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Effect of Fenugreek against Induced Sub Acute Thiacloprid Toxicity in Rats

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Abstract

The study was carried out to investigate efficacy of fenugreek against general toxicity induced by sub acute exposure of thiacloprid in rats. Wistar rats were divided in four groups having six rats in each group. Group I served as control (corn oil administered as vehicle). Group II served as thiacloprid treated group (@ 22.5 mg/kg b.wt. orally), in Group III (experimental group) thiacloprid along with fenugreek extract (@500 mg/kg.b.wt.orally) and in case of Group IV (experimental group) fenugreek seeds extract (@500 mg/kg.b.wt.orally)were administered. The thiacloprid treated groups showed clinical signs *viz* lacrimation, piloerection, diarrhoea and dyspnea and significantly decreased absolute and relative body weight, higher relative liver and kidney weight, decreased relative spleen weight and altered hemo-biochemical profile. Fenugreek treatment showed improvement in absolute and relative body weight and organ weight and hemo-biochemical profile. The ethanolic extract of fenugreek has the potential to restore the general toxicity, serum biochemical and hematological parameters caused by sub-acute thiacloprid toxicity.

Keywords: Fenugreek, Rats, Sub-acute Toxicity, Thiacloprid

1. Introduction

The discovery of neonicotinoid insecticide was an important milestone in the history of modern insect control. There are three types of neonicotinoid insecticides with chloropyridinylmethyl (imidacloprid or IMI, thiacloprid or THIA, acetamiprid or ACE and nitenpyram or NIT), chlorothiazolylmethyl (thiamethoxam or TMX and clothianidin or CLO) or tetrahydrofuranylmethyl (dinotefuran or DIN) substituents1. The neonicotinoids are the fastest growing chemical class of insecticides, accounting for over 15% of the total insecticide market. Their tremendous success are based on their unique chemical and biological properties, such as broad spectrum insecticidal activity, low application rates, excellent systemic characteristics, favourable safety profile and a new mode of action[1].

Thiacloprid, a neonicotinoid insecticide belonging to new group of active ingredients, the cyanoamidines is neurotoxic[2] and is effective on contact and also via stomach action. It produces its action by binding agonistically to the nAChRs in the CNS of insects[3]. The selective toxicity of neonicotinoid insecticides appear to be related to receptor subtypes. Thiacloprid is presently the most important commercial product because of its high efficacy against insects. It has outstanding potency and systemic action for crop protection against piercing-sucking pests and is also highly effective for flea control on cats and dogs[2]. In 2007, thiacloprid was registered in more than 50 countries for use in agriculture, horticulture etc.

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. Ayurveda and Unani Medicine are widely practiced in India.

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Fenugreek *(Trigonella foenum-gracum)* is one of the most promising medicinal herbs, known from ancient times, having nutritional value too. Its green leaves and seeds are used for multipurpose. 100 g of seeds provide more than 65% of dietary fibre. Due to high fibre content it has an ability to change food texture. It is well known for its gum, fibre, alkaloid, flavonoids, saponin and volatile contents. In various medicinal applications, it works as antidiabetic, anticarcinogenic, remedy for hypoglycemia, antioxidant, antibacterial agent, gastric stimulant, and anti-anorexic agent. In modern food technology, it is used as food stabilizer, adhesive and emulsifying agent due to its fibre, protein and gum content. Its protein is found to be more soluble (91.3%) at alkaline pH of 11[4].

Since efficacy of fenugreek against toxicity induced by thiacloprid is scarcely available in literature, therefore the present work was conducted with the objective to evaluate the effect of fenugreek against general toxicity induced by sub-acute exposure of thiacloprid in rats.

2. Materials and Methods

All the experimental procedure, housing and management of the rats were strictly carried out according to the recommendation and approval of the Institutional Animal Ethical Committee (IAEC).

2.1 Experimental Animals

Adult male albino rats weighing 120-140 g were used for the study. A total of 24 rats were used in the study. Rats were housed in polyacrylic cages in a group of 6 rats per cage in the Department of Veterinary Pharmacology and Toxicology. Bedding material (wheat straw) was changed on alternate day. The animals were provided with feed and water *ad libitum* and maintained at room temperature with a natural light-dark cycle. Rats were acclimatized to laboratory conditions for 7 days before the experiment was conducted.

2.2 Herbal Extract

2.2.1 Collection of Plant Materials

Fenugreek seeds procured from grocery.

2.2.2 Extraction

The seeds of fenugreek were powdered and extracts were prepared by rotatory vacuum evaporator. The percentage yield of ethanolic extract of fenugreek seed was 13% which was greater than aqueous extract so the said extract were used in the experiment. According to Nathiya *et al.*[5] ethanolic extract of fenugreek seeds have highest anti-oxidant activity.

2.3 Thiacloprid

Technical grade Thiacloprid (96.8% pure) was procured from commercial firm and was administered @ 22.5 mg/kg b.wt. orally. The desired concentration was prepared in corn oil. The same dose of thiacloprid was used by Aydin[6] in rats to generate oxidative stress and other toxicity which showed better results.

2.4 Experimental Design

Rats were randomly divided into four groups (6 rats/group). Group I served as control in which corn oil (acting as vehicle of thiacloprid) was administered. Group II served as thiacloprid treated group (@ 22.5 mg/kg b.wt.orally). In case of Group III (experimental group) thiacloprid along with fenugreek seeds extract (@500 mg/kg.b.wt.orally) were administered. In case of Group IV (experimental group) fenugreek seeds extract (@500 mg/kg.b.wt.orally) was administered. Administration of thiacloprid and extract was done for 28 days.

2.5 Body Weight

Body weight of each rat was recorded on day 0 and at an interval of one week till the completion of experiment.

