

The protective effect of fenugreek seeds extract supplementation on glucose and lipid profile in thioacetamide induced liver damage in rats

Syeda Nuzhat Fatima Zaidi^{1*} and Javeria Masood²

Department of Biochemistry Clinical and hematology Research lab, Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan

Abstract: The present study was designed to determine the hepatoprotective effects of fenugreek seeds extract supplementation on thioacetamide induced liver damage in rats. For this purpose 24 male Sprague Dawley rats were distributed into 4 groups (n=60). Group I remained untreated throughout the study. Group II and Group III received thioacetamide intraperitoneally 200mg/kg body weight on alternative days for 8 weeks. Group III and Group IV received 2ml of 2% Fenugreek seeds extract orally daily from 4th week till 8th week of the study. Next day after completion of study, the rats were weighted and decapitated; blood was collected for plasma for glucose and lipid profile analysis. Marked reduced plasma glucose level and HDL level and increased triglyceride, cholesterol, LDL, VLDL and total lipid level were observed in test group. The Fenugreek seeds extract supplementation significantly increased plasma glucose and HDL level and remarkably decreased triglyceride, cholesterol, LDL, VLDL and total lipid level in Test+Supplement group whereas glucose and lipid profile were reduced in supplement group showing supplement is hypoglycemic and hypolipidemic. The results of this study confirmed that treatment with Fenugreek seeds extract successively ameliorates the altered glucose and lipid profile in liver damage induced by thioacetamide in male rats.

Keywords: Fenugreek seeds, hepatoprotective effects, triglyceride, cholesterol,

INTRODUCTION

Plants are an enrich origin of bioactive constituents possess desirable health advantageous and are conventionally known to be helpful in preventing chronic disorders (Yogalakshmi *et al.*, 2010). Fenugreek seeds (*Trigonella foenum-graecum*) belongs to plant family Leguminosae. Few countries grow Fenugreek seeds like India, Africa, Egypt, England and Morocco (Khosla *et al.*, 1995). The seed constituents are moisture, protein, fat, saponins, and dietary fibers (Ikeuchi *et al.* 2006). It commonly utilize as additive for preserving food, glutinous as well as emulsifier substance because it contains high content of gum, protein as well as fiber. Fenugreek seeds protein found more solubilize in alkaline state (Meghwal and Goswami, 2012). Fenugreek seeds is an oldest medicinal plant, utilized traditionally to cure problems involving abdominal ailments, gout, swelling, recover injury, flatulence reliever, restorative, aphrodisiac effects, abnormal lipid levels as well as elevated blood sugar levels (Khosla *et al.*, 1995). It is an excellent origin of soluble dietary fiber (SDF) and its utilization has previously been shown to cause a remarkable decrease in serum and liver cholesterol levels (Sharma, 1986). Fenugreek Seeds assisted not merely decreasing blood glucose levels from its high phytochemicals concentration but it has assisted in decreasing low density lipoprotein cholesterol and triglyceride levels also (Shani *et al.*, 1974). Polyphenols present in fenugreek seeds perform an important role in mitigating lipid irregularities and

managing collagen contents in alcohol-induced liver injury (Sowmya and Rajalakshmi, 1999). And recently quercetin, one of the recognized flavonoids in seeds of fenugreek was found capable in preventing hepatocytes against oxidative injury prompted by ethanol in rats (Liu *et al.*, 2011).

Thioacetamide is highly effectual compound for liver damaging and cancer development. For this reason, it frequently utilized to develop liver cancer in an animal model (Sarker and Sil, 2007). Thioacetamide prompts liver injury through S-oxide, a metabolic transformed compound (thioacetamide-S-dioxide), unstable, responsive intermediate; this commences hepatic cell destruction as well as production of responsive oxidative agents via bonding covalently with hepatic macromolecules (Baskaran, *et al.*, 2010). In addition to, TAA causes hepatocellular injury via thioacetamide sulphene and sulphone metabolism, through vital mechanism which includes CYP450E1-intervened biological modification (Ramaiah *et al.*, 2001). This study intent to evaluate hepatoprotective effects of Fenugreek seeds extract supplementation on glucose and lipid profile in thioacetamide prompted hepatic damage in male Sprague Dawley rats.

