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Fenugreek seeds attenuate thioacetamide induced liver damage

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Abstract: The intention to conduct this study was to evaluate the hepatoprotective effects of Fenugreek seeds' extract supplementation in thioacetamide induced liver damage in male Sprague Dawley rats. For this study, 24 male Sprague Dawley rats (200-264gm) were distributed randomly into four groups. Group I remained untreated as control rats, group II received thioacetamide (200mg/Kg b.w i.p, administered on alternative days for 8 weeks), group III received thioacetamide (200mg/Kg b.w i.p administered on alternative days for 8 weeks) as well as 2ml of 2% extract of fenugreek seeds (orally administered daily from 4th week till 8th week of the experiment. Group IV only received 2ml of 2% extract of Fenugreek seeds daily for 4 weeks respectively. At the end of the experiment, blood was sampled to obtain plasma that was used for the analysis of liver markers and liver was used for analysis of antioxidant enzymes (catalase and SOD). Increase in total bilirubin, direct bilirubin, ALT and ALP levels, catalase activity and decrease in SOD activity was found in TAA-treated groups which assured liver damage. Whereas, treatment with Fenugreek seeds extract restored the altered levels of total bilirubin, direct bilirubin, ALT, ALP, catalase and SOD activities in the Test + Supp group. The results of this study confirmed the hepatoprotective role of Fenugreek seeds extract in thioacetamide induced liver damage.

Keywords: Thioacetamide, fenugreek seeds, liver biomarkers, liver antioxidant enzymes.

INTRODUCTION

Drug Induced Liver Injury (DILI) can influence both parenchymal and non-parenchymal cells of liver resulting in a wide variety of pathological conditions, inclusive of acute and chronic hepatocellular hepatitis, fibrosis, cirrhosis, cholestasis, steatosis, also the sinusoidal and hepatic artery/vein damage (Larrey, 2000). Biostimulation of a drug produces reactive species which causes the dysfunction of mitochondria, catabolism of cytoskeleton, and disruption in cell membrane. However, biostimulation can affect the protein transport, causing the disturbance in bile flow and bilirubin secretion (Yun *et al*., 1993; Beaune *et al*., 1987; Trauner *et al*., 1998). TAA goes through a two-step biostimulation via a mixed-function oxidase system, namely, through CYP and/or flavin-containing monooxygenase (FMO) systems to sulfine (sulfoxide) and sulfene (sulfone) intermediates (Akhtar and Sheikh, 2013; Staňková *et al*., 2010; Kang *et al*., 2008; Chilakapati *et al*., 2005). *In vivo* administration to rodents caused a highly selective liver injury inclusive of cirrhosis, fibrosis, the hepatic necrosis/apoptosis as well (Staňková *et al*., 2010; Hajovsky *et al*., 2012). Liver injury follows the intervention of microsomal CYP2E1 to TA sulfoxide (TASO), and then to TA-S,S-dioxide ($TASO_2$), which eventually modifies amine-lipids and proteins to cause cellular necrosis (Chilakapati *et al*., 2005; Hajovsky *et al*., 2012; Koen *et al*., 2014).

Generally the term used to indicate the combination of liver biochemical tests are Liver Function tests (LFT's), involving serum aminotransferases, alkaline phosphatase (ALP), and bilirubin.(Green and Flamm, 2002). The aminotransferases (previously known as transaminases) are present in hepatocytes and are the susceptive indicator of liver damage. They are helpful in detection of acute hepatopathies, like hepatitis (Franklin and Mitchell, 2012). ALT is present in highest concentration in hepatocytes and in a minute concentration in other tissues. ALT is located only in cytoplasm (Franklin and Mitchell, 2012).

Alkaline phosphatase belongs to a family of zinc metalloenzymes that catalyze the hydrolysis of various organic phosphate esters at a neutral pH (Mukherjee and Gollan, 2011). ALPs are located in the canalicular membrane of hepatocytes, the osteoblasts membrane of bone, brush borders of small intestinal mucosal cells, the proximal convoluted tubules of kidneys, placenta, and white blood cells. Serum ALP increases when the disruption in hepatocyte canalicular membrane, causing the displacement from canalicular membrane to basolateral (ie, sinusoidal) surface of hepatocyte and discharge into serum (Franklin and Mitchell, 2012). The liver injury pattern can be distinguished depending upon the ratio of serum ALT to ALP, both are expressed as multiples of normal upper range (Woreta and Alqahtani, 2014). Bilirubin is majorly derived from haemoglobin in senescent erythrocytes, even though the other heme-comprising proteins make a remarkable contribution (Chowdhury *et al*., 1989). The synthesis of bilirubin initially takes place in reticuloendothelial cells of liver and spleen (Franklin and Mitchell, 2012). Both hepatocellular and cholestatic liver may result in

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increased serum bilirubin levels (Polson and Lee, 2005; Miyake *et al*., 2012).