2.6 Blood Collection

Experimental rats were anaesthetized by diethyl ether 24 hrs after the last treatment/dose. Blood samples were collected directly from the posterior vena cava and heart in tubes containing sodium-EDTA and without sodium-EDTA from all the rats. Serum was separated and stored at -20°C for various biochemical estimations. After the blood collection rats were sacrificed.

2.7 Organ weights

At the time of sacrifice, vital organs viz. liver, kidney, brain, spleen, thymus, heart and lung were excised free from surrounding tissues blotted with tissue paper and examined for gross pathology. All these organs were weighed individually then after absolute body weight gain and relative organ weight were calculated.

Absolute body weight gain (g) = final body weight (g) – initial body weight (g).

Relative organ weight (g% or g/10gm) = organ weight × 100/body weight.

2.8 Serum Biochemical

Serum was separated from the blood and stored at -20°C for estimations of the activity of ALT and AST, LDH and creatinine kinase. These were estimated by using commercially available diagnostic kits (Span Diagnostics

Ltd; India). LDH was estimated by kit of Crest Biosystems, India.

2.9 Haematological Studies

Various hematological parameters viz. Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined using automated hematology analyzer.

2.10 Statistical Analysis

The observations were made in duplicate and averaged (n = 6). The data were analyzed using SPPSS software version 20.0. The data were subjected to Analysis of Variance (one way ANOVA), least significant difference (Snedecor and Cochran, 1989) and Duncan's multiple range tests to determine significant differences among means of different treatments. A significance level of 0.05 was chosen.

3. Results and Discussions

3.1 General Toxicity Parameter

3.1.1 Clinical Signs and Symptoms

There was no mortality in the groups. Body coat was roughened and feed intake was reduced in thiacloprid treated group in comparison to control. The rats in the group exhibited salivation, lacrimation, piloerecton, diarrhoea and dyspnoea. All the other groups exhibited normal activities and behaviour during the study period.

3.1.2 Effect on Body Weight

The body weight recorded at weekly intervals are shown in Table 1 and Figure 1 .The rate of gain in body weight for thiacloprid alone and in combination with fenugreek treated group were significantly affected ($p<0.05$). The average body weight of thiacloprid treated rats was significantly less ($p<0.05$) on 1st week onwards as compared to all other groups viz. Groups I, III and IV.

Table 1. Effect of extract of fenugreek on weekly recorded Body weights (g.) against toxicity induced by sub-acute exposure of thiacloprid in rats (Mean ± S.E.)

Group	0 day	1st week	2nd week	3rd week	4th week
I.	$132.17^{a} \pm 2.03$	$144.09^{ab} \pm 2.51$	$156.96^{b} \pm 2.39$	$168.19^{b} \pm 2.96$	$179.39^{b} \pm 2.81$
II.	$136.01^{b} \pm 1.48$	$144.48^{ab} \pm 1.46$	$153.19^{ab} \pm 1.28$	$161.91^{a} \pm 1.51$	$168.86^{a} \pm 1.80$
III	$135.40^{ab} \pm 2.61$	$145.10^{b} \pm 2.73$	$152.41^{a} \pm 2.71$	$164.86^{ab} \pm 2.96$	$174.92^{ab} \pm 2.94$
IV	$133.27^{a} \pm 2.27$	$142.97^{a} \pm 2.67$	$155.89^{b} \pm 2.45$	$167.15^{b} \pm 2.12$	$178.71^{b} \pm 2.67$

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

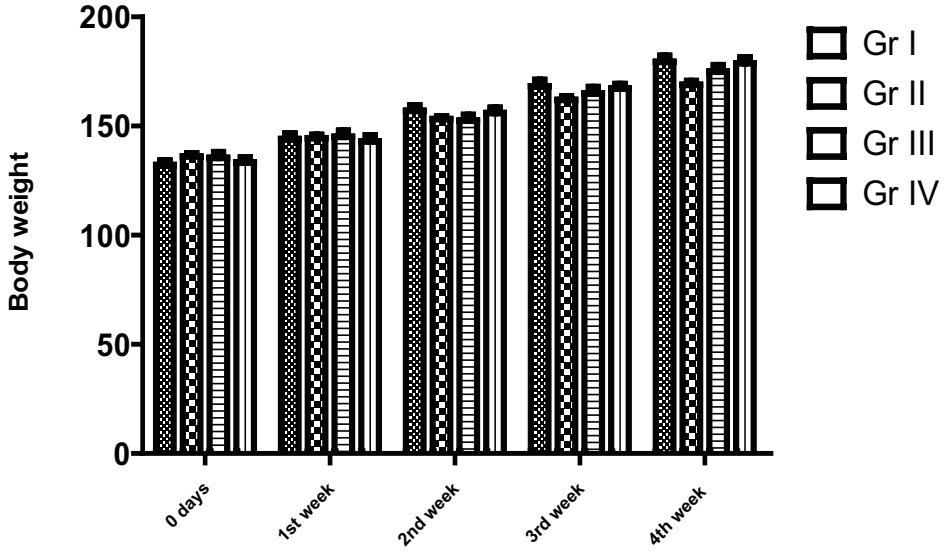


Figure 1. Effect of extract of fenugreek on weekly recorded body weights (g.) against toxicity induced by sub-acute exposure of thiacloprid in rats (Mean ± S.E).

3.1.3 Effect on Absolute Weight Gain (g) and Relative Weight Gain (g%)

The mean value of absolute weight gain (g) and relative weight gain (g%) in each group observed are presented in Table 2 and Figure 2. Absolute and relative body weight gain were significantly ($p<0.05$) lower in Group II as compared to Group I and Group IV, while in the Group III it was significantly ($p<0.05$) higher as compared to Group II but significantly lower than control. On the other hand, no significant ($p<0.05$) difference in absolute and relative body weight gain was observed among Group III.