MATERIALS AND METHODS

Experimental animals

Male Sprague Dawley rats; weight was about 200-264 grams bought from animal house Agha Khan University Hospital, Karachi, Pakistan. Animals were caged separately and kept in a temperature maintained room 23±

*Corresponding author: e-mail: snfzaidi@gmail.com

4°C. One week before the start of an experiment rats had freely access to water and standard diet to adapt laboratory environmental changes.

Ethical guideline

This experiment was performed as per guidance provided to institution by Ethical Review Board and foreign approved protocol for Laboratory use and animal care in research (Health research extension Act of 1985).

Drug preparation

Thioacetamide solubilized in isotonic solution (Saline) and dose of 200mg/kg body weight was injected intraperitoneally on alternative days.

Preparation of fenugreek seeds extract

Fenugreek seeds were obtained from the local market of Karachi, Pakistan and processed in our research lab. The seeds were cleaned by removing extraneous matter and finely powdered in a grinding machine. The 2gm of powder were mixed in 100ml of distilled water, vortexed for 10 min then centrifuge. The supernatant was collected and 2ml of extract was given to each rat daily for four weeks. The extract was freshly prepared every day.

Study design

Twenty four male Sprague Dawley rats were distributed into four groups (n=6). The time period of this study was 8 weeks. Following treatments were being given to every group.

Group I (Control Group) was untreated throughout the experiment.

Group II (TAA Treated Group) was given thioacetamide (200mg/kg body weight) intraperitoneally injected on alternative days for 8 weeks.

Group III(TAA + Supplement Treated Group) was given Thioacetamide (200mg/kg body weight) intraperitoneally injected on alternative day and was also given 2ml of 2% extract of fenugreek seeds every day from 4th week to 8th weeks of an experiment.

Group IV (Supplement Treated Group) was given 2ml of 2% extract of fenugreek seeds every day for four weeks (from 4th week to 8th weeks).

After the completion of an experiment, all rats were decapitated and blood was taken in tubes containing heparin. Plasma was obtained after centrifuging the tubes at 3000 rpm for 10 minutes. Liver were kept at -70°C for antioxidant analysis.

Analysis of glucose and lipid profile

Glucose and Lipid profile were estimated by commercially prepared Merck Kits using MicroLab-300.

STATISTICAL ANALYSIS

Data is represented in Mean±Standard Deviation. Statistical Analysis determines differences among the mean values of experimental groups and was done by One Way ANOVA of SPSS Version 22. Significant level was considered as P> 0.05.

RESULTS

Treatment effects of thioacetamide and fenugreek seeds extract supplementation on body weights of control, test, test+supplement and supplement group

fig No.1: Marked reduced body weight were noted in Test group showing growth retardation as they were subjected to Thioacetamide for eight weeks whereas Test + Supplement group significantly regained body weight as they were subjected to Thioacetamide as well as fenugreek seeds extract supplement as compared to test group. However supplement group was only subjected to fenugreek seeds extract reduced body weight as compared to control group showing supplement is weight reducing and control group gained body weight throughout the study because they were untreated throughout the study.

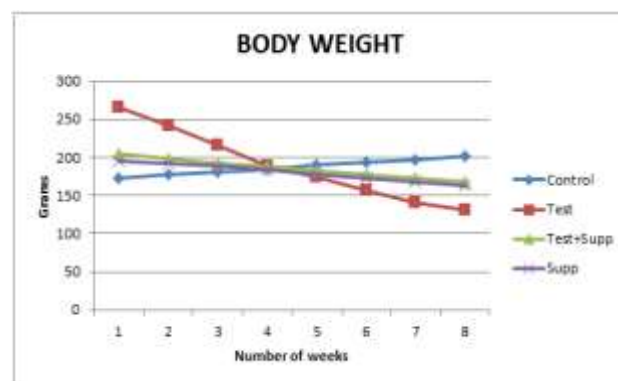


Fig. 1: Effect of Thioacetamide and Fenugreek seeds extracts supplementation treatment on control, Test, Test+Supplement and Supplement group.

