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Influence of Dietary Supplementation of Fenugreek (*Trigonella foenum-graecum* L.) on Serum Biochemical Parameters of Rats Fed High Cholesterol Diet

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ABSTRACT

Thirty-two rats were divided into four groups (8 rats/each) named as 1, 2, 3 and 4. Group 1 kept on basal diet and served as a control group. Whereas, group 2 kept on the basal diet mixed with 1% cholesterol (high cholesterol diet). Groups 3 and 4 kept on the high cholesterol diet mixed with Fenugreek seed powder 5 and 10%, respectively. Total cholesterol, low-density lipoprotein cholesterol (LDL-c) and alanine transaminase (ALT) activity were significantly increased (32.4±0.2; 12.2±1.5 mg dL^{-1}; 26.5±0.6 IU L^{-1}) whereas, high-density lipoprotein cholesterol (HDL-c) concentration was decreased significantly (11.9±1.6 mg dL^{-1}) in rats fed high cholesterol diet compared to control group (28.1±0.1; 6.0±1.3; 22.7±0.4; 13.9±1.5), respectively. Inclusion of both concentrations of Fenugreek in high cholesterol diet reduced serum total cholesterol (30.5±0.1; 29.5±0.5) and ALT activity (22.1±0.5; 21.7±0.7), respectively compared to rats fed high cholesterol diet and control groups. Histopathological findings revealed fatty changes in liver of rats fed high cholesterol diet which recovered by dietary supplementation of Fenugreek seed powder. Only, inclusion of lower concentration of Fenugreek in diet of rats fed high cholesterol reduced LDL-c concentration (6.2±1.2) and increased HDL-c concentration (15.0±1.0) compared to rats fed high cholesterol diet and the control group. Conclusively, inclusion of low dose of Fenugreek (5%) in ration of rats was recommended than the high dose (10%).

Key words: Biochemistry, serum, lipoproteins, Fenugreek, enzymes

INTRODUCTION

Hyperlipidemia is the main risk factor for development of atherosclerosis and heart attack (Wang *et al*., 1997). Atherosclerosis is caused by accumulation of cholesterol or cholesterol esters in intema of large and medium sized arteries as abdominal aorta, coronary and cerebral arteries (Yoshikawa *et al*., 1997) forming atheroma (Varshney and Sharma, 1996). The atheroma causes narrowing of arterial lumen, damage to underlying tissues, ulceration and calcification (Goldstein and Brown, 1990). Successful trials were exhibited to reduce mortality caused by chronic heart diseases by lowering serum cholesterol levels (McNamara, 2000). Synthetic hypolipidemic

drugs are used extensively with recognized effect. However, high price and side effects are the main reasons for limitation of its uses (Thomas, 2003). Therefore, natural products are the safe and cheap alternatives.

The leaves and seeds of Fenugreek (*Trigonella foenum graecum*) were used during Hippocrates and ancient Egyptian times (Jensen, 1992) for preparation of extract and powder, respectively for medicinal purposes (Muralidhara *et al*., 1999; Al-Sultan and El-Bahr, 2015). Trigonellin and saponins were recorded as the main active ingredients of Fenugreek (Ribes *et al*., 1987; Sauvaire *et al*., 1991). Fenugreek has been reported in literature as antihyperlipidemic (Al-Habori *et al*., 1998; Basch *et al*., 2003; Shrivastava *et al*., 2009). In addition, the hypocholesterolemic effect of Fenugreek was reported in rats (Sharma, 1984, 1986; Awal *et al*., 1999; Moosa *et al*., 2006), dogs (Valette *et al*., 1984) and humans (Madar and Odes, 1990; Sharma *et al*., 1990). Moreover, the hypoglycemic effect of Fenugreek was also documented (El-Soud *et al*., 2007; Basch *et al*., 2003). Parallel with the recent increased interest in alternative/herbal medicine for the prevention and treatment of various illnesses including hypercholesterolemia and because Fenugreek is used daily by many people, therefore, this study is undertaken to evaluate scientifically the effect of feeding two doses of the whole Fenugreek seeds powder mixed with high cholesterol diet on selected biochemical parameters with special references to lipid and lipoproteins profile using rats as experimental animals.

MATERIALS AND METHODS

Kits, chemicals and plant: Diagnostic kits for serum total proteins, albumin, total lipid, triglyceride, total cholesterol, High-density Lipoprotein Cholesterol (HDL-c), Low-density Lipoprotein Cholesterol (LDL-c), Very Low-density Lipoprotein Cholesterol (VLDL-c), Alanine Aminotransferase (ALT) and Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), urea, uric acid and creatinine were purchased from ELIPSE, United diagnostic industry, UDI, Dammam, Saudi Arabia). Pure cholesterol (Cat# C3045) was purchased from Sigma-Aldrich, USA. Routine chemicals and solvents used in the study were of highest grade and commercially available. Fenugreek seeds were purchased from local market at Al-Ahsa, Saudi Arabia. The seeds were identified by botanists of College of Agricultural Sciences, King Faisal University, Saudi Arabia.

Animals and treatment: A total of thirty two albino rats (200-250 g) were obtained from the Laboratory House of College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia and acclimatized for 10 days before starting the experiment. All animals were housed in standard cages, fed with standard laboratory diet and tap water *ad libitum*. The experimental animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity and kept on a 12h light/12 h dark cycle. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by ethics of Scientific Research Committee of King Faisal University, Saudi Arabia.

Induction of hypercholesterolemia: One gram of pure cholesterol powder was added to each 99 g of basal diet (1%) except the control for induction of hypercholesterolemia according to Sharma (1984) and Pandya *et al*. (2006).

Experimental groups and protocol: Rats were fed on standard diet and divided randomly into 4 groups (named as 1, 2, 3 and 4), comprising 8 rats in each group.

Group 1: Rats fed the basal diet without any additives and served as a control group
Group 2: Rats fed the high cholesterol diet 1% (1 g/99 g of basal diet) (Sharma, 1984; Pandya *et al*., 2006)
Group 3: Rats fed the high cholesterol diet mixed with Fenugreek seed powder 5% (5 g/95 g cholesterol diet) (Helmy, 2011)
Group 4: Rats fed the high cholesterol diet mixed with Fenugreek seed powder 10% (10 g/90 g cholesterol diet); double to the dose used by Helmy (2011)

Samples collection: Blood samples were collected after two weeks following treatment so as to confirm the induction of hypercholesterolemia. At the end of the experiment, the animals were overnight fasted were sacrificed under light ether anesthesia. Blood samples were collected by cardiac puncture and 5 mL of blood samples were received in plain vacutainers. Sera were harvested and stored at -20°C until time of analysis. Liver tissues were collected also and cut in small pieces and immersed in neutral buffered formalin for 24 h for histopathological examination.

Biochemical analysis: Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of total proteins (EP56-660), albumin (EP03-570), Glucose (EP37L-660), ALT (EP07-500), AST (EP15-500), creatine kinase, CK (EP28-310), BUN (EP20-420), uric acid (EP61-620), creatinine (EP33K-660), TAG (EP59-660), cholesterol (EP24-660), HDL-c (EP41HD), calcium (EP22-660), phosphorus (EP46-660) magnesium (EP50-660) and chloride (EP27-500) on ELIPSE full automated chemistry analyzer (Rome, Italy). The concentrations of the biochemical constituents were calculated according to the manufacture instruction. Very Low Density Lipoprotein Cholesterol (VLDL-c) was calculated by division of TAG by 5 while LDL-c level was calculated by subtracting the values of sum HDL-c and VLDL-c from total cholesterol value (Bauer, 1982).

Histopathological examination: The fixed liver tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques (Bancroft and Gamble, 2002). The effect of high cholesterol diet induced fatty degeneration was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E), using standard techniques.

Statistical analysis: All data was presented as Mean±Standard Error of Mean by using student t-test. All tests were performed using computer package of the statistical analysis system (SAS., 2002).

RESULTS

Biochemical analysis: The effect of Fenugreek on glucose, total proteins and lipid profile of rats fed high cholesterol diet and treated with two doses of Fenugreek (5 and 10%) are presented in Table 1. These findings indicated that, total cholesterol was increased in the rats fed high cholesterol diet (32.4±0.2 mg dL^{-1}) compared to the control group (28.1±0.1). However, inclusion of Fenugreek in the high cholesterol diets (5 and 10%) reduced serum total cholesterol (30.5±0.1; 29.5±0.5), respectively compared to the rats fed high cholesterol diet (32.4±0.2) and toward the normal control values (28.1±0.1). These findings indicated that, triacylglycerol was increased in rat fed high cholesterol diet (41.6±1.4 mg dL^{-1}) compared to the control group

Table 1: Glucose, protein and lipid profiles of rats fed high cholesterol diet and treated with two doses of Fenugreek (5 and 10%)

	Groups			
Parameters	1	2	3	4
Glucose (mg dL^{-1})	93.2±25	120.0±25	85.3±25	108.7±19
Total proteins (g dL^{-1})	4.7±0.5	4.5±0.4	4.6±0.4	4.9±0.4
Albumin (g dL^{-1})	3.4±0.3	3.5±0.5	3.5±0.3	3.5±0.3
Globulins (g dL^{-1})	1.3±0.1	1.0±0.2	1.1±0.1	1.4±0.4
Triglycerides (mg dL^{-1})	40.9±1.6	41.6±1.4	40.7±2.2	40.5±2.0
Total cholesterol (mg dL^{-1})	28.1±0.1	32.4±0.2*	30.5±0.1**	29.5±0.5**
HDL-c (mg dL^{-1})	13.9±1.5	11.9±1.6*	15.0±1.0**	11.9±1.5*
LDL-c (mg dL^{-1})	6.0±1.3	12.2±1.5*	6.2±1.2**	10.5±2.0*
VLDL-c (mg dL^{-1})	8.2±1.2	8.3±1.1	9.3±1.1	8.1±1.0

Each value represents the Mean±Standard Deviation of 8 rats. HDL-c: High-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol, VLDL-c: Very low-density lipoprotein cholesterol, *Mean values are significantly ($p<0.05$) different compare to the control (group 1). **Mean values are significantly ($p<0.05$) different compare to cholesterol treated rats (group 2). Group 1: Rats fed basal diet and served as control group, Group 2: Rats fed basal diet mixed with cholesterol powder 1%, Group 3: Rats fed basal diet mixed with cholesterol powder 1 and 5% Fenugreek seed powder. Group 4: Rats fed basal diet mixed with cholesterol powder 1 and 10% Fenugreek seed powder

Table 2: Liver function biomarkers of rats fed high cholesterol diet and treated with two doses of Fenugreek (5 and 10%)

	Groups			
Parameters (IU L^{-1})	1	2	3	4
ALT	22.7±0.4	26.5±0.6*	22.1±0.5**	21.7±0.7**
AST	71.4±5.1	64.4±4.9	70.0±4.0	68.5±5.0
CK	510.4±10.1	493.0±11.2	505.0±10.1	496.8±10.1

Each value represents the Mean±Standard Deviation of 8 rats. ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, ACP: Acid phosphatase, CK: Creatine kinase, *Mean values are significantly ($p<0.05$) different compare to the control (group 1). **Mean values are significantly ($p<0.05$) different compare to cholesterol treated rats (group 2). Group 1: Rats fed basal diet and served as control group, Group 2: Rats fed basal diet mixed with cholesterol powder 1%, Group 3: Rats fed basal diet mixed with cholesterol powder 1 and 5% Fenugreek seed powder, Group 4: Rats fed basal diet mixed with cholesterol powder 1 and 10% Fenugreek seed powder

(40.9±1.6). However, inclusion of Fenugreek in the high cholesterol diets (5 and 10%) did not affect serum triacylglycerol level (40.7±2.2; 40.5±2.0), respectively compared to the rats fed high cholesterol diet (41.6±1.4 mg dL^{-1}) and toward the normal control values (40.9±1.6). The data presented in Table 1 also indicated that, serum HDL-c concentration was decreased (11.9±1.6 mg dL^{-1}) and LDL-c concentration was increased (12.2±1.5 mg dL^{-1}) in rats fed the high cholesterol diet, compared to control groups (13.9±1.5; 6.0±1.3), respectively. Interestingly, the low dose of Fenugreek (5%) induced elevation of HDL-c concentration only (15.0±1.0) and reduction of LDL-c (6.2±1.2) compared to its concentration in serum of rats fed the high cholesterol diet (11.9±1.6; 12.2±1.5) towards the control group (13.9±1.5; 6.0±1.3). Whereas, the values of total proteins, albumin, glucose and VLDL-c concentrations were non-significantly affected in all experimental groups. As shown in Table 2, ALT activity was increased in the rats fed the high cholesterol diet (26.5±0.6 IU L^{-1}) compared to the control groups (22.7±0.4). However, inclusion of Fenugreek in the high cholesterol diets (5 and 10%) reduced serum ALT activity (22.1±0.5; 21.7±0.7), respectively compared to the rats the fed high cholesterol diet (26.5±0.6) and toward the normal control values (22.7±0.4). The activities of AST and CK remained unchanged in all experimental groups. As presented in Table 3, kidney functions were not disturbed in all experimental groups as reflected by the estimated values of BUN, uric acid, creatinine. Electrolytes concentrations (calcium, phosphorus, magnesium and Chloride) were not changed in all experimental groups compared to the control (Table 4).