Generally free radicals of various forms are produced at a low rate in cells to assist the changes in various physiological functions. An integrated antioxidant system in body quenched these free radicals (Anandjiwala *et al*., 2008).In tissues and cells, the oxidative stress results from an increased production of free radicals or reactive oxidative agents and the reduced capability of antioxidant defense (Matti *et al*., 2001). Thioacetamide is an effectively selective hepatotoxin, which has been extensively utilized to cause acute and chronic liver damage (Bruck *et al*., 2001). Oxidative damage has been known as the principal mechanism in thioacetamide prompted liver injury, the oxidative metabolism of thioacetamide from the hepatic FAD monooxygenase system and cytochrome P-450 monooxygenase system generates reactive oxidative species particularly the highly reactive compound. Thioacetamide-S-dioxide targets tissue macromolecules like lipids, protein, and DNA- prompted tissue oxidative damage and necrosis. It has been observed that thioacetamide induced tissue damage and necrosis/apoptosis via the several processes associated with protein, DNA and lipid peroxidation (Balkan *et al*., 2001).

In different laboratories various experimental studies are in progress to analyze the preventive effects of various natural antioxidant compounds. Herbs are usually considered safe and verified to be efficacious against various human diseases and their medicinal application has been rising gradually in developed countries (Heeba and Abd-Elghany, 2010). Natural antioxidants encourage the defense of endogenous antioxidants to reactive oxidative agents and reestablish the optimal balance through neutralizing the reactive agents (V. Lobo *et al*., 2010) .Fenugreek, *Trigonella foenum-graecum* Linn. (Fabaceae) is a broadly growing herb in India, Egypt, and countries of the Middle East. Fenugreek seeds contain an array of phyto-constituents like carbohydrate (mucilaginous fiber, galactomannan), proteins, alkaloids (trigonelline and choline), flavonoid glycosides (vicenin 1), furostanol glycosides (trigoneoside Ib), free amino acids (4-hydroxyisoleucine, arginine, lysine, histidine), saponins (diosgenin, and yucca genin), vitamins and mucilage (Taylor *et al*., 2002). It has the ability to cure different ailments like diabetes, hyperlipidemia, inflammation, and ulcers have been well reported in Ayurveda (Amit *et al*., 2017). Presence of Furostanol and flavonoid glycosides in fenugreek seeds are known to be accountable for its anti-inflammatory, antioxidant, anabolic, androgenic and metal chelating characteristics (Kawabata *et al*., 2011; Aswar *et al*., 2010; Kenny *et al*., 2013; Uma Devi *et al*., 2000; Uma Devi and Satyamitra, 2004; Uma Devi, 2001; Vrinda and Uma Devi, 2001). Fenugreek seed possesses 45-60% carbohydrates, majorly mucilaginous fiber (galactomannans); 20-30% proteins rich in lysine and tryptophan, 5-10% fixed oils (lipids), pyridine-type alkaloids mostly trigonelline (0.2-0.36%), choline (0.5%), gentianine, and carpaine, flavonoids, free amino acids, calcium and iron, saponins, glycosides, cholesterol and sitosterol, vitamins A_1,B_2,B_3,B,C and D, nicotinic acid and volatile oils (Blumenthal *et al*., 2000; Shang *et al*., 1998). Trigonella foenum-graecum L. is utilized in folk medicine because of its hepatoprotective, hypoglycemic, expectorant and laxative properties (Umesh *et al*., 2014).

Therefore, the aim to conduct this study was to determine the hepatoprotective effects of fenugreek seeds extract supplementation on thioacetamide induced liver damage in rats.

MATERIALS AND METHODS

Experimental animals

Male Sprague Dawley rats weighing (200-264 gm) purchased from an animal house of Aga Khan University Hospital, Karachi, Pakistan. Animals were acclimatized to the laboratory conditions one week before the start of an experiment and caged separately in a temperature controlled room (23±4°C). Rats had free access to water and a standard rat diet.

Ethical guideline

The experiments were conducted according to the ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for Laboratory use and care in animal research (Health research extension Act of 1985). All the experimental work and clinical estimations were carried out in animal house and clinical biochemistry and hematology research lab, department of biochemistry, Federal Urdu University, Karachi.

Drug preparation

Thioacetamide was dissolved in 0.9% isotonic solution (Saline) and was administered intraperitoneally (200mg/kg b.w) on alternative days.

Preparation of fenugreek seeds extract

Fenugreek seeds were obtained from the local market of Karachi, Pakistan. The seeds were cleaned by removing extraneous matter and finely powdered in a grinding machine. The 2gm of powder were mixed in 100ml of distal water, vortexed for 10 min then centrifuged. 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 randomly distributed into four groups (n=6). The experimental period consisted of 8 weeks. Following treatments were being given to every group.

Group I (Control Group) remained untreated.
Group II (TAA Treated Group) received thioacetamide at a dose of 200mg/ kg b.w., i.p, administered on alternative days for 8 weeks.
Group III (TAA + Supplement Treated Group) received thioacetamide at a dose of 200mg/kg b.w., i.p administered on alternative day and also received 2ml of 2% extract of fenugreek seeds every day from 4th week till 8th weeks of an experiment.
Group IV (Supplement Treated Group) received 2ml of 2% extract of fenugreek seeds every day for four weeks (from 4th week till 8th week).

At the end of the experimental period, the rats were decapitated and blood was collected in heparin containing tubes and was centrifuged at 3000 rpm for 15 minutes to obtain plasma and liver was preserved at -70ºC for antioxidant analysis.

Assessment of liver biomarkers
LFTs (plasma total and direct bilirubin, plasma ALT, plasma ALP) were estimated through microlab-300 by using commercially available Merk reagent kits.

Assessment of antioxidant status
Preparation of post mitochondrial supernatant
Liver homogenate was prepared by taking 1gm of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get post mitochondrial supernatant which was used to assay tissue SOD and Catalase activity.

Estimation of superoxide dismutase activity (kono et al., 1978)
Levels of SOD in the cell free supernatant were measured by the method of Kono *et al*., 1978. Briefly, 1.3ml of Solution A (0.1mM EDTA containing 50mM Na_2CO_3 , pH=10.0), 0.5ml of Solution B (90 µM NBT-nitro Blue Tetrazolium Dye) and 0.1ml of solution C (0.6% TritonX-100 in solution A), 0.1 ml of Solution D (20mM Hydroxylamine hydrochloride, pH=6.0) were mixed and the rate of NBT reduction was recorded at 560nm on Spectrophotometer. 0.1ml of the supernatant was added to the test and reference cuvette, which does not contain solution D. Finally, the percentage inhibition in the rate of NBT reduction was recorded in U/gram tissue. One enzyme unit was expressed as inverse of the amount of protein (mg) required inhibiting the reduction rate by 50% in one minute. The activity was calculated using the % inhibition in grams of tissue and expressed in Unit/g tissue.

Estimation of tissue catalase activity
Catalase activity was analyzed by the method of Sinha, *et al*., 1972). In a clean glass tube, the assay mixture consisted of 1.96ml of Phosphate buffer (0.01M, pH=7.0), 1.0ml of hydrogen peroxide (0.2M) and 0.04ml (10%) of homogenate in a final volume of 3.0ml. 2ml of dichromate acetic acid reagent (5% of 50ml dichromic acid + 150ml of glacial acetic acid) was added in 1ml of reaction mixture, boiled for 10min, cooled, changes in absorbance were recorded at 570nm on spectrophotometer. The concentration values were calculated from absorption measurements as standard absorption in mM/g tissue (Sinha *et al.,* 1972).

STATISTICAL ANALYSIS

The presented data is expressed as Mean ± Standard Deviation and was analyzed by One Way ANOVA using SPSS (Statistical Package for Social Sciences) Version 22 to determine the differences among the mean values of experimental groups. P<0.05 was considered as Significant.

RESULTS

Effect of treatment on body weight of control and treated groups
Fig. 1 shows a significant increase in body weight of control group rats. On other hand, a significant decrease was found in body weight of test group rats as compared to control group. Treatment with Fenugreek seeds mildly increased the body weight of the Test+Supp group as compared to the test group. However, treatment with Fenugreek seeds extract reduced the Body weight of supplement group rats as compared to control group rats.

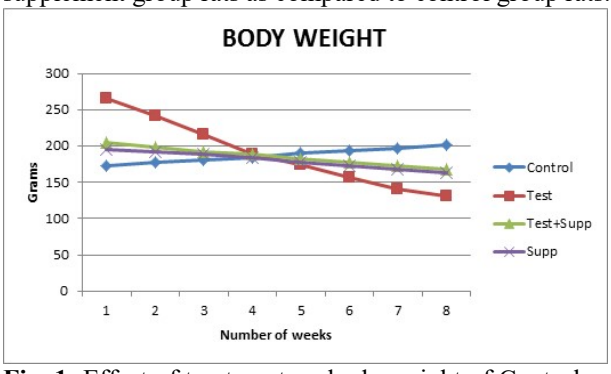

Fig. 1: Effect of treatment on body weight of Control and treated groups.

Effect of treatment on liver weight of control and treated groups
Fig. 2 and table 1 shows a significant increase in liver weight of test group (8.25±0.68*) as compared to the Control group. Treatment with fenugreek seeds extract decreased the liver weight of Test+Supp group (7.6±0.26*) as compared to the test group. However, treatment with Fenugreek Seeds extract decreased the liver weight of supplement group (7.08±0.37*) as compared to the Control group (P<0.05).

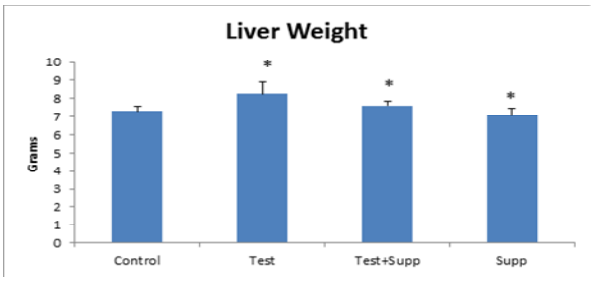

Fig. 2: Effect of treatment on liver weight of Control and treated groups.

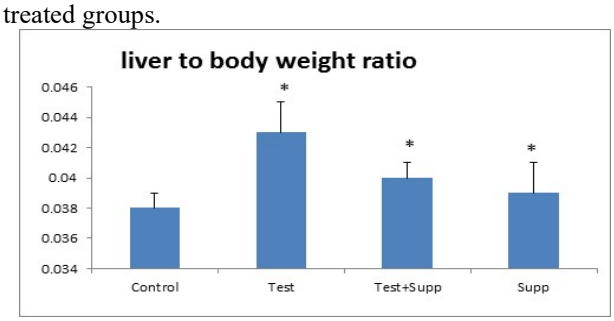

Fig. 3: liver to body weight ratio of Control and treated groups

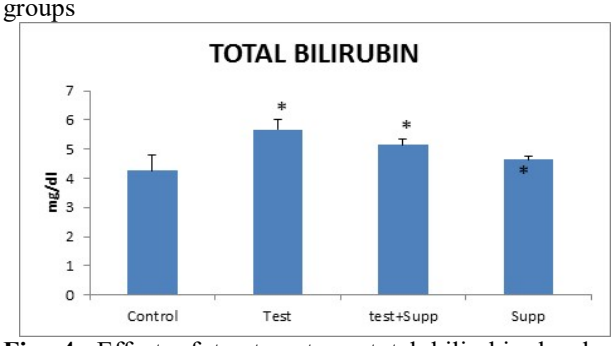

Fig. 4: Effect of treatment on total bilirubin level of control and treated groups

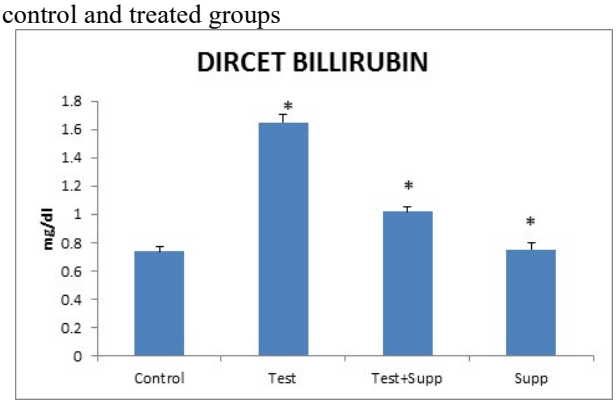

Fig. 5: Effect of Treatment on Direct Bilirubin Level of Control and treated groups

Effect of treatment on liver to body weight ratio of control and treated groups

Fig. 3 & table 1 shows a significant increase in liver to body weight ratio of test group (0.043±0.002*) as compared to the control group. Treatment with fenugreek seeds extract decreased the liver to body weight ratio of Test + Supp group (0.040±0.001*) as compared to the test group. However, treatment with fenugreek seeds extract increased the liver to body weight ratio of supplement group (0.039±0.002*) as compared to control group ($P<0.05$).

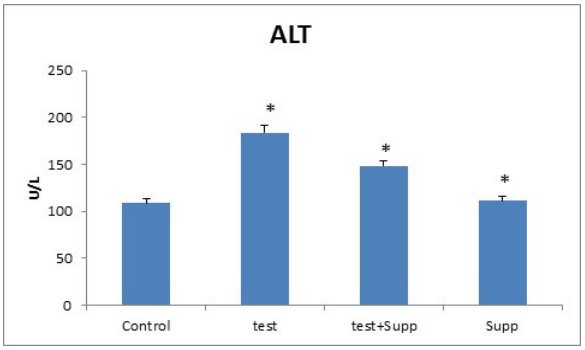

Fig. 6: Effect of treatment on ALT level of Control and treated groups

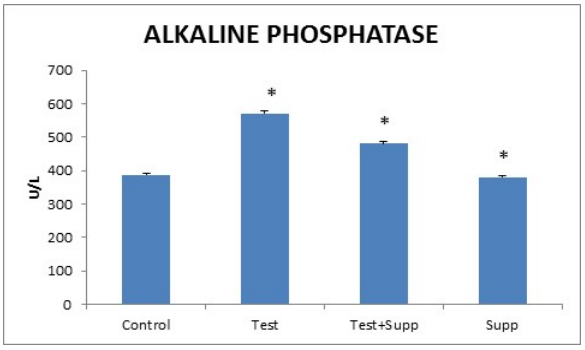

Fig. 7: Effect of treatment on ALP level of Control and treated groups

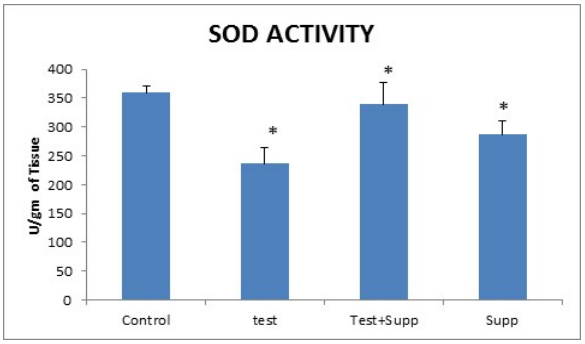

Fig. 8: Effect of treatment on SOD activity of control and treated groups

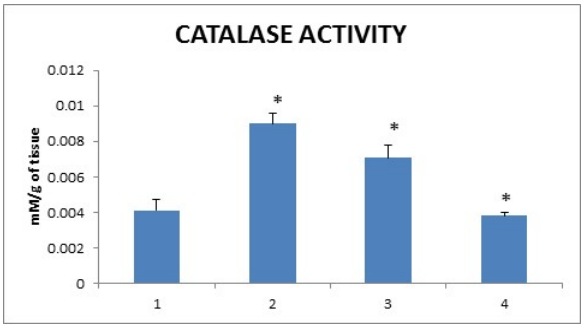

Fig. 9: Effect of treatment on Catalase activity of control and treated groups.

Table 1: liver weight and liver to body weight ratio of control and treated groups

Parameters	Control	Test	Test+Supp	Supp	F-Value
Liver Weight	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

Values are Mean±Standard Deviation. Significant differences between Control, Test Test+Supplement, Supplement groups by SPSS One-Way ANOVA. *P>0.0

Table 2: Liver function tests (LFT's)

Parameters	Control	TEST	TEST+SUPP	SUPP	F-VALUE P>0.05
Total Bilirubin (mg/dl)	4.26±0.54	5.66±0.35*	5.05±0.18*	4.64±0.11*	19.97
Direct Bilirubin (mg/dl)	0.736±0.037	1.65±0.054*	1.02±0.03*	0.751±0.048*	533.13
ALT (U/L)	108.5±5.4	183.83±7.19*	148.3±5.46*	111.16±4.4*	232.1
ALP (U/L)	386.6±6.31	571.16±7.75*	480.83±6.52*	379.8±4.70*	1169.3

Values are Mean ± Standard Deviation. Significant differences between Control, Test, Test + Supplement, Supplement groups by SPSS One-Way ANOVA. *P>0.05

Table 3: Antioxidant enzymes

Parameters	Control	TEST	TEST+SUPP	SUPP	F-VALUE P>0.05
SOD (U/gram)	359.3±12.02	236.7±27.0*	339.5±37.7*	286.5±23.2*	25.71
CATALASE (mM/gram)	0.0041±0.0006	0.009±0.0006*	0.00708±0.0007*	0.0038±0.00021*	107.6

Values are Mean ± Standard Deviation. Significant differences between Control, Test Test+Supplement, Supplement groups by SPSS One-Way ANOVA. *P>0.05

Effect of treatment on total bilirubin level of control and treated groups

Fig. 4 and table 2 shows a significant increase in total bilirubin level of test group (5.66±0.35*) as compared to the Control group. Treatment with fenugreek seeds extract reduced the total bilirubin level of Test + Supp group (5.05±0.18*) as compared to the test group. However, treatment with Fenugreek Seeds extract mildly increased the total bilirubin level of Supplement group (4.64±0.11*) as compared to the Control group (P<0.05).

Effect of treatment on direct bilirubin level of control and treated groups

Fig. 5 & table 2 shows a significant increase in direct bilirubin level of test group (1.65±0.054*) as compared to the control group. Treatment with fenugreek seeds extract reduced the direct bilirubin level of test + Supp group (1.02±0.03*)as compared to the test group. However, treatment with Fenugreek Seeds extract mildly increased the direct bilirubin level of Supplement group (0.751±0.048*) as compared to the control group (P<0.05).

Effect of treatment on alt level of control and treated groups

Fig. 6 & table 2 shows a significant increase in ALT level of test group as compared to control group (183.83±7.19*). Treatment with fenugreek seeds extract reduced the ALT level of Test+Supp group (148.3±5.46*)as compared to the test group. However, treatment with Fenugreek Seeds extract mildly increased the ALT level of supplement group (111.16±4.4*) as compared to the Control group (P<0.05).

Effect of treatment on alp level of control and treated groups

Fig. 7 & table 2 shows a significant increase in ALP level of test group (571.16±7.75*) as compared to the control group. Treatment with fenugreek seeds extract reduced the ALP level of Test + Supp group (480.83±6.52*) as compared to the test group. However, treatment with Fenugreek Seeds extract mildly reduced the ALP level of supplement group (379.8±4.70*) as compared to the control group (P<0.05).

Effect of treatment on sod activity of control and treated groups

Fig. 4 & table 2 shows a significant decrease in SOD activity of test group (236.7±27.0*) as compared to the control group. Treatment with fenugreek seeds extract significantly increased the SOD activity of Test+Supp group (339.5±37.7*) as compared to the test group. However, treatment with Fenugreek Seeds extract significantly reduced the SOD activity of supplement group (286.5±23.2*) as compared to the control group (P<0.05).

Effect of treatment on catalase activity of control and treated groups

Fig. 5 & table 2 shows a significant increase in Catalase activity of test group (0.009±0.0006*) as compared to the control group. Treatment with fenugreek seeds extract decreased the catalase activity of Test + Supp group (0.009±0.0006*) as compared to the test group. However, treatment with Fenugreek Seeds extract mildly reduced the catalase activity of supplement group as compared to Control group(0.0038±0.00021*)(P<0.05).

DISCUSSION

Drugs utilized in therapy of hepatopathies are sometimes inadequate and may have severe adverse effects. Thus, it is essential to search alternative drugs for the treatment of hepatopathies in order to substitute the currently used drugs of dubious efficacy and safety (Ozbek *et al*., 2004). The lack of reliable liver safety drugs in allopathic medical practices, natural products, herbs and dietary antioxidants perform a role to manage several hepatic ailments (Bjelakovic *et al*., 2011; Buraimoh *et al*., 2011).The present study demonstrated that the maximum increases in body weight were noted in control rats. The remarkable rise in body weight of control rats may be the indication of the normal growth pattern of rodents. On the contrary, the minimum increases in body weight were observed in TAA treated rats along-with a remarkable increase in liver/body ratio. These observations were also demonstrated from other studies (Wong *et al*., 2012; Kadir *et al*., 2013; Kabiri *et al*., 2014; Zargar, 2014; Al-Attar and Shawush, 2015; Al-Attar *et al*., 2016).

TAA is a powerful hepatotoxic drug. It is metabolized from Cytochrome P450 enzyme located in the liver and is altered to oxidative chains of toxic substances called TAAS-oxide and TAAS-dioxide (Kadir *et al*., 2011). TAA is renowned to cause centrilobular hepatic necrosis, liver cirrhosis, liver cancer, and bile duct proliferation (Spira and Raw, 2000).

The plasma concentration of total bilirubin and activities of liver marker enzymes ALP, ALT and AST are well-established markers of hepatotoxicity (Hauser *et al*., 2003). An elevated concentration of total bilirubin in plasma (TBILI) and ALP activity is related to hepatobiliary damage and hepatic cholestasis (Singh *et al*., 2011). Bilirubin is located in the liver, bile, intestines and the reticuloendothelial cells of the spleen, Although, ALP is associated with the hepatic cell membrane (Cullen, 2005). The plasma total bilirubin and ALP activity are known to increase in conditions associating hepatobiliary injury and discharge of ALP from hepatocytes (Ramaiah, 2007). In addition to, serum total bilirubin, also the direct bilirubin, serves as a gold standard for hepatopathies (Honmore *et al*., 2015). In liver parenchyma cells, glucuronidation takes place where bilirubin forms conjugates with glucuronic acid in the presence of catalytic converting catalyzed enzyme glucuronyltransferase. The resultant product is secreted in bile. Therefore, an increased level of total bilirubin and direct bilirubin in serum revealed injury to hepatic parenchymal cells (Chakrapani and Satyanarayana, 2013). The presence of high plasma activities of ALT and AST is an established marker of hepatocellular damage in human and animal studies (Giannini *et al*., 2005). In this study, a marked rise in plasma ALT activity was observed indicating its discharge from damaged hepatocytes (Amacher, 1998).

Studies have suggested that few phytochemicals are found to regulate cytochrome P450 enzymes and showed the hepatoprotective effects (Chiu *et al*., 2006; Bhadauria *et al*., 2007). Fenugreek seeds are an enrich origin of many active phytochemicals like saponins, coumarin, fenugreek line, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline, which are deliberated to account many of its assumed therapeutic effects (Ahluwalia *et al*., 1996).

These results are in accordance with (Meera *et al*., 2009) they achieved that the above enzyme levels are normalized in rat's liver through the plant drugs, prove the hepatoprotective effect of T. foenum-graecum which may be able to encourage accelerated regeneration of hepatic cells by decreasing the discharge of these enzymes into circulation. The results indicated that fenugreek significantly prevented the increased liver marker enzyme activity, indicating an amelioration of liver functional status by the fenugreek.

Fenugreek seeds extract is documented to prevent the CCL_4 and H_2O_2-prompted hepatotoxicity through normalizing all the marker enzymes accelerating the regeneration of hepatic cells (Meera *et al*., 2009). (Thirunavakkuarasu *et al*., 2003) have documented that the aqueous extract of fenugreek inhibits ethanol and prompts toxicity in the liver by decreasing the activities of serum AST, ALT and ALP. Currently, (Kaviarasan *et al*., 2006) have demonstrated that the ethanolic extract of fenugreek powder inhibited ethanol-caused toxicity in chang liver cells and its preventive effect is similar to the standard hepatoprotective agent silymarin. Fenugreek Polyphenolic extract was found to prevent against ethanol-caused liver damage through normalizing the markers of liver damage (ALT, AST, ALP, LDH and GGT), enhancing hepatocyte viability and decreasing the apoptotic nuclei (Kaviarasan *et al*., 2007).

Disturbance in the balance among produced oxidants and antioxidants in favor of oxidants result in Oxidative stress. This is usually caused by an increased formation of reactive oxygen species (ROS) and the reduced activity of antioxidant systems. It might be said that the reduced levels of SOD, GPx, indicate serious injury of the liver. Therefore, activity of antioxidants and prevention of free radical formation are crucial to inhibit hepatopathies. The body has the powerful defense mechanisms to inhibit and neutralize oxidative agents. This activity is finely attained by regulating endogenous antioxidants like glutathione peroxidase, superoxide dismutase, and catalase. These enzymes form dependent support systems contrary to ROS. In normal conditions, the antioxidant system detoxified the ROS which has both enzymatic and non-enzymatic antioxidants. SOD is an especial antioxidant for the modulation of anyone superoxide to hydrogen peroxide which is detoxified by catalase (Singh *et al*., 2014). These enzymes are located in the peroxisomes of

almost all aerobic cells. Decreased superoxide dismutase (SOD) enzyme activity is a susceptive marker of hepatocellular injury (Palanivel *et al*., 2008). CAT prevents the cells from the harmful effects of hydrogen peroxide via catalyzing into molecular oxygen and water without the formation of reactive species (Nehru and Anand, 2005). TAA is a renowned hepatotoxicity, hepatotoxicity as a result of its metabolic alteration to free radical products: thioacetamide sulfoxide and thioacetamide-S, S-dioxide which damages microsomal lipids resulting in their peroxidation and formation of reactive oxidative agents like H_2O_2, superoxide anion O_2^- and the hydroxyl radical. Reactive oxidative agent affects the antioxidant defense mechanism, diminishes the SOD activity which causes liver damage, development of cirrhosis, and liver cancer (Poli, 2000).

Polyphenol and flavonoids have been called as hepatoprotective plants (Sharma *et al*., 2012). Fenugreek is an enriched source of a variety of flavonoids that impart the significant antioxidant potentiality to it. (Gupta and Nair., 1999) have reported that fenugreek seeds are rich in flavonoids (>100mg/g). Later (Shang *et al*., 1998) have isolated five different flavonoids namely, vitexin, tricin, naringenin, quercetin and tricin 7-O-bD-glucopyranoside from fenugreek seeds. In the present study the fenugreek seeds extract revealed remarkable antiradical and anti-oxidative properties. The quenching activity of phenolic substances is accredited to the active hydrogen-donating potentiality to the hydroxyl substitutions (Bors *et al*., 1996). The presence of phenolic groups in the phytochemicals mainly naringenin and quercetin in fenugreek seeds might be accountable for $^{-}$OH radical quenching activity (Kaviarasan *et al*., 2007). In their study, consumption of fenugreek seed powder resulted in reduced catalase activity and increased superoxide dismutase activity following the H_2O_2 generation (Sakr and Abo-El-Yazid, 2012). (Mitchell *et al*., 1973) revealed that fenugreek seeds are rich in polyphenols which encourage the antioxidant capability (Mitchell *et al*., 1973). Polyphenols, particularly flavonoids, are among the most effective herbal antioxidants. Polyphenols can form complexes with reactive metals such as iron, zinc and copper and decrease their absorption (Nwanna and Oboh, 2007). Moreover, polyphenols function as quenching free radicals or neutralize these elements (Ara and Nur, 2009). These results were in line with decreased oxidative stress documented in other studies and revealed the fenugreek seed powder could remarkably ameliorate antioxidant activities in thioacetamide-prompted hepatotoxicity and inhibit the enhanced lipid peroxidation in rats (Zargar, 2014). The main flavonoid quercetin, it is present in FGS was shown a preventive activity against CCl_4 prompted hepatotoxicity (Pavanato *et al*., 2003).Thus, the present study confirmed hepatoprotective role of fenugreek seeds in thioacetamide induced liver toxicity.

CONCLUSION

Fenugreek seeds extract successfully attenuates thioacetamide induced liver damage by restoring altered levels of liver biomarkers and tissue antioxidant enzymes.

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