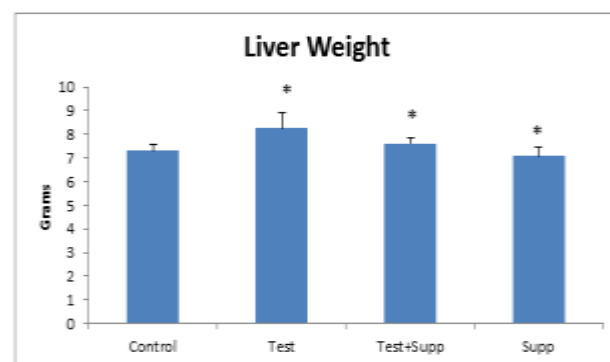


Fig. 2: Effect of Thioacetamide and Fenugreek Seeds Extract Supplementation Treatment on Liver Weight of Control, Test, Test+Supplement and Supplement Group.

Effects of thioacetamide and fenugreek seeds extract supplementation on liver weight and liver to body weight ratio of control, test, test+supplement and supplement group

An increase Liver Weight and Liver to Body Weight Ratio was observed increased in Test group after 8 weeks thioacetamide administration as compared to control group (table 1, figs. 2 & 3). However, liver weight and liver to Body Weight ratio was observed decrease in Test+Supp group as compared to test. A slightly decrease liver weight was observed in supplement group as compared to control group where as an increase liver to body weight ratio was observed as compared to control (P>0.05).

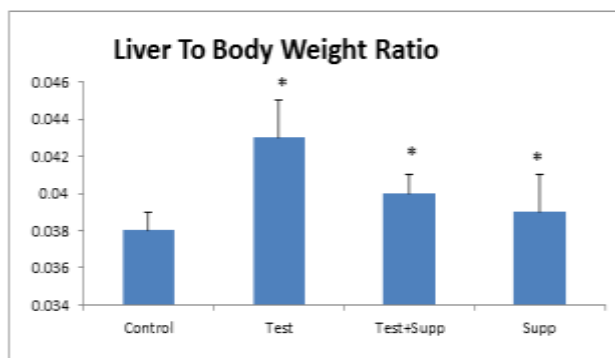


Fig. 3: Effect of thioacetamide and fenugreek seeds extract supplementation on liver to body weight ratio of control, Test, Test+Supplement and supplement group.

Effect of thioacetamide and fenugreek seeds extract supplementation treatment on plasma glucose level of control, test, test+supplement and supplement group

Level of plasma glucose was reduced in Test group as compared to control group (table 2 and fig.4). Fenugreek seeds extract supplementation in Test + Supplement group increased Glucose level as compared to test. While Fenugreek seeds extract Supplementation treated group reduced glucose level as compared to control (P>0.05).

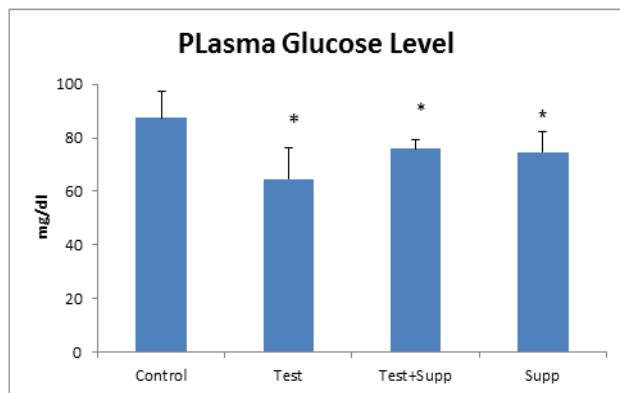


Fig. 4: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma glucose level of control, Test, Test+Supplement and supplement group.

Effect of thioacetamide and fenugreek seeds extracts supplementation on lipid profile of control, test, test+supplement and supplement group

Plasma Triglyceride levels were significantly increased in test group as compared to control (fig. 5 and table 3). The fenugreek seeds extract supplementation reduced these elevated levels in Test + Supplement group as compared to tests. However, alone supplement treated group mildly reduced triglyceride levels as compared to control (P>0.05).