Table 3: Kidney markers of rats fed high cholesterol diet and treated with two doses of Fenugreek (5 and 10%)

	Groups			
Parameters (mg dL^{-1})	1	2	3	4
BUN	8.0±1.3	7.0±1.2	7.0±1.2	8.0±1.1
Creatinine	0.2±0.1	0.1±0.1	0.2±0.1	0.2±0.1
Uric acid	1.0±0.1	1.1±0.1	1.0±0.1	0.9±0.1

Each value represents the Mean±Standard Deviation of 8 rats. BUN: Blood urea nitrogen, Group 1: Rats fed basal diet and served as control group, Group 2: Rats fed basal diet mixed with cholesterol powder 1%, Group 3: Rats fed basal diet mixed with cholesterol powder 1 and 5% Fenugreek seed powder, Group 4: Rats fed basal diet mixed with cholesterol powder 1 and 10% Fenugreek seed powder

Table 4: Electrolytes profile of rats fed high cholesterol diet and treated with two doses of Fenugreek (5 and 10%)

	Groups			
Parameters	1	2	3	4
Calcium (mg dL^{-1})	5.1±0.5	5.5±0.4	4.9±0.4	5.2±0.4
Phosphorus (mg dL^{-1})	2.3±0.5	2.8±0.5	2.8±0.5	2.8±0.5
Magnesium (mg dL^{-1})	4.0±0.3	4.4±0.2	4.1±0.2	4.0±0.3
Chloride (mEq L^{-1})	44.4±5.0	53.9±6.0	47.2±4.0	48.6±3.0

Each value represents the Mean±Standard Deviation of 8 rats. Group 1: Rats fed basal diet and served as control group, Group 2: Rats fed basal diet mixed with cholesterol powder 1%, Group 3: Rats fed basal diet mixed with cholesterol powder 1 and 5% Fenugreek seed powder. Group 4: Rats fed basal diet mixed with cholesterol powder 1 and 10% Fenugreek seed powder