Table 2. Effect of extract of fenugreek on absolute weight gain (g) and relative weight gain (g%) against toxicity induced by sub-acute exposure of thiacloprid in rats

Group	Initial body wt. (g)	Final body wt. (g)	Absolute Body wt. gain (g)	Relative Body wt. gain (g%)
I.	132.17 ± 2.03	179.39 ± 2.81	47.22^{c} ± 1.05	35.5^{c} ± .67
II.	136.01 ± 1.48	168.86 ± 1.80	32.85^{a} ± 0.66	23.16^{a} ± .40
III.	135.40 ± 2.61	174.92 ± 2.94	39.51^{b} ± 1.38	28.83^{b} ± 1.3
IV.	133.27 ± 2.27	178.71 ± 2.67	45.44^{c} ± 1.20	34.09^{c} ± .84

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

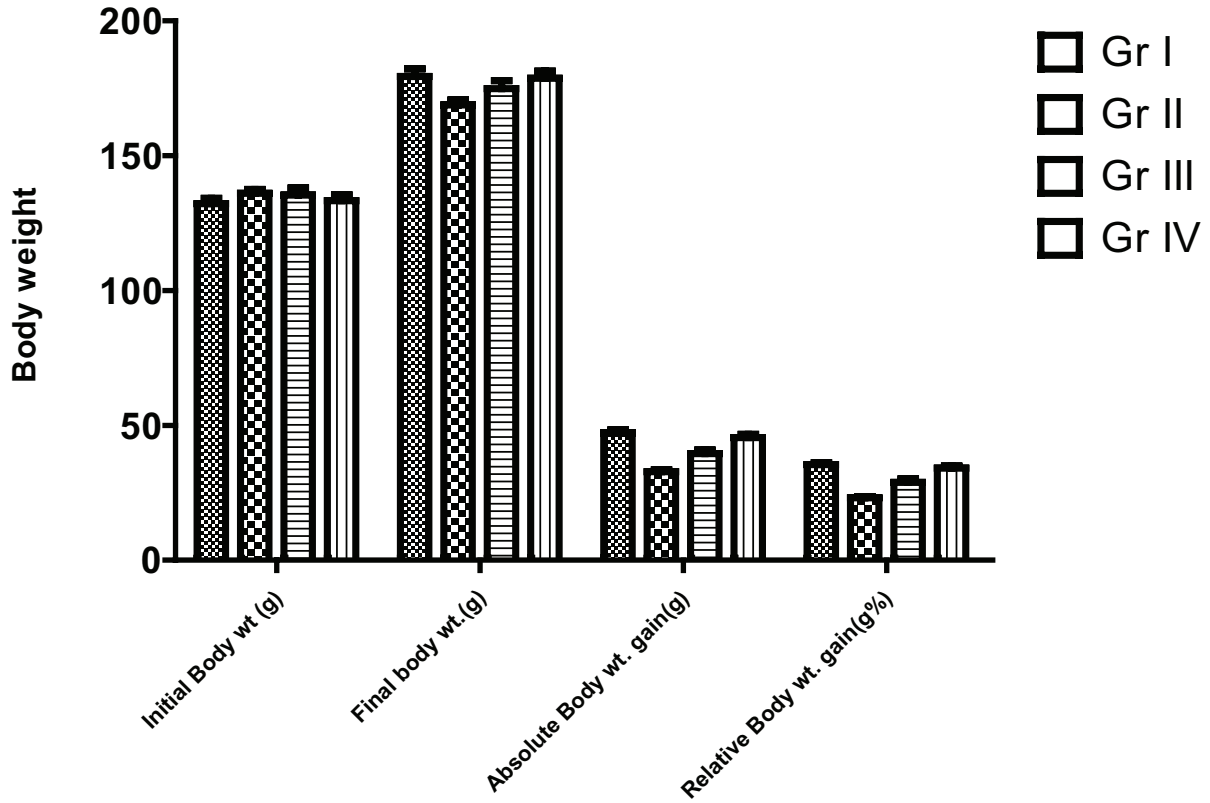


Figure 2. Effect of extract of fenugreek on weekly recorded body weights (g.) against toxicity induced by sub-acute exposure of thiacloprid in rats (Mean ± S.E).

Goyal *et al.*[7] carried out a toxicity study of thiacloprid in the digestive tract of birds and reported that thiacloprid acts as an irritant to the intestinal membrane and further opined that this could reduce food consumption. A similar response of the thiacloprid in rat intestine cannot be ruled out and might have resulted in reduced food intake in animals subjected to subacute exposure of thiacloprid. Anand *et al.*[8] reported similar findings in case of acetamiprid toxicity. Fenugreek also helps to improve body weight by its free radical scavenging and potent antioxidant activities[9].

3.1.4 Effect on Absolute Organ Weight

The absolute organ weight after 28 days of experiment was recorded and is presented in Table 3 and Figure 3. Statistically significant ($p<0.05$) increase in liver weight was observed in Group II and III in comparison to Groups I and IV. In Group III liver weight was significantly ($p<0.05$) reduced as compared to the Group II. The absolute weight of kidney was significantly ($p<0.05$) higher in Groups II and III compared to Groups I and IV, while in Group III kidney weight was significantly lower ($p<0.05$) compared to Group II. No significant change was observed on absolute weight of brain ($p<0.05$) in groups. A statistically significant ($p<0.05$) decrease was observed in absolute spleen weight in Groups II and III compared to control and Group IV, while in Group III the absolute spleen weight was significantly ($p<0.05$) higher compared to Group II. The relative weights of thymus, heart and lungs were also significantly ($p<0.05$) higher in different treatment groups compared to control.