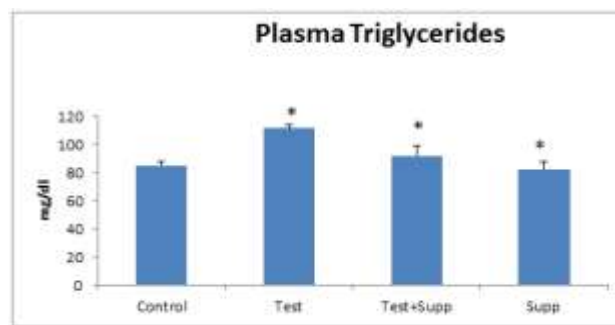


Fig. 5: Effect of Thioacetamide and fenugreek seeds extract supplementation on plasma triglyceride level of control, test, Test+Supplement and Supplement Group:

Levels of plasma cholesterol were significantly elevated in Test group as compared to control (fig. 6 and table 3). These elevated levels were reduced in Test+Supplement group as compared to test. While in supplement treated group the cholesterol level was mildly reduce as compared to control (P>0.05).

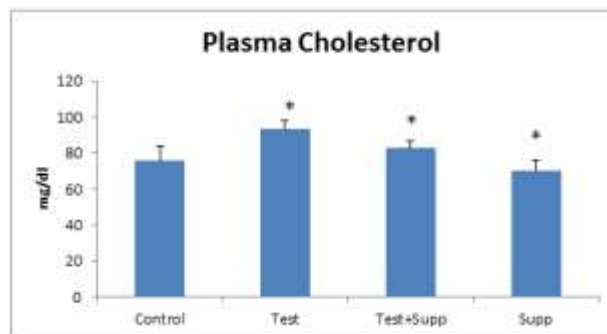


Fig. 6: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma cholesterol level of control, test, Test+Supplement and supplement group:

Plasma LDL level was elevated in Test group as compared to Control (fig. 7 and table 3). The fenugreek seeds extract supplementation reduced this level in Test+Supplement group as compared to test. However, LDL level was mildly reduced in Supplement treated group as compared to control (P>0.05).

Plasma HDL level was markedly reduced in test group after 8 weeks TAA administration as compared to control (fig. 8 and table 3). The HDL level was significantly

increased in Test + Supplement group as compared to control. However, HDL level was almost normal in supplement treated group as compared to control ($P>0.05$).

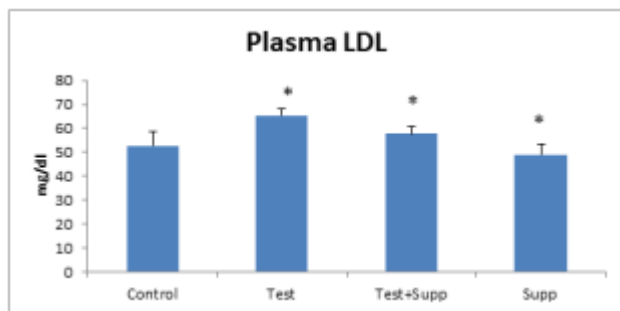


Fig. 7: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma ldl level of control, Test, Test+Supplement and supplement group:

Plasma VLDL level was elevated in test group as compared to Control (fig. 9 and table 3). In Test+Supplement group, this elevated level was significantly reduced as compared to test. However, the VLDL level was mildly reduced in supplement group as compared to control ($P>0.05$).

