPATIC ENZYMES IN MALE WISTAR RATS

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ABSTRACT

Background: Inappropriate handling and use of pesticides, i.e. triazophos without any plan in agriculture have caused hepatotoxicity. The need for pesticide products for agricultural efficiency is clear, but the volume of production indicates that the occurrence of accidental exposure and as a result life threatening incidents are great.

Objective: To determine the effects of Flaxseeds on hepatic enzymes and steatosis in hepatocytes induced by Triazophos pesticide.

Methods: In this study 24 Wistar albino rats of age: 6-8 weeks and weight 150-200gms were segregated into four groups comprising six rats each (Random number Generator). Group A was labelled as control group. Groups B, C and D were labelled as experimental. All groups were given standard rat feed and water *ad libitum*. Group B was given powdered flaxseeds (800mg/kg b.w.) once daily for 21 consecutive days, Group C was given triazophos (8.2mg/kg b.w.) once daily for 21 consecutive days and Group D was given triazophos (8.2mg/kg b.w.) and flaxseeds (800mg/kg b.w.) once daily for 21 consecutive days. Livers of Albino rats were dissected out and their frozen sections were stained with Sudan III to evaluate steatosis. Blood samples were collected from the tail of rats for biochemical evaluation of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), which were assessed for biochemical changes on 1st, 7th, 14th and 21st day. On day 22, 24 hours after administration of last dose, rats of control as well as of experimental groups were sacrificed under deep anesthesia.

Results: A statistically significant notable increase with a p value of 0.001, was observed in the hepatic enzymes and steatosis in livers of adult male albino rats. Additionally, Flaxseeds have significant ability to ameliorate the effects of Triazophos pesticide.

Conclusion: Use of Flaxseeds in our daily routine can decrease the drastic effects of pesticides on the liver.

Key Words: Flaxseeds, Triazophos pesticide, steatosis, Wistar albino rats.

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INTRODUCTION

Food occupies the central domain of human lives. Improving diet quality has been a target of public health policy¹. The demand for pesticide products and the produced concentration for agricultural efficiency are obvious, but the intensity for mishandling and accidental exposure has drastically inflicted the human environment². Triazophos, an organophosphorus pesticide, is the most commonly used pesticide. The

field workers handling this chemical, their families and even the people consuming fruits and vegetables with triazophos residues, are the ones at risk³. Liver, being the metabolic organ, could be at risk due to the chronic intake of pesticide residues on fruits and vegetables and it shows subsequent histological as well as pathological drastic changes in liver extending from mid-zonal area to the complete liver lobule⁴.

Plants in different forms are used as traditional medicine in 80% of population worldwide⁵. Flaxseeds are becoming popular alternatives to pharmacological treatments by providing omega-3 fatty acids, high fiber, minerals and proteins with anti-inflammatory and antioxidant properties⁶. Flaxseeds, also called linseeds, were first cultivated in the ancient Netherland and China. Currently, they are mainly harvested in China, India, Ethiopia, and United States⁷. Flax plants (botanical name: *Linum usitatissimum*) of white flower variety were firstly found in Pakistan during 2011⁸.

Primary antioxidants donate a hydrogen atom, while secondary antioxidants bind metal ions (which are able to catalyze oxidative processes), absorb UV radiation, inhibit enzymes, engulf reactive oxygen radicals and decompose hydroperoxides⁹. Experimental findings of this study showed that centrilobular necrosis and scattered fatty changes extending from mid-zonal area to the complete liver lobule are alleviated due to antioxidant effect of flaxseeds.

METHODS

This animal study was executed at Histology laboratory of Chemical Pathology laboratory and Animal house of Postgraduate Medical Institute (PGMI) Lahore. Advanced Studies and Research Board of University of Health Sciences, Lahore, and Ethical Committee of PGMI approved the protocol for this experiment.

Animals: Healthy adult male Wistar rats, of the same age group and weighing between 170-200 g, were picked up for this study. The rats were acclimatized to a well-ventilated room maintained at temperature of $25.0 \pm 2.0^\circ\text{C}$, under control conditions of humidity ($60^\circ \pm 10\%$) and with a 12-hour light and dark cycle. They were fed with a standard rat diet and water ad libitum throughout the acclimatization period.

Test chemicals: Triazophos is a pesticide and was weighed on electronic weighing scale in pharma department of AMC/PGMI. It was given to the rats after dissolving in distilled water. Flaxseeds were purchased from Alfateh grocery store. These are easily available in raw form and then were freshly grinded. **Experimental design:**

24 adult albino Wistar male rats were divided randomly into 4 equal groups, 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.

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

Groups	No of Animals (n)	Day of Sacrifice	Specifications for 21 consecutive days	Route of administration and Frequency
A Control	6	22 nd	Standard rat feed and distilled water by oral gavage/day	Oral gavage Once daily
B Experimental	6	22 nd	Powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Oral gavage Once daily
C Experimental	6	22 nd	Triazophos by oral gavage (8.2mg/kg b.w.)/day	Oral gavage Once daily
D Experimental	6	22 nd	Triazophos (8.2mg/kg b.w.) and powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Oral gavage Once daily

n = Number of rats in each group.

Histological study: The livers of all experimental animals were carefully dissected out, washed with cold normal saline and placed in gauze soaked in normal saline and slides were prepared following fixed frozen sections with Sudan III (for steatosis). The Sudan III stain demonstrates accumulation of unsaturated esters and triglycerides under bright field microscope. Blood was drawn from the tails of albino rats for enzyme biochemistry. Kits for AST, ALT and ALP were obtained from Human Company, Germany. Tests were

performed in Biochemistry lab of Lahore General Hospital by using Spectra Pro XL (automatic biochemistry analyzer). Structural and biochemical changes were assessed.

RESULTS

We have decided a grading criterion for Steatosis on a scale of 0–3 as follows: an absence of steatosis is grade 0, <30% of hepatocytes affected is grade 1, 30–70% of hepatocytes affected is grade 2, >70% of hepatocytes

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affected is grade 3¹⁰. A statistical analysis showed that group A indicated no steatosis. While in group B, 1 (16.7%) animal. In group C, 4 (66.7%) animals had

moderate steatosis while 2 (33.3%) animals had severe steatosis. In group D, only 2 animals had mild steatosis. (Table 02; Figure 1, 2, 3, 4).

Table 02: Comparison of steatosis among groups.

	Steatosis				Total
	Absent	Mild	Moderate	Severe	
Group A (Control)	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (100.0%)
Group B (Flax seed)	5 (83.3%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	6 (100.0%)
Group C (Triazophos)	0 (0.0%)	0 (0.0%)	4 (66.7%)	2 (33.3%)	6 (100.0%)
Group D (Triazophos + Flax seed)	4 (66.7%)	2 (33.3%)	0 (0.0%)	0 (0.0%)	6 (100.0%)
p-value [#]			< 0.001		

Fisher's Exact Test

ALT, AST, ALP (U/L): The mean ALT, AST, ALP levels in all groups is mentioned in table 11. Data was normally distributed and it also fulfills one way ANOVA test for comparison of the mean enzyme levels

(ALT, AST, ALP) among groups. It was also observed that the mean ALT, AST, ALP levels among groups were significantly different (p-value < 0.001) (Table 3).

Table 3: Comparison of ALT levels among groups.

Parameters	Group A (Control)	Group B (Flax seed)	Group C (Triazophos)	Group D (Triazophos + Flax seed)	p value [#]
ALT (U/L)	24.6 ± 2.6	26.3 ± 5.1	125.5 ± 5.5	71.9 ± 17.5	< 0.001
AST (U/L)	49.6 ± 2.6	49.6 ± 2.1	139.2 ± 16.7	89.2 ± 21.7	< 0.001
ALP (U/L)	77.1 ± 5.9	77.2 ± 5.1	179.0 ± 23.6	106.0 ± 20.6	< 0.001

One way ANOVA

p value (<0.001) is considered statistically significant

DISCUSSION

Many previous studies have already reported the hepatotoxicity by triazophos (when administered 8.2mg/kg orally) produced due to reactive oxygen species as a result of lipid peroxidation¹¹. Similarly, the preventive role of flaxseeds has been proven and reported in many studies (Sawant and Bodhankar in 2016 observed effect of 200,400 and 800mg/kg flaxseeds orally)¹².

A marked increase in serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels was observed in rats of group C after the administration of triazophos (8.2mg/kg orally for 21 days). These results are similar to those of Ghaffar study¹³, who demonstrated hemato-biochemical damage caused by triazophos (0.010, 0.015 and 0.200 ppm for 6 days) in fresh water fish (*Labeo rohita*). Similar observations have been also made by Mossa in 2015¹⁴, who reported that oxidative stress caused by pesticide fipronil significantly increased ALT, AST and ALP level. He noticed the effect of this pesticide in male albino rats at three amounts (0.1mg/L, 1mg/L and 10mg/L) dissolved in drinking water for 45days. These enzymes were elevated and it could be a reason for production of reactive oxygen species and increased lipid peroxidation. Rats of group D treated with both triazophos (8.2mg/kg

and flaxseeds (800mg/kg) orally for 21 days showed marked decrease in ALT, AST and ALP near to normal values. Another study Abdou and Hassan (2014) had given diet containing omega-3 fatty acids to their rats orally at doses of 125mg/kg and 260mg/kg body weight for 10 days. Abdou and Hassan (2014) exhibited that there was a decrease in serum ALT, AST and ALP levels because of the protection by omega3 fatty acid¹⁵. The degree of steatosis in the hepatocytes was assessed through scoring criteria in present study. Group A (control) and B (given 800mg/kg b.w orally) showed only mild or no adipocytes. Group C fed with 8.2mg/kg triazophos orally for 21 days and showed increased number of adipocytes (stained with sudan III) scattered in hepatocytes. It ranged from moderate to severe steatosis. These results are in accordance with histopathological lesions observed in Bhushan study¹⁶. In his study total lipid count, cholesterol, free fatty acids and phospholipids all were increased on administration of pesticides. Rats of group D in this study were fed on 800mg/kg flaxseeds and 8.2mg/kg triazophos orally for 21 days simultaneously and steatosis was decreased. Wang in 2016 also showed the same results, flaxseeds attenuated the alcohol induced hepatic lipid accumulation by lipolysis and changed the composition of fatty acids¹⁷.

Figure 1: Photomicrograph of the liver tissue from the control Group A showing normal hepatocytes (yellow arrow), no steatosis. Sudan III. X 40.

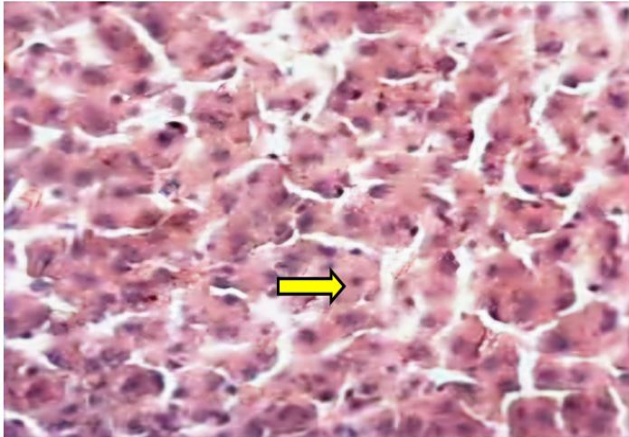


Figure 2: Photomicrograph of the hepatic tissue from the Group B treated with flaxseed showing normal hepatocytes (yellow arrow), no steatosis (silver arrow) and Sudan III. 40X.

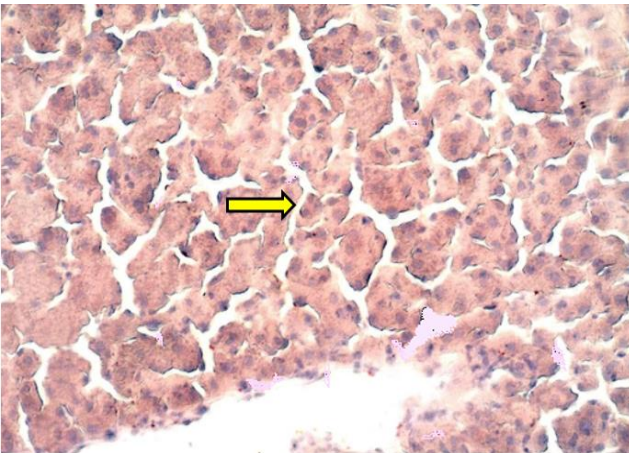


Figure 3: Photomicrograph of the liver section from group C treated with triazophos showing steatosis (silver arrow) of grade III (severe). Sudan III. X 10. Inset shows X 40.

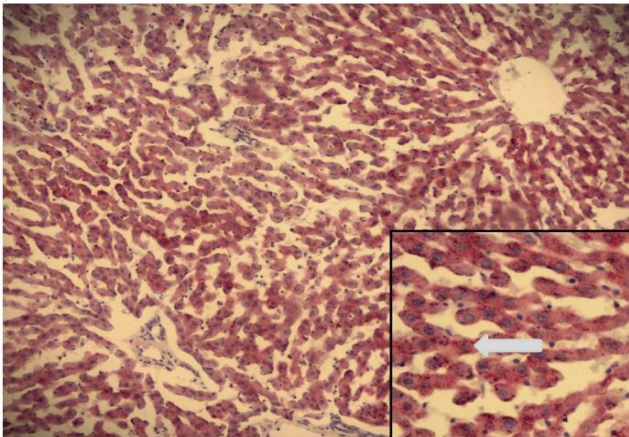
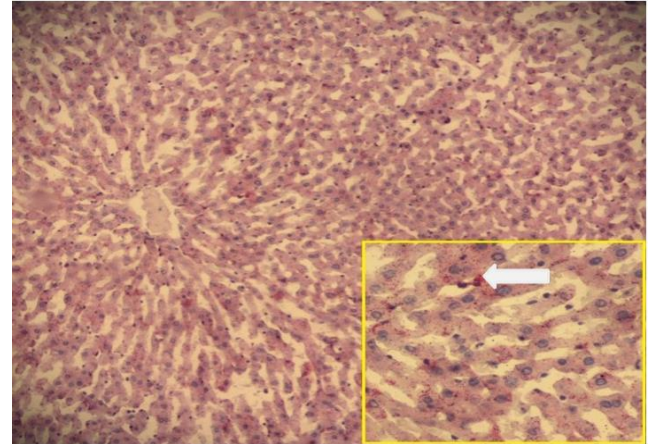


Figure 4: Photomicrograph of the liver from group D treated with both reagents showing steatosis (silver arrow) of grade I (mild). Sudan III. X 10. Inset shows X 40.



Limitations: Further studies should be done to evaluate hormonal assay and changes in gene/molecular level.

CONCLUSION

The findings of this study demonstrate that flaxseeds can minimize hepatotoxicity induced by triazophos. It is anticipated that this research will raise awareness about the benefits of powdered form of flaxseed powder into daily routine, as well as highlight the need to limit the unrestricted use of triazophos in crops.

ETHICAL APPROVAL

Ethical approval of article was granted by the Ethics Committee of Postgraduate Medical Institute, Lahore Dated: May 08, 2018.

AUTHOR'S CONTRIBUTIONS

AN: Conception of study and final drafting of the article.

SN: Critically revising the article for further addition of intellectual content.

SS, MS, SA & GPW: Data analysis and collection and for important changes in the main content.

All Authors: Approval of the final version of the manuscript to be published

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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