Table 3. Effect of extracts of fenugreek on absolute organ weight against toxicity induced by sub-acute exposure of thiacloprid in rats

Group	Organ weight (g)						
	Liver	Kidney	Spleen	Brain	Thymus	Heart	Lungs
I	3.62a ± .03	0.48a ± .03	0.42c ± .03	0.80a ± .03	0.198a ± .004	0.565a ± .004	0.741a ± .004
II	4.95c ± .02	0.88c ± .02	0.19a ± .02	0.87a ± .02	0.255b ± .006	0.666b ± .015	0.831c ± .007
III	4.62b ± .03	0.72b ± .03	0.25b ± .02	0.84a ± .02	0.195a ± .002	0.713c ± .004	0.796b ± .005
IV	3. 95a ± .03	0.56a ± .02	0.39c ± .02	0.81a ± .02	0.196a ± .012	0.523a ± .005	0.736a ± .003

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

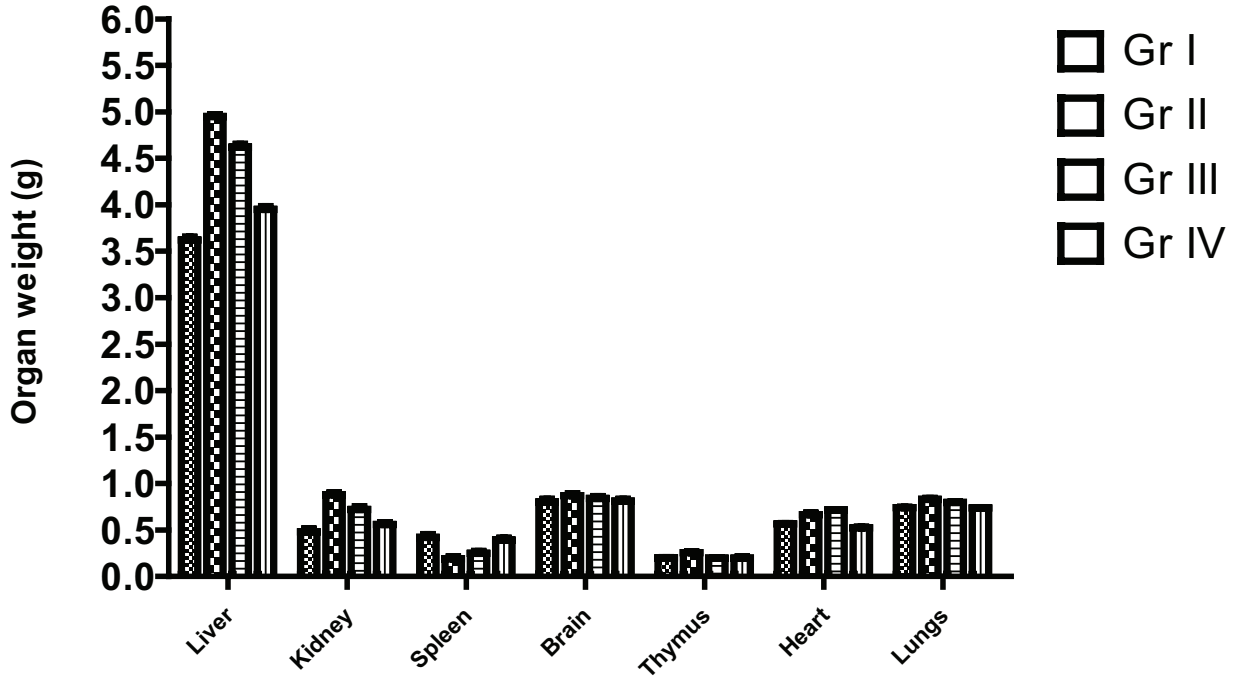


Figure 3. Effect of extracts of fenugreek on absolute organ weight against toxicity induced by sub-acute exposure of thiacloprid in rats.

3.1.5 Effect on Relative Organ Weight

Effect of extract of fenugreek on relative organ weight against toxicity induced by sub-acute exposure of thiacloprid in rats is presented in Table 4 and Figure 4. The relative weight of liver and kidney were significantly ($p<0.05$) higher in Groups II and III as compared to control group and Group IV. While in Group III liver and kidney weights were significantly ($p<0.05$) decreased in comparison to Group II. The relative weight of spleen was statistically ($p<0.05$) decreased in Group II as compared to control group and Group IV. While in Group II there was significant ($p<0.05$) increase in spleen weight compared to Group II. No significant change was observed in relative organ weight of brain.

In toxicological studies, relative organ weights are important criteria for evaluation of organ toxicity.[10-11]. Generally in subacute toxicity studies relative weight of affected organ increases. A statistically significant increase was observed in weight of liver and kidney in thiacloprid treated groups. The results were in accordance with Bhardwaj *et al.*[12] where a significant higher relative liver weight was reported at 20 mg/kg b.wt./day imidacloprid dose. Liver is the main organ of imidacloprid metabolism. Thus, physiologically the liver could be affected directly by thiacloprid during the period of exposure. The findings are also in agreement with the study of Srivastava *et al.*[13] where a significant higher weight of kidney was observed after exposure to liquid mosquito repellent containing allethrin (3.6% w/w).

Table 4. Effect of extract of fenugreek on relative organ weight by sub acute exposure of thiacloprid in rats

Group	Relative Organ Weight (g)			
	Liver	Kidney	Spleen	Brain
I.	$2.02^{a} \pm 0.03$	$0.273^{a} \pm 0.021$	$0.22^{c} \pm 0.03$	$0.493^{a} \pm 0.018$
II.	$2.93^{c} \pm 0.03$	$0.526^{c} \pm 0.012$	$0.10^{a} \pm 0.02$	$0.521^{a} \pm 0.017$
III	$2.64^{b} \pm 0.05$	$0.418^{b} \pm 0.021$	$0.19^{b} \pm 0.02$	$0.486^{a} \pm 0.013$
IV	$2.21^{a} \pm 0.03$	$0.313^{a} \pm 0.016$	$0.21^{c} \pm 0.03$	$0.453^{a} \pm 0.013$

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$).

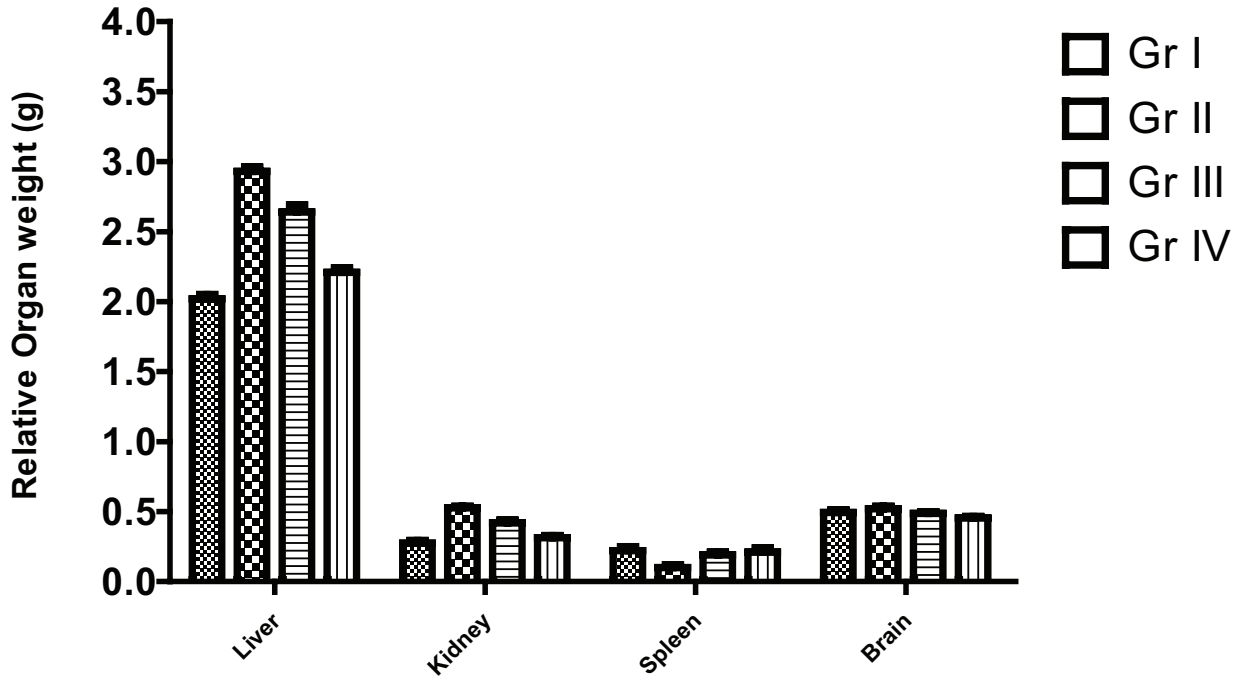


Figure 4. Effect of extract of fenugreek on relative organ weight by sub acute exposure of thiacloprid in rats.

In the present study, fenugreek co-treatment significantly lowered the relative liver and kidney weight as compared to thiacloprid group. The findings were in agreement with Kaviavasan and Anuradha[14] who investigated the effect of fenugreek against toxicity induced by ethanol. Fenugreek restored the levels of markers of liver injury due to presence of hepatoprotectiveagent, silymarin.Verma *et al.*[15] investigated the nephroprotective effect of fenugreek in type-2 diabetic patient.

Spleen weight was reduced in thiacloprid treated rats compared to control. The decrease in spleen weight may be due to damage of spleen cells reflected as decreased weight of the organ[16]. The results were in agreement with study of Gatne *et al.*[17], where it was observed that the spleen of mice exposed to imidacloprid are indicative of tissue destruction and injury reflecting imidacloprid induced death of lymphocytes and also showed disintegration of white pulp in the spleen of imidacloprid treated rats at a dose of 160 mg/kg b.wt . Anand *et al.*[8] reported significantly decreased relative spleen weight due to acetamiprid toxicity which was restored by administration of extract of Panax. Fenugreek treatment significantly increased spleen weight. The results were in agreement with Choudhary *et al.*[18] who reported spleen protective effect of fenugreek.

3.2 Serum Biochemical Parameter

3.2.1 Effect on ALT, AST, LDH, (IU/L), Creatinine Kinase (mg/dl) Enzymes Activity in Serum

Effect of extract of fenugreek on ALT, AST, LDH, (IU/L), creatinine kinase (mg/dl) enzymes activity against toxicity induced by sub-acute exposure of thiacloprid in male albino rats are presented in Table 5 and Figure 5. Thiacloprid treatment in Group II and III resulted insignificantly ($p<0.05$) higher serum ALT and AST activities compared to the control and Group IV. While, in Group III ALT and AST activities were significantly ($p<0.05$) lower compared to Group II. The activities of LDH and creatinine kinase were also found to be significantly ($p<0.05$) higher in Groups II and III compared to control group, while in Group III it was significantly ($p<0.05$) lower compared to the Group II.

Table 5. Effect of extract of fenugreek on ALT, AST, LDH (IU/dl) and creatinine kinase (mg/dl) in serum against toxicity induced by sub-acute exposure of thiacloprid in rats

Group	Serum biochemical			
	ALT	AST	LDH	Creatinine Kinase
I.	$38.67^{a} \pm .47$	$97.31^{a} \pm 1.67$	$237^{a} \pm 3.67$	$302.21^{a} \pm 1.75$
II.	$79.1^{d} \pm .81$	$191.41^{c} \pm 3.17$	$288^{c} \pm 4.52$	$379^{d} \pm 1.03$
III.	$58.43^{c} \pm 1.89$	$153.74^{b} \pm 1.21$	$242.79^{a} \pm 2.01$	$367.94^{c} \pm 3.02$
IV.	$48.75^{b} \pm 1.17$	$153.62^{b} \pm 1.44$	$239.45^{a} \pm 2.32$	$360.25^{b} \pm .80$

Values are Mean ± S.E; n = 6; values bearing common superscripts do not differ significantly ($p<0.05$).

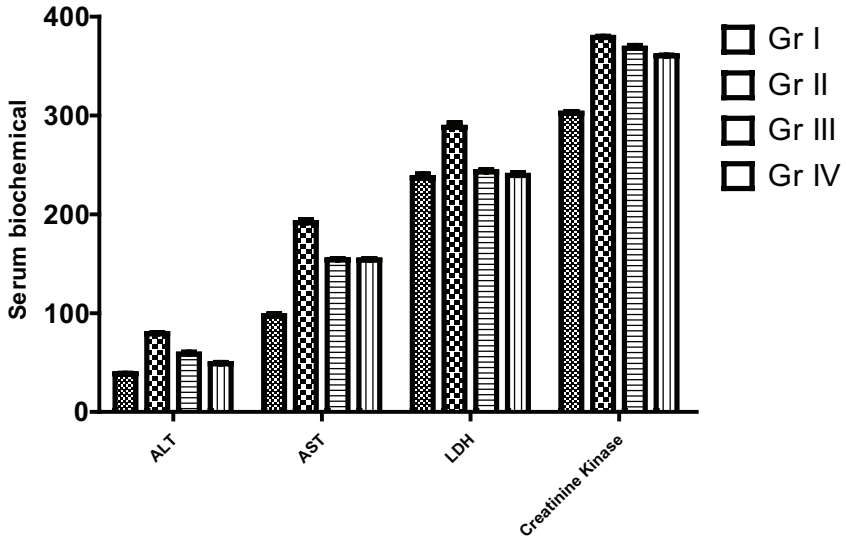


Figure 5. Effect of extract of fenugreek on ALT, AST, LDH (IU/dl) and creatinine kinase (mg/dl) in serum against toxicity induced by sub-acute exposure of thiacloprid in rats.

Increase in the level of serum ALT and AST, the marker enzyme for liver function in thiacloprid group is indicative of its toxic effect on organ whereas increase in LDH and creatinine kinase may be associated to its effect on heart. In the present study, the increase in AST, ALT and LDH after thiacloprid could be attributed to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane. The findings were in accordance with Hendawi *et al.*[19] who reported that sub-acute thiacloprid intoxication at 22.5 mg/kg body wt. caused significant increase in Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) related to hepatic injury. In the present study fenugreek co-treatment in thiacloprid administered rats reduced the serum levels of liver bio-marker enzymes. The findings in present study were similar to Azza *et al.*[20] who observed a similar decrease in AST and ALT enzyme activity in the animals that received fenugreek aqueous extract suggesting that fenugreek has hepatoprotective effect.

3.3 Haematological Parameters

3.3.1 Effect on TEC, Hb and PCV

Effect of extract of fenugreek on haematological parameters (TEC, Hb and PCV) against toxicity induced by sub-acute exposure of thiacloprid in male albino rats are presented in Table 6 and Figure 6. In Group II TEC values was significantly ($p<0.05$) decreased as compared to control group while in Group III, it was towards amelioration as compared to the Group II. Hb and PCV levels were also found to be significantly ($p<0.05$) decreased in Group II in comparison to the control, while in Group III they were significantly ($p<0.05$) increased in comparison to Group II. Group IV was comparable to control.

Table 6. Effect of extract fenugreek on haematological parameters (TEC, Hb and PCV) against toxicity induced by sub-acute exposure of thiacloprid in rats

Hematological parameters			
Group	**TEC (x10⁶/µL)**	**Hb(g/dL)**	**PCV(%)**
I.	$6.68^{c} \pm 0.24$	$13.48^{c} \pm 0.18$	$42.84^{c} \pm 0.25$
II.	$5.73^{a} \pm 0.28$	$10.53^{a} \pm 0.83$	$32.26^{a} \pm 2.59$
III.	$5.89^{b} \pm 0.27$	$11.40^{b} \pm 0.53$	$35.26^{b} \pm 1.65$
IV.	$6.53^{c} \pm 0.18$	$13.23^{c} \pm 0.48$	$41.53^{c} \pm 0.95$

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$).

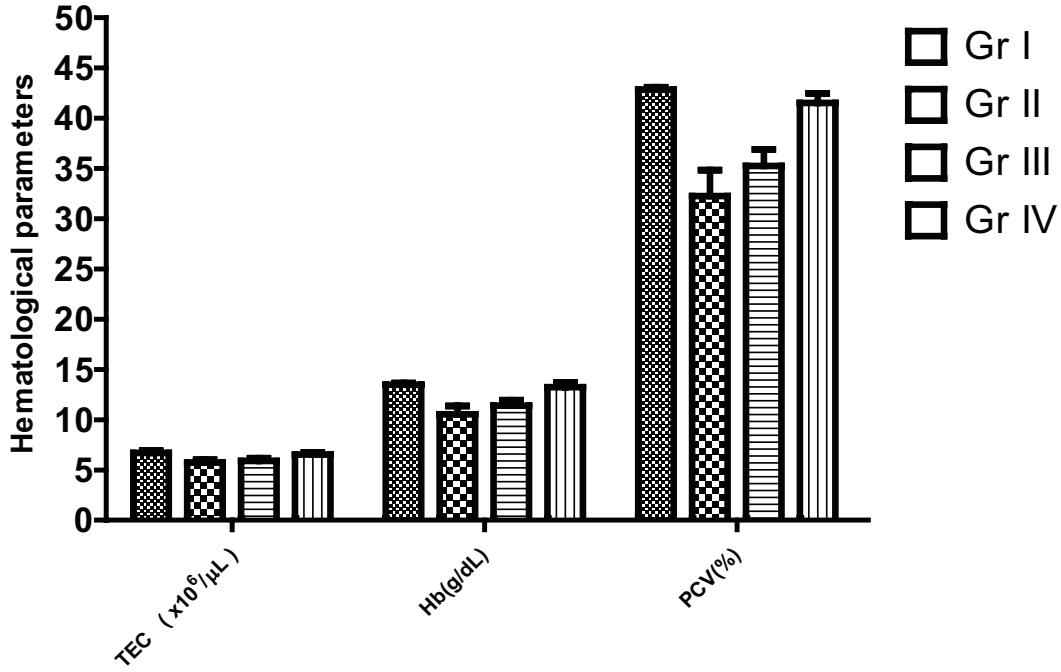


Figure 6. Effect of extract fenugreek on haematological parameters (TEC, Hb and PCV) against toxicity induced by sub-acute exposure of thiacloprid in rats.

3.3.2 Effect on TLC, MCV, MCH and MCHC

Effect of extract of fenugreek on haematological parameters (TLC, MCV, MCH and MCHC) against toxicity induced by sub-acute exposure of thiacloprid in rats is presented in Table 7 and Figure 7. TLC levels were found to be significantly ($p<0.05$) higher in Groups II and III compared to control, while Group III had shown no -significant ($p<0.05$) decrease in TLC values compared to the Group II. Group IV was comparable to control.

MCV levels were found to be lower in Groups II and III compared to control, while in Group III its level was non-significantly ($p<0.05$) higher as compared to the Group II. No significant changes in values of MCH, MCHC were observed in various treatment groups of animals.

Table 7. Effect of extract fenugreek on TLC, MCV, MCH and MCHC against toxicity induced by sub-acute exposure of thiacloprid in rats

Serum biochemical				
Group	**TLC($x10^3/\mu L$)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**
I.	$7.87^a \pm 0.22$	$64.13^c \pm 1.72$	$20.17^a \pm 0.64$	$30.39^a \pm 0.54$
II.	$11.35^b \pm 0.68$	$56.32^a \pm 3.02$	$18.37^a \pm 0.17$	$32.40^a \pm 0.93$
III.	$10.40^b \pm 0.48$	$58.36^b \pm 2.96$	$19.45^a \pm 0.47$	$31.80^a \pm 1.23$
IV.	$8.36^a \pm 0.39$	$63.54^c \pm 1.67$	$20.31^a \pm 0.83$	$30.22^a \pm 1.32$

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly ($p<0.05$)

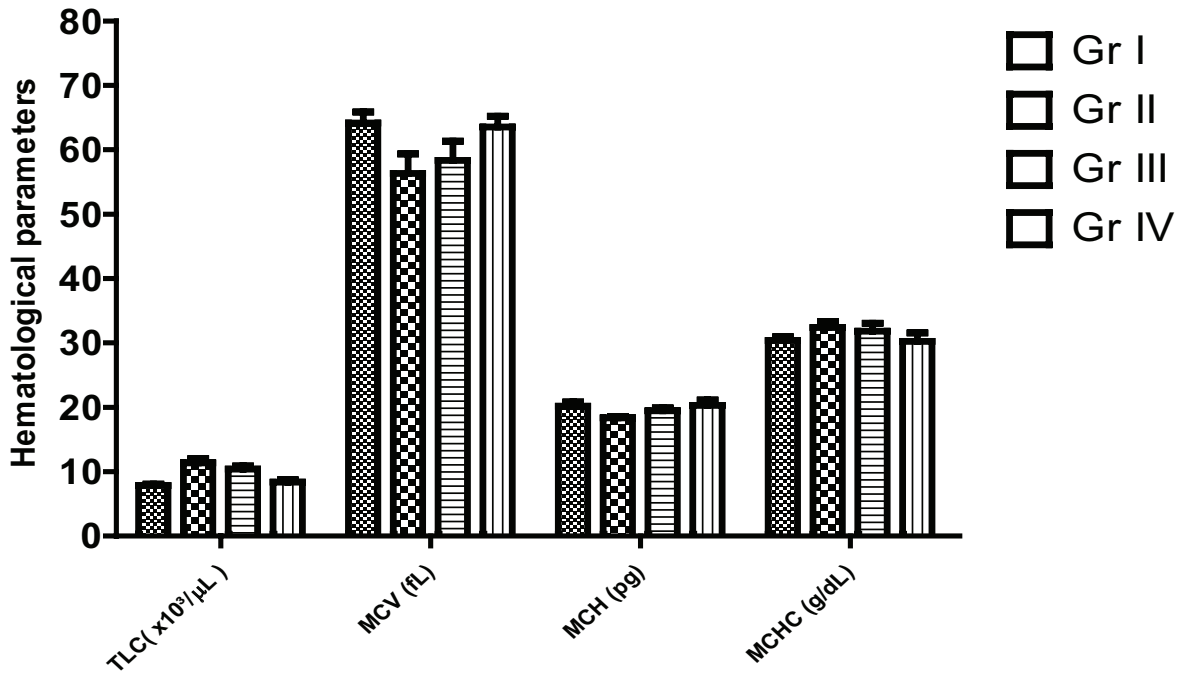


Figure 7. Effect of extract fenugreek on TLC, MCV, MCH and MCHC against toxicity induced by sub-acute exposure of thiacloprid in rats.

3.3.3 Effect on DLC

Effect of extract of fenugreek on differential leukocyte count against toxicity induced by sub-acute exposure of thiacloprid in rats is presented in Table 8 and Figure 8. There were significant ($p<0.05$) increase in the lymphocytes and neutrophils level in Groups II and III compared to control. While Group III showed significant ($p<0.05$) decrease in lymphocytes and neutrophils values compared to Group II. Monocyte, eosinophil and basophil were also significantly ($p<0.05$) higher in treatment groups compared to control and Group IV.

Table 8. Effect of extract of fenugreek on DLC (x10^6/µL) against toxicity induced by sub-acute exposure of thiacloprid in rats

DLC (x10^3/µL)					
Group	**Neutrophil**	**Lymphocyte**	**Monocyte**	**Eosinophil**	**Basophil**
I.	1.96^a ± 0.05	5.44^a ± 0.15	0.133^a ± 0.02	0.157^a ± 0.03	0.015 ^{a} ± 0.002
II.	2.83^b ± 0.17	7.82^b ± 0.47	0.192^b ± 0.01	0.225^b ± 0.01	0.022 ^{b} ± 0.003
III.	2.47^b ± 0.09	7.1^b ± 0.27	0.169^b ± 0.01	0.199^b ± 0.01	0.019 ^{b} ± 0.005
IV	1.87^a ± 0.16	5.66^a ± 0.33	0.138^a ± 0.03	0.149^b ± 0.02	0.018^a ± 0.003

Values are Mean ± S.E; n = 6; Values bearing common superscripts within a column do not differ significantly (p<0.05)

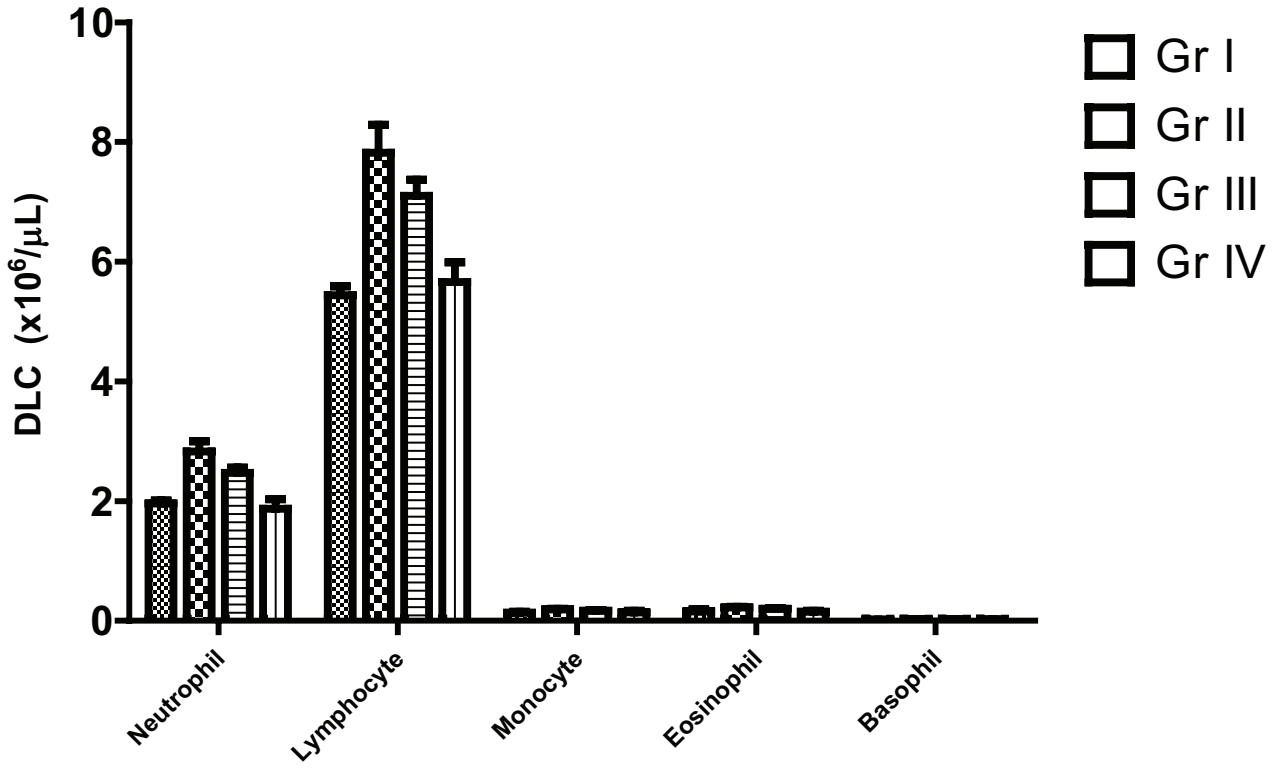


Figure 8. Effect of extract of fenugreek on DLC (x10^6/µL) against toxicity induced by sub-acute exposure of thiacloprid in rats.

The Hb concentration and PCV generally provide an accurate reflection of the extent to which the circulating red cell mass is reduced[21]. The decrease in Hb concentration suggested that thiacloprid might have interfered with erythropoiesis. The observed results were in agreement to that of Kaur[22] who investigated similar changes in haematological parameter after the administration of imidacloprid @ 0.5 mg/kg/day for 150 days in cow calves. The increase in leucocyte count during treatment has been attributed to the occurrence of internal haemorrhage and to the effects on bone marrow.

Erythrocytopenia and hypohemoglobinemia in the present study were indicative of anemic conditions. Similar findings were reported by Kaur[22] after the oral administration of imidacloprid in cow calves for 150 days. The findings in the present study were in agreement with Singla and Sandhu[23] who reported a significant decline in values of PCV, Hb, TEC and significant increase in TLC in *Gallus domesticus* on thiacloprid administration @ 1 mg/kg/day for 90 days. There was significant increase in TEC, Hb, PCV, MCV and MCH, MCHC in fenugreek co-treatment group. The results

were in accordance with Singh *et al.*[24] also investigated effect of fenugreek on haematological parameters in broiler chick.

4. Conclusion

The study suggests that ethanolic extract of fenugreek has the potential to restore the general toxicity caused by sub-acute thiacloprid exposure in rats.

5. Acknowledgement

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6. Conflict of Interest

Authors declare here that they do not have conflict of interest in publication of manuscript.

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