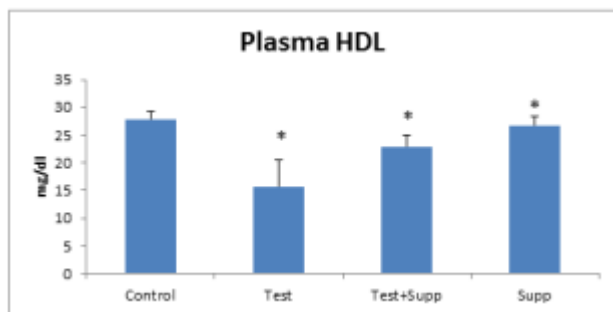


Fig. 8: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma hdl level of control, Test, Test+Supplement and supplement group:

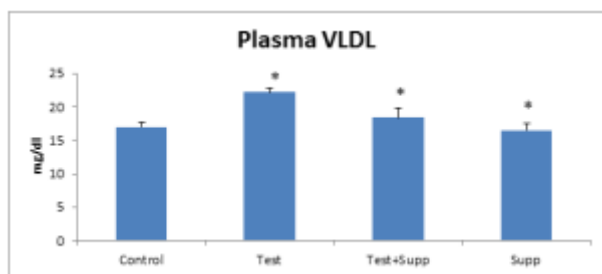


Fig.9: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma vldl level of control, test, Test+Supplement and supplement group:

The plasma Total lipid level was significantly increased in Test group after 8 weeks thioacetamide administration as compared to control (fig. 10 and table 3). In Test+Supplement, the fenugreek seeds extract supplementation remarkably reduced Total Lipid levels as compared to test. Whereas, the alone fenugreek seeds

extract supplementation remarkably reduced the lipid levels in Supplement group as compared to control ($P>0.05$).

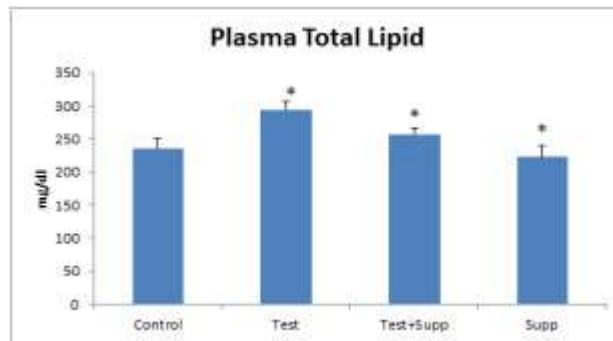


Fig.10: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma total lipid level of control, test, Test+Supplement and supplement group:

DISCUSSION

In present study, the prolonged TAA application to rats produced visual and quantifiable responses which can observe in modulation of liver and body weights (Sunitha *et al.*, 2001). A decrease in body weight would mean that catabolism has continued. The reduced body weight is the result of TAA administration, was the effect of direct toxicity or indirect toxicity producing liver injury. The liver enlargement in TAA treated rats indicate hepatic lesions and liver damage related to the toxicological effects of TAA (Rizzo *et al.*, 1997). A present decrease blood plasma levels of glucose, HDL, decreased hepatic glycogen, and total protein alongwith an increase triglycerides, cholesterol, LDL of plasma, whereas hepatic total lipid shows metabolic protein disruption alongwith carbohydrate and lipid prompted from TAA toxicity (Trennery and Waring, 1983; Galisteo *et al.*, 2006; Jain and Singhai, 2011). The changed glucose level is attributed to the pattern associated with TAA-toxicity which causes temporary effect on liver glycogen producing glycogenolysis basically. The elevated glucose can also influence β -cells of pancreas and insulin secretion, which led to increase an effect after hepatotoxic dose causing lowered hepatic glycogen subsequently producing significant hyperglycemia. Therefore, serum insulin was raised serum glucose levels afterward showed a reduction by reason of the raised serum insulin, this describes the hypoglycemia noticed on 8th week in TAA treated group (Ebrahim *et al.*, 2004). Moreover, TAA-prompted unusual lipid anabolism or catabolism involves disease state such as carcinoma. The membrane or tissue lipid peroxidation causes ejaculation of lipids in bloodstream resultantly cause hyperlipidemia (Sako *et al.*, 2004).

The present study observation of extract supplementation of fenugreek seeds in decreasing the adipose tissue and body weight are in accordance with an evaluation in

Table 1: Treatment effects of thioacetamide and fenugreek seeds extract supplementation on liver weights and liver to body weight ratios of control, test, test + supplement and supplement groups.

Parameters	Control	Test	Test+Supplement	Supplement	F-Value P>0.05
Liver weight (grams)	7.3±0.25	8.25±0.68*	7.6±0.26*	7.08±0.37*	8.14
Liver to body weight ratio	0.038±0.001	0.043±0.002*	0.040±0.001*	0.039±0.002*	6.601

Table 2: Effect of thioacetamide and fenugreek seeds extracts supplementation treatment on glucose level of control, test, test+supplement and supplement group.

Parameters	Control	Test	Test+Supplement	Supplement	F- Value P>0.05
Glucose	87.5±10	64.66±11.5*	75.8±3.4*	74.5±7.79*	6.82

Table 3: Effect of thioacetamide and fenugreek seeds extract supplementation on plasma lipid profile of control, test, test+supplement and supplement group.

Parameters	Control	Test	Test+Supplement	Supplement	F- Value P>0.05
Triglycerides (mg/dl)	85±3.16	111.3±2.65*	91.83±7.30*	82.3±5.08*	42.75
Cholesterol (mg/dl)	75.5±8.50	93.3±4.41*	82.6±4.17*	70.16±6.14*	16.39
LDL (mg/dl)	52.85±5.95	65.33±3.08*	57.86±2.92*	49.11±4.30*	16.38
HDL (mg/dl)	27.91±1.31	15.75±4.8*	22.91±2.03*	26.75±1.69*	52.16
VLDL (mg/dl)	17±0.63	22.2±0.53*	18.36±1.46*	16.46±1.01*	42.15
Total lipid (mg/dl)	236±14.9	294.6±12.9*	257.1±9.5*	222.6±16.7*	31.06

Values are mean±standard deviation. The significant differences among control, test, test+supplement, supplement groups were analyzed by SPSS One-Way ANOVA. P>0.05

previous study by Toshiaki Handa *et al.*, 2005. The fenugreek seeds predicted mechanism in lowering whole body as well as fatty tissue weight probably is 1) Fenugreek wipes out carbohydrate from the body before it comes into circulation leading weight reduction 2) Fenugreek seeds have large fraction of solvable fiber. This fiber makes sticky substance (alike the gaurgum) whose effect is in decelerating food digestion as well as absorption from intestine and therefore produces a feeling of abdominal satiety, hence represses hunger ultimately prompts weight reduction (Geeta *et al.*, 2011). The normoglycemic effects of fenugreek seeds extract may be because of the major presence of 4-isohydroxy leucine which encourages secretion of insulin from pancreas (Al-Habori and Reman, 1998). The normoglycemic effect can also be mediated by encouraging synthesis of insulin and/or enhancing β -pancreatic cells' secretion by Langerhans (Puri *et al.*, 2002).

Prevention of lipid anabolism is stimulated through membrane bound elements like sterol regulation element binding protein-1 (SREBP-1) transcriptionally (Foretz *et al.*, 1999) meanwhile, medicating these hypercholesterolemic male rats with fenugreek seeds have significantly ameliorated lipid profile by lowering cholesterol, triglycerides, low density lipoprotein as well as very low density lipoproteins and enhances HDL. The result is similar with the (Makni *et al.*, 2008). Fenugreek has lecithin which solubilizes cholesterol and has a lipotropic (fat soluble) material, which solubilizes fat

depots, inhibits cumulate of fats and water retention (Blumenthal *et al.*, 1998). Fenugreek seed enhances removal of bile acid and cholesterol in feces. This is a secondary reaction among bile acids and saponins, which give rise to micelles formation too big for uptake through gastrointestinal tract. Other effects are seeds fiber fraction enriched mucilage decreases cholesterol synthesis in liver. These two mechanisms cause the cholesterol reduction (Narender *et al.*, 2006). Seed has nearly 4-10% moisture, 6-8% fat, 18-30% protein and 48-55% fiber (Sauvaire *et al.*, 1976; Sharma 1986; Vats *et al.*, 2003; Srinivasan, 2006). Perhaps the major effect of dietary fibers on bile acids in the small intestine is to lower the efficiency of enterohepatic circulation resulting in an increase in fecal elimination (Dongowski and Lorenz, 2004).

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