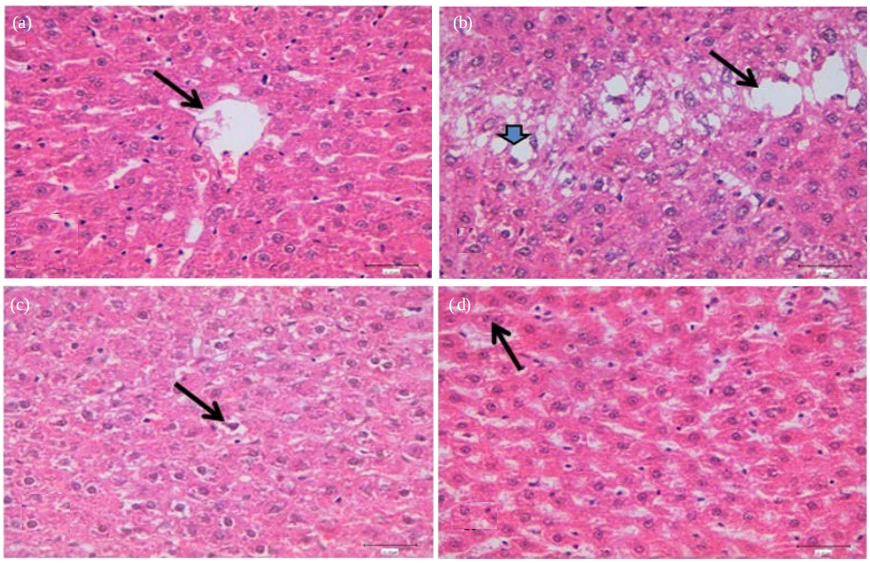

Fig. 1(a-d): Histopathological examination of rat's liver (a) Liver of control rats showing normal central vein and regular hepatic cords (arrow). HE bar = 40 µm. (b) Liver of cholesterol treated rats showing fatty degeneration (arrowhead) and fatty cysts (arrow). HE bar = 40 µm (c) Liver of Fenugreek 5% treated rats showing mild vacuolar degeneration of hepatocytes (arrow). HE bar = 40 µm and (d) Liver of Fenugreek 10% treated rats showing regenerating hepatocytes with binuclear cells (arrow). HE bar = 40 µm

Histopathological examination: Liver of the control rats showed central veins surrounded by polygonal cells arranged in regular cords separated from each other by sinusoids (Fig. 1a). Liver of the cholesterol treated rat showed varying degrees of vacuolar degeneration and differences in size, shape and staining affinity. The majority of vacuolar degeneration was fatty degeneration which extended to outward appearance of fatty cysts (Fig. 1b). The resulting irregularity of the liver cell plates is termed lobular disarray. Liver of the rats received high cholesterol diet and treated

with Fenugreek seed powder 5% showed moderate degree of recovery, in which the foremost hepatocytes still revealed mild degree of vacuolar degeneration (Fig. 1c). The remaining hepatocytes display a larger nucleus and an expanded eosinophilic cytoplasm (signs of regeneration). Liver of rats received high cholesterol diet and treated with Fenugreek seed powder 10% showed complete degree of recovery, in which the majority of hepatocytes display signs of regeneration, a large nucleus, mitotic figures and binuclearity (Fig. 1d). Concomitantly, the liver cell plates restored their architecture.

DISCUSSION

Hyperlipidemia is characterized by elevated level of triacylglycerol and cholesterol and it is the leading factor for atherosclerosis and heart diseases. Medicinal plants provides valuable therapeutic agent in traditional and modern times. The investigation of hypolipidemic activity of medicinal plants could be a valuable strategy in the discovery of new safe and cheap drugs. Fenugreek were used as antihyperlipidemic (Al-Habori *et al*., 1998; Basch *et al*., 2003; Shrivastava *et al*., 2009), antihypercholesterolemic (Sharma *et al*., 1990; Awal *et al*., 1999; Moosa *et al*., 2006) and antidiabetic (Basch *et al*., 2003; El-Soud *et al*., 2007). However, publications regarding the effect of different levels of seeds powder as feed additives are lacking. Therefore, the current study has been undertaken to investigate the effect of Fenugreek seeds powder as feed additives on biochemical parameters and histopathological picture with special reference to lipid profile of rats fed high cholesterol diet. The present study reported that, there were no significant differences in serum total proteins, albumin, globulins and glucose of all experimental groups. The current findings disagree with previous report (Chauhan *et al*., 2012; Korish and Arafah, 2013) which demonstrated that, rats fed high fat and cholesterol diet exhibited significant increase in serum glucose concentration. Moreover, other reports stated by Eidi *et al.* (2007) and Xue *et al.* (2007) demonstrated that, administration of ethanolic and aqueous extracts of Fenugreek, respectively decreased serum glucose level in diabetic rats. This conflict might be attributed to the different dose, plant preparation, route of administration and time of the experiment. Until now, no literature demonstrated the effect of Fenugreek seed powder or extract on serum proteins (total, albumin and globulin). The significant increase of total cholesterol and LDL-c concentrations and significant decreased of HDL-c concentrations in rats fed high cholesterol diet compared to the control group observed in the current study is in accordance with Al Hamedan (2010), Chauhan *et al*. (2012), Belguith-Hadriche *et al*. (2013) and Korish and Arafah (2013) in rats fed on the same cholesterol diet. In line with the present study, recent report (Belguith-Hadriche *et al*., 2013) demonstrated that, only the ethyl acetate extract of Fenugreek reduced total cholesterol and LDL-c concentration and increased HDL-c concentration in rats fed high cholesterol diet. Additionally, other reports (Eidi *et al*., 2007; Xue *et al.,* 2007) demonstrated that, administration of ethanolic and aqueous extracts of Fenugreek, respectively decreased triglycerides, total cholesterol, LDL-c and increased HDL-c concentrations in diabetic rats. Previous reports (Elmnan *et al.,* 2012) also reported that, Fenugreek seeds powder reduced total cholesterol and LDL-c concentration and increased HDL-c concentration in dose dependent manner (0.25-0.75%) in rats. In addition, other report (Moosa *et al*., 2006) demonstrated that oral administration of 25 g of Fenugreek seed produced significant reduction of total cholesterol and LDL-c without any changes in HDL-c concentration in hypercholesterolemic patients. The hypocholesterolemic effect of Fenugreek might be attributed to significant content of saponins which are known to have

hypocholesterolemic effect (Sharma, 1986; Sharma *et al*., 1990; Al-Habori *et al*., 1998). It is well known that, saponins form insoluble complex with lipids (Rao *et al*., 1996). Therefore, It has been suggested that, saponins prevent the absorption of lipid either directly in the intestine or bind to bile acids during enterohepatic reabsorption mechanism (Madar and Odes, 1990). The crude fibers contents of Fenugreek seeds are one of the most hypocholesterolemic agent (Madar and Odes, 1990; Elmnan *et al.,* 2012) because the presence of soluble fiber may block cholesterol absorption from the intestine (Lanksy *et al*., 1992). It is well known that, protein quality and quantity plays an important role on cholesterol levels. Crude fiber and saponins contents of Fenugreek seed were found to reduce cholesterol absorption (Malinow *et al*., 1977). Plants proteins decreased the cholesterol level and exerts a lipid lowering capacity (Sharma, 1986). The current study demonstrated that, high cholesterol in diet did not change the triglyceride concentration in rats. These findings disagree with the previous reports (Das *et al*., 1997; Al Hamedan, 2010; Chauhan *et al*., 2012; Korish and Arafah, 2013) demonstrating that high fat and cholesterol diet induced hyperlipidemia which is related to the enhanced de-esterification of the abundant free fatty acids and decreased lipoproteins (Jensen, 2008). Biochemical changes and alterations in enzyme activities induced a stress on liver function. In the present study, there was an increase in ALT activity of rats fed the high cholesterol diet compared to the control. The supplementation of Fenugreek in the high cholesterol diet caused a reduction in these enzyme activity compared to the cholesterol fed rats. Based on this result, the present study can argue that Fenugreek may have hepatoprotective effect. In line with this result, recent work (Murugesan *et al.,* 2012) demonstrated that, oral administration of Fenugreek improved liver function toward normal values in rats that disturbed by isoproterenol. Also, administration of ethanolic extract of Fenugreek decreased ALT and AST activities in streptozotocin-induced diabetic rats (Eidi *et al*., 2007). Hepatoprotective effect of Fenugreek was supported by histopathological findings which revealed fatty changes in liver of rats fed high cholesterol diet and significant recovery when Fenugreek seed powder has been administered. The current histopathological findings (Fig. 1) regarding the effect of high cholesterol diet on liver tissues is in accordance with previous work of Korish and Arafah (2013), in human. To the authors information, the current study demonstrated the first histopathological report regarding the protective effect of Fenugreek on hepatic fatty changes induced by administration of high cholesterol diet in rats. The kidney function was not affected in all rats of all experimental groups as reflected by the unchanged values of BUN, creatinine and uric acid. However, urea, uric acid and creatinine values were decreased when Fenugreek ethanolic extract was administered orally in streptozotocin diabetic rats (Eidi *et al*., 2007). Electrolytes concentrations (calcium, phosphorus, magnesium and Chloride) were not changed in all experimental groups compared to the control (Table 4). The effect of either Fenugreek or cholesterol on electrolytes concentrations was not mentioned before.

CONCLUSION

The present study demonstrated that, inclusion of Fenugreek in the high cholesterol diets improved liver function of rats as indicated by biochemical and histopathological analysis. The hypocholesterolemic effect of Fenugreek was conducted at lower concentration (5%), however higher concentration of the seed was not beneficial. Therefore, inclusion of Fenugreek in the ration of rats above 5% as hypocholesterolemic drug is not recommended.

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