Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/sarn2874

PROTECTIVE ROLE OF FLAXSEEDS (*LINUM USITATISSIMUM L.*) ON TRIAZOPHOS INDUCED BODY WEIGHT GAIN IN MALE WISTAR RATS

Arooj Nawaz[1*], Amber Shami[2], Mehreen Salahuddin[3], Lubna Shahper[4], Huma Jawad[5], Saba Saleem[6]

[1]Assistant Professor, Anatomy Department, Azra Naheed Medical College, Lahore
[2]Assistant professor, Anatomy Department, Central Park Medical College. Lahore
[3]Assistant Professor, Anatomy Department, FMH College of Medicine and Dentistry, Lahore
[4]Assistant professor, Anatomy Department, Services Institute of Medical Sciences, Lahore
[5]Assistant professor, Anatomy Department, Al-Aleem Medical College, Lahore
[6]Associate Professor, Anatomy Department, Central Park Medical College, Lahore

Corresponding author: Arooj Nawaz
[*]Assistant Professor, Anatomy Department, Azra Naheed Medical College, Lahore, Email ID: aroojsonia1@gmail.com

Abstract:
Background: Improper and unplanned handling and application of pesticides, i.e. triazophos in agriculture have caused hepatotoxicity. The need of pesticides to enhance agricultural efficiency is evident, but the high volume of production suggests a significant risk of accidental exposure and potentially life-threatening incidents.
Objective: To determine the effects of Flaxseeds on liver and body weight gain induced by Triazophos pesticide.
Methods: In the current study 24 Wistar albino rats of age: 6-8 weeks and weight 150-200gms were segregated into four equal groups comprising six rats each by Random number Generator. Group A was kept 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.
All the selected rats were weighed before the start of the experiment and at the end of the experiment before sacrificing them. Livers of Albino rats were dissected out and their sections were prepared and stained with standard Hematoxylin and Eosin stains to evaluate sinusoidal congestion. Blood samples were taken from the tail of the rats for biochemical evaluation of liver enzymes. On day 22, exactly after 24 hours of administration of the last dose, rats of both the groups, control as well as experimental, were sacrificed under deep anesthesia.
Results: A statistically significant increase with a p value of 0.001, was observed in the body weight as well as liver weight gain in adult male albino rats. Additionally, the antioxidant effect of Flaxseeds has significant ability to ameliorate the effects of Triazophos pesticide.
Conclusion: Daily use of Flaxseeds in our diet can decrease the drastic effects of pesticides on the liver.

Key Words: Flaxseeds, Triazophos pesticide, sinusoidal congestion, Wistar albino rats

INTRODUCTION

Food holds a fundamental place in human existence. Enhancing diet quality has consistently been a key objective within public health policy frameworks[1.] Pesticides used for agricultural and additional domestic purposes generate unhealthy biological effects against the selected group but also affect the health of unselected groups including human beings[2]. Although the implementation of pesticides has presented remarkable profitable advantages by increasing the food production and fibers and the deterrence of vector borne transmission, evidence suggests that utilization of pesticide has drastically inflicted the human environment[3]. Pesticides are one of the main sources which can cause production of free radicals and are a serious cause of decline of health. Membranes of lipids, nucleic acids, proteins and carbohydrates are damaged by pesticides and as a result these cause carcinomas, disorders of the nervous system, respiratory diseases, genetic variations, hepatic and renal diseases, diabetes mellitus, cardiovascular diseases, disorders related to immune system, premature aging, and ocular diseases[4].

Ample studies have recommended that nutrition plays a vital role in control or at least delay of development of late complications. A few basic foods have been 8 proved to contain antioxidant, anti-inflammatory and hypolipidemic properties. Flaxseed is an important food that is filled with fatty acids (omega-3) and antioxidants and has a less amount of carbohydrates. An extensive number of studies indicated that flaxseeds are useful for managing diabetes mellitus[5]. Flaxseeds in powdered form contain more nutritional value than the other forms. In raw form husk has less content and it is not digested easily. In oil form, processing method can affect the composition badly, contents are altered depending upon the method of extraction and it is proved through studies[6].

MATERIAL AND METHOD

An experimental animal study was executed at the Histology laboratory and Animal house of Postgraduate Medical Institute (PGMI) Lahore. The study protocol was approved by Advanced Studies and Research Board of University of Health Sciences, Lahore, and Ethical Committee of PGMI. Rats were grouped by using random number generator and were weighed on 1st day of the experiment and then on last day (22^{nd} day) before sacrifice. The livers from all experimental groups were also weighed on last day and carefully dissected, washed with cold normal saline, and then preserved in 10% neutral buffered formalin for fixation.

ANIMALS:

Healthy adult male Wistar rats of the same age group of (weight between 170-200 g), were chosen for this study. The rats were acclimated in a well-ventilated room maintained at an ambient temperature of 25.0 ± 2.0°C, with controlled humidity levels (60% ± 10%) and a 12-hour light/dark cycle was maintained. They were provided with a standard diet of rats and water ad libitum throughout the experiment.

Test chemicals:

Triazophos is a pesticide, which was dissolved in distilled water and given to the rats. Flaxseeds were purchased from Alfateh grocery store in raw form and then were freshly grinded. Both were weighed on electronic weighing scale in pharma department of AMC/PGMI.

Experimental design:

24 adult albino Wistar male rats were divided by random number generator into 4 groups, as group A, group B, group C and group D, each group had 6 rats. Details of all the groups are in the following table.

Groups	**No of Animals (n=24)**	**Day of Sacrifice**	**Specifications for 21 consecutive days**	**Route of administration and Frequency**
A Control	6	22nd	Standard rat feed and distilled water by oral gavage/day	Oral gavage Once daily
B Experimental	6	22nd	Powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Oral gavage Once daily
C Experimental	6	22nd	Triazophos by oral gavage (8.2mg/kg b.w.)/day	Oral gavage Once daily
D Experimental	6	22nd	Triazophos (8.2mg/kg b.w.) and powdered flaxseeds (800mg/kg b.w. in 0.5ml distilled water)/day	Oral gavage Once daily

Table 1: Showing detail of the Animal Groups and Experimental Intervention.
n = Number of rats in each group.

All the data was analyzed in Statistical Package of Social Sciences (SPSS) version 22. Shapiro Wilk test was used to assess the normality of distribution for the given data. One-way ANOVA was used to observe the differences in means across the three groups. Post-hoc Tukey test was conducted for pairwise comparisons between the individual groups. *P*-value of less than 0.05 was considered statistically significant.

RESULTS

The animals were weighed at the beginning and completion of experiment (Table. 2). Rats were examined to assess the state of their health; they lived healthy throughout the time of experiment. As data was normally distributed, one way ANOVA test was applied to compare the body weight at the start & end of the experiment and %age weight gain among groups. It was conceived that there was no remarkable difference in the mean body weight of animals among groups at the start of experiment (p-value = 0.419). Significant difference was observed in mean body weight among groups at the end of experiment (p-value = 0.003). At the end of the experiment, %age weight gain in groups was also significant, p-value < 0.001 (Table. 2, Fig. 1).

Table 2: Comparison of initial body weight, final body weight and %age weight gain among groups.

Parameters	**Group A (Control)**	**Group B (Flax seed)**	**Group C (Triazophos)**	**Group D (Triazophos + Flax seed)**	**pvalue#**
Weight of the animal at the start of experiment(g)	193.7 ± 2.3	193.3 ± 3.3	194.5 ± 2.3	195.7 ± 2.3	0.419
Weight of the animal at the end of experiment(g)	207.8 ± 2.8	207.0 ± 4.1	213.7 ± 2.4	211.5 ± 2.1	0.003
% age weight gain	7.3 ± 1.1	7.1 ± 0.7	9.9 ± 0.2	8.1 ± 0.3	< 0.001

#One way ANOVA

p value is considered statistically significant

For multiple comparisons, post hoc Tukey test was used which showed that percentage weight gain in group C was significantly higher as compared to remaining all groups. However, no obvious difference was found in percentage weight gain among groups A, B and D. (Table 3)

Table 3: Pair wise comparison of percentage weight gain among groups.

Multiple Comparison						
	S. No.	**Group**	**Group**	**Mean Difference**	**Std. Error**	**p-value**
Percentage weight gain	**1**	A	B	0.28333	0.39627	0.890
			C	-2.53333*	0.39627	< 0.001*
			D	-0.76667	0.39627	0.246
	2	B	C	-2.81667*	0.39627	< 0.001*
			D	-1.05000	0.39627	0.068
	3	C	D	1.76667*	0.39627	0.001

post hoc Tukey test
*The mean difference is significant at the 0.05 level.

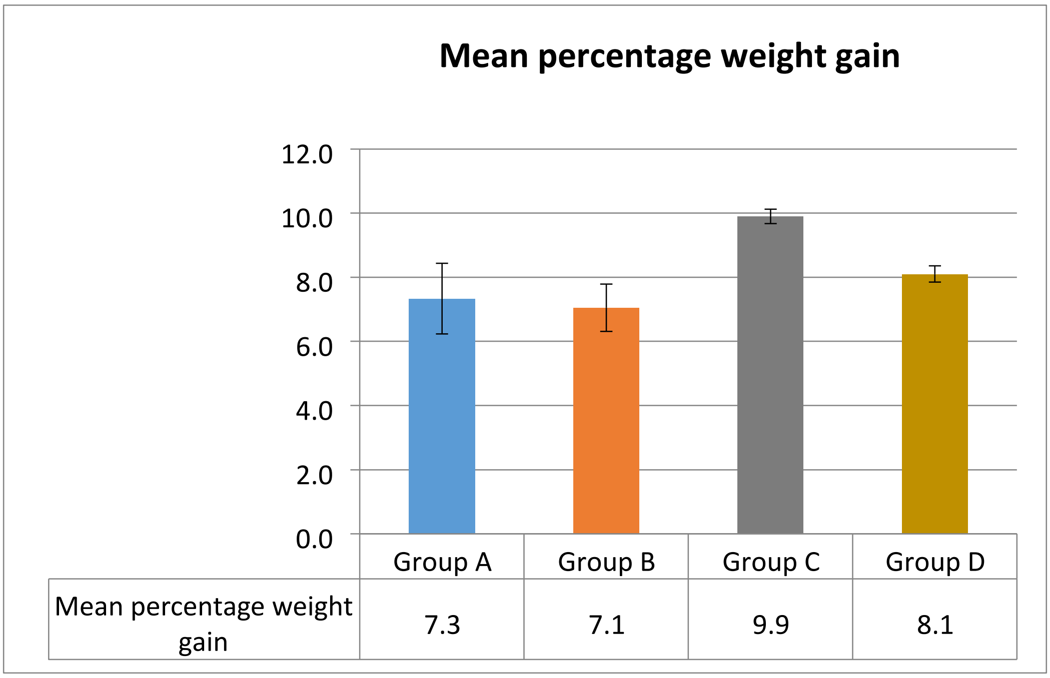

Figure 1: Bar chart showing comparison of percentage weight gain among groups.

Weight of Liver (g):
The weight of liver in all groups was measured (Table. 4). Data was not normally distributed as assessed by Shapiro Wilk test; therefore, Kruskal Wallis test was applied to compare the weight of liver among groups. It was found that the average weight of liver in all groups were considerably different (p-value = 0.002) (Table. 4, Fig. 2).

Table 4: Comparison of weight of liver among groups.

Parameters	Group A (Control)	Group B (Flax seed)	Group C (Triazophos)	Group D (Triazophos + Flax seed)	pvalue#
Weight of liver (mg)	5.83 ± 0.75	5.75 ± 0.42	7.92 ± 0.49	5.50 ± 0.55	0.002*

#Kruskal-Wallis Test
*p value ≤ 0.05 is considered statistically significant

For multiple comparisons, Mann Whitney test with Bonferroni correction was used which showed that weight of liver in group C was consequentially higher as compared to groups A, B and D. However, no remarkable difference was found in weight of liver among groups A, B and D (Table 5).

Table 5: Pair wise comparison of weight of liver among groups.

Multiple Comparison						
	S. No.	**Group**	**Group**	**Test statistics**	**Std. Error**	**pvalue#**
Weight of liver (g)	**1**	A	B	0.417	3.891	> 0.999
			C	-10.917	3.891	0.030*
			D	2.500	3.891	> 0.999
	2	B	C	-11.333	3.891	0.021
			D	2.083	3.891	> 0.999
	3	C	D	13.417	3.891	0.003*

#Mann Whitney test
*p value ≤ 0.05 is considered statistically significant

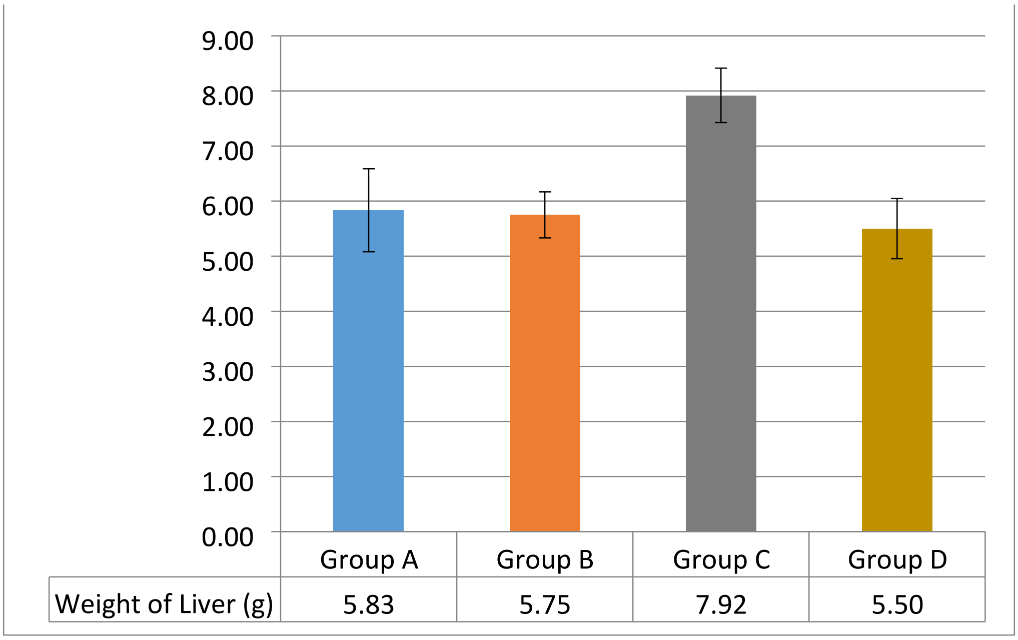

Figure 2: Bar chart showing comparison of weight of liver among groups.

Relative Tissue Weight Index (RTWI):

The mean RTWI of all groups is mentioned in table 6. Data was normally distributed as assessed by Shapiro Wilk test. One way ANOVA test was applied to compare the mean RTWI among groups. It was found that the mean RTWI among groups were remarkably different (p-value < 0.001) (Table 6; Fig 3).

Table 6: Comparison of relative tissue weight index among groups.

Parameters	Group A (Control)	Group B (Flax seed)	Group C (Triazophos)	Group D (Triazophos + Flax seed)	pvalue#
Relative Tissue Weight Index	2.80 ± 0.37	2.80 ± 0.24	3.7 ± 0.27	2.58 ± 0.24	< 0.001

#One way ANOVA
*p value is considered statistically significant

For multiple comparisons, post hoc Tukey test was used which showed that RTWI in group C was considerably inflated as compared to remaining all groups. However, no considerable difference was found in RTWI among groups A, B and D. (Table 7)

Table 7: Pair wise comparison of relative tissue weight index among groups.

		Multiple Comparison				
	S. No.	Group	Group	Mean Difference	Std. Error	p-value
Relative Tissue Weight Index	1	A	B	0.00000	0.16575	>0.999
			C	-0.90000*	0.16575	< 0.001*
			D	0.21677	0.16575	0.569
	2	B	C	-0.90000*	0.16575	< 0.001*
			D	0.21677	0.16575	0.569
	3	C	D	1.11667*	0.16575	< 0.001*

*The mean difference is significant at the 0.05 level.

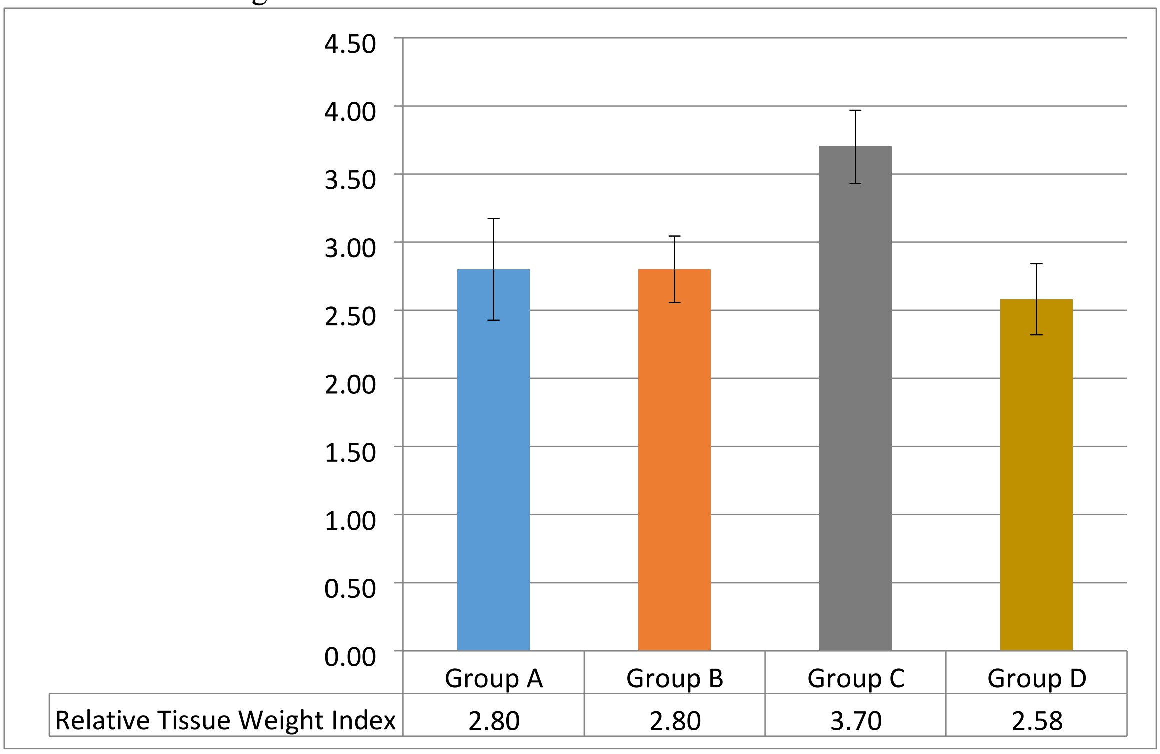

Figure 3: Comparison of size of Relative Tissue Weight Index among groups.

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.

Discussion
Numerous previous studies have documented the hepatotoxic effects of triazophos, particularly when administered orally at a dose of 8.2 mg/kg. These are basically produced due to reactive oxygen species because of lipid peroxidation[7]. At the same time, the protective effects of flaxseeds have been well-documented in numerous studies. For instance, Sawant and Bodhankar in 2016 observed beneficial outcomes following the oral administration of flaxseeds at doses of 200, 400, and 800 mg/kg.[8].
The body weight of rats was measured at the beginning of experiment and at the completion of experiment, while the liver weight was seen at the end of experiment.
Weight of body at the completion of experiment was compared with the control group A. Notable difference in the weight of the liver and body was seen in the rats of all the groups. Group B given flaxseed showed a decrease in body weight. According to Schumann in 2010 the group of hen being given flaxseeds 100g/kg orally and the result suggested that dietary flaxseed decreased hepatic fat content and reduced body weight[9]. Another study of Vijaimohan in 2006 investigated the effect of flaxseed (1g/kg orally) in the rats (these were given high fat diet), it alleviated plasma cholesterol, triglycerides, phospholipids, free fatty acids, high-density lipoprotein (HDL), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein and as a result significantly lowered the gain in body and liver weight[10].
Group C treated with triazophos showed a remarkable rise in the weight of liver and body. Sharma and Sangha in 2016 observed mild elevation in the body and liver weight at a dose of 8.2mg/kg oral triazophos[11].
Current study in group D treated with triazophos (8.2mg/kg) and flaxseed (800mg/kg) showed mild reduction in the body weight nearly equivalent to control group A. While there was an insignificant decrease in weight of liver. Sherma and sangha in 2014 with the same dose of triazophos[12] and Hendawi in 2016 with flaxseeds perceived the same results[13]. On the other hand, Toor in 2013 observed the opposite effect to that of present study[14]. He perceived that the imidacloprid (1/10th of LD_{50} of pesticide orally for 4 weeks) was given to rats. After 4 weeks rats showed decreased body as well as liver weights.
Limitations: Further studies are needed to assess hormonal assays and investigate changes at the gene or molecular level.
Acknowledgement: The authors wish to express their gratitude to the staff of the Anatomy Department at PGMI, Lahore, for their invaluable technical support.
Conflict of interest: Nothing to declare.
Financial Support: Nil.

References:
1. Griffith R, O'Connell M, Smith K. The importance of product reformulation versus consumer choice in improving diet quality. Economica. 2017 Jan;84(333):34-53.
2. Dharmender Sharma DS, Sangha GK. Triazophos induced oxidative stress and histomorphological changes in liver and kidney of female albino rats.
3. Yadav IC, Devi NL, Syed JH, Cheng Z, Li J, Zhang G, Jones KC. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: A comprehensive review of India. Science of the Total Environment. 2015 Apr 1;511:123-37.

4. Touré A, Xueming X. Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. Comprehensive reviews in food science and food safety. 2010 May;9(3):261-9.
5. Mani UV, Mani I, Biswas M, Kumar SN. An open-label study on the effect of flax seed powder (Linum usitatissimum) supplementation in the management of diabetes mellitus. Journal of dietary supplements. 2011 Aug 4;8(3):257-65.
6. Rubilar M, Gutiérrez C, Verdugo M, Shene C, Sineiro J. Flaxseed as a source of functional ingredients. Journal of soil science and plant nutrition. 2010 Jul;10(3):373-7.
7. Sharma D, Sangha GK, Khera KS. Triazophos induced oxidative stress in female albino rats. Int. J. Adv. Res. 2014;2(1):746-54.
8. Sawant SH, Bodhankar SL. Flax lignan concentrate reverses alterations in blood pressure, left ventricular functions, lipid profile and antioxidant status in DOCA-salt induced renal hypertension in rats. Renal Failure. 2016 Mar 15;38(3):411-23.
9. Schumann BE, Squires EJ, Leeson S. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplement on hepatic and plasma characteristics relevant to fatty liver haemorrhagic syndrome in laying hens. British Poultry Science. 2000 Sep 1;41(4):465-72.
10. Vijaimohan K, Jainu M, Sabitha KE, Subramaniyam S, Anandhan C, Devi CS. Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. Life sciences. 2006 Jun 27;79(5):448-54.
11. Sharma, D. and Sangha, G. K., 2016. Effects of Glucosinolates Rich Broccoli extract against Triazophos induced Toxicity in Wistar Rats. *Int J Biomed Sci.*, ***5***(4): 111.
12. Sharma D, Sangha GK. Effects of glucosinolates rich broccoli extract against treetops induced toxicity in Wistar rats. A Randomized Control Study. J Biomedical Sci. 2016;5(4).
13. Hendawi MY, Alam RT, Abdellatief SA. Ameliorative effect of flaxseed oil against thiacloprid-induced toxicity in rats: hematological, biochemical, and histopathological study. Environmental Science and Pollution Research. 2016 Jun;23:11855-63.
14. Toor HK, Sangha GK, Khera KS. Imidacloprid induced histological and biochemical alterations in liver of female albino rats. Pesticide biochemistry and physiology. 2013 Jan 1;105(1):1-4.