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Glycemic Reaction of Glimepiride Combined with Popular Egyptian Antidiabetic Drinks of Fenugreek and Coffee in Diabetic Rats

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Abstract: Sulfonylureas (SUs) are valuable in the treatment of diabetes mellitus, when it is mixed with herb each can alter the others pharmacokinetics profile. The interaction can be beneficial or sometimes contraindicated. The study aimed to investigate the interaction of some dietary drinks with respect to their traditional uses, to that of the antidiabetic drug as glimepiride. Seventy adult male albino rats divided into main two groups, ten rats were considered as a negative control group (NCG) and the others were administered a single dose of Alloxan (120 mg/kg body weight) that induced with diabetes, then divided into the following groups, positive control group (PCG), glimepiride group (GG) were administered orally antidiabetic drug as glimepiride (4 mg/kg orally once daily). Another diabetic rats given each different drinks plus antidiabetic agent glimepiride, which administered orally (by stomach tube) for 30 days. Rats were divided according to the type of drink given into fourth subgroups, as follow: glimepiride plus 0.5 g fenugreek group (GFG1), glymepiride plus 1 gm fenugreek group (GFG2), glymepiride plus 0.5 gm coffee group (GCG3) and glymepiride plus 1 gm coffee group (GCG4). Serum total cholesterol, triglycerides, HDLc, VLDLc, LDLc, ALT, AST, ALP, urea, creatinine, albumin, glucose and insulin was estimated. The results illustrated that after 6 weeks of experimental duration, the mean comparable in both GG and GFG1 to NCG had insignificant change for FBG (98.33±2.52, 104.90±11.62 and 97.27±11.91, respectively), whereas for other treated groups had been a significant decline. While at long period, GFG2 had the best effect that did not differ significantly to another studied drink at different doses as compared to GG and GFG1. It was concluded that the inter-positive effect between antidiabetic glimepiride and fenugreek or coffee drink on glycemic profiles was stated.

Key words: Sulfonylureas, diabetes mellitus, dietary drinks, antidiabetic drug

INTRODUCTION

Diabetes is a chronic disorder associated with high blood glucose level, either due to less production of insulin by the pancreas or due to inability of body cell to respond to the insulin produced. Based on this, there are two types of diabetes: Type I and Type II. The pathophysiology of type 2 diabetes mellitus (T2DM) is characterized by relative decrease in insulin secretion and/or insulin resistance (Fujimoto, 2000). In 2014 the global prevalence of diabetes* was estimated to be 9% among adults aged 18+years (WHO, 2012). WHO projects that diabetes will be the 7th leading cause of death in 2030 (Mathers and Loncar, 2006). The United Kingdom Prospective Diabetes Study (UKPDS) showed that ≥50% loss of β cells had occurred by the time of diagnosis; therefore, β cell secretagogues are useful for achieving sufficient glycemic control (Endocrinologic and Metabolic Drugs Advisory Committee, 2013). Sulfonylureas (SUs) are widely used in the management of T2DM as insulin secretagogues and are named for their common core configuration. They are classified as first-and second-generation SUs. Glimepiride is the newest second-generation SUs and is sometimes classified as a third-generation SUs because it has larger substitutions than other second-generation SUs. The United States Food and Drug Administration (FDA) approved glimepiride for the treatment of T2DM as monotherapy as well as in combination with metformin or insulin. Treatment with glimepiride as monotherapy results in a 1.5-2.0% reduction in HbA1c (Shukla *et al*., 2004). Glimepiride may be taken either immediately before breakfast or 30 min after breakfast with similar results (Overkamp *et al*., 2002). Maximal glucose-lowering activity and insulin level in T2DM patients is achieved within 2-3 h of taking glimepiride and can last for 24 h (Massi-Benedetti, 2003).

Although SUs are valuable in the treatment of diabetes mellitus, their use is restricted by their limited action, pharmaco-kinetic properties, secondary failure rates and accompanying side effects. So many people often combine the herbal remedies with oral hypoglycemic agent. Trigonellafoenum-graecum is one such plant that has been extensively used as a source of antidiabetic compounds in different model systems (Rai *et al*., 2012). Studies on different experimental models have proved that fenugreek has strong antidiabetic properties (Kumar *et al*., 2012). Human studies have also confirmed the glucose and lipid-lowering ability of

fenugreek. A considerable increment of the area of insulin immuno reactive cells has been observed (Hamden *et al*., 2010). The therapeutic potential of fenugreek is primarily due to the presence of saponins, 4-hydroxyisoleucine and trigonelline, an alkaloid and a high-fiber content (Vats *et al*., 2002). Coffee is a highly popular drink that is traditionally used to complement meals, as well as for hedonistic and psycho stimulant purposes. It is estimated that throughout the world, 80% of the adult population consume coffee beverages (Sridevi *et al*., 2011). Epidemiological studies have suggested that regularly drinking coffee prevents chronic diseases and especially metabolic disorders, such as type 2 diabetes. Previous results have shown that coffee had antidiabetic effects and suggested that it could ameliorate hyperglycemia by improving the insulin resistance. When the drug is taken orally it travels through the digestive system in mostly the same way as food and herbs taken. So, when it is mixed with herb, each can alter the others pharmacokinetics profile. The interaction can be beneficial or sometimes contraindicated also. This paper is an attempt to outline the interaction of herbal medicine with respect to their traditional uses, to that of the antidiabetic drug as glimepiride.

MATERIALS AND METHODS

Fenugreek: In the present study, fenugreek seeds (100 g) were washed in distilled water repeatedly, then put into 1000 ml distilled water and boiled for 0.5 h to obtain the decoction. Then the whole content, decoction and seeds were minced. The mixture was cooled for 1 h at room temperature till used for the following experiment.

Coffee preparation: The coffee was prepared in the traditional fashion by using two tablespoons of freshly ground medium roasted coffee beans (20 to 25 g) per 90 to 100 ml distilled water. This was left to boil for three minutes, a moderately low heat is used so that the coffee does not come to the boil too quickly, left at room temperature for two minutes then served with no additives such as sugar or cardamom. This method of preparation accords with the common style of making homemade coffee. Each samples were subjected to chemical analysis in order to determine: Moisture, protein, fat, fiber, ash and chromium according to Jorhem method (2000). Total phenols content and flavones also were determined in addition to the identification of phenolic compounds by HPLC according the folinciocalteu method of Meda *et al*. (2005).

Experimental design: Seventy adult male white albino rats, Sprague Dawley Strain, 8 weeks age, weighing (100±10 g) were used in this experiment, were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt. All rats were fed on basal diet (casein diet) prepared according to American Institute of Nutrition (AIN) (1993) for 7 consecutive days. After this adaptation period, ten rats were considered as a negative control group (NCG) and the others were induced with diabetes (It was induced by administered rats a single dose of Alloxan, that was obtained from SIGMA Chemical Co., Cairo, Egypt, 120 mg/kg body weight) that divided into the following groups (ten rats in each group), Positive control group (PCG) was fed on the standard diet during the study period. Glimepiride group (GG), the diabetic rats were fed on the standard diet and administered orally antidiabetic drug as Glimepiride. Glimepiride was provided as Diabenor drug (1 tablet contain 3 mg Glimepiride), an oral antidiabetic drug for NIDDM, that was procured purchased from famous pharmacy. Glimepiride was administered as suspension in freshly prepared 0.5% w/v carboxy methyl cellulose sodium salt. Glimepiride was administered as (4 mg/kg orally once daily) a treatment of diabetic rats (Haritha *et al*., 2013). Another diabetic rats given each different drinks plus antidiabetic agent glimepiride, which administered orally (by stomach tube) for 30 days. Rats were divided according to the type of drink given into fourth subgroups, as follow: glimepiride plus 0.5 g fenugreek group (GFG1), glymepiride plus 1 gm fenugreek group (GFG2), glymepiride plus 0.5 gm coffee group (GCG3) and glymepiride plus 1 gm coffee group (GCG4).

Blood sampling: After fasting for 12 h, blood samples in initial times and after three weeks were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of experiment after four weeks. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 30 min, then centrifuged for 10 min at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen at -20°C till analysis.

Biological evaluation: During the experimental period the net food intake was daily recorded, while body weight was weekly recorded. The net food intake and gained body weight were used for the calculation of feed efficiency ratios (FER) as follow:

$$\text{FER (\%)} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}} \times 100$$

Biochemical analysis: Serum total cholesterol was determined according to the colorimetric method described by Thomas (1992), Serum triglycerides was determined by enzymatic method using kits according to the Fossati (1982), HDLc was determined according to the method described by Lopez (1977). VLDLc was calculated in mg/dl according to Lee and Nieman (1996) using the Friedwald's formula:

$$\text{VLDLc (mg/dl)} = \frac{\text{Triglycerides}}{5}$$

LDLc was calculated in mg/dl according to Lee and Nieman (1996) as follows: LDLc (mg/dl) = Total cholesterol-HDL-c-VLDL-c. Determination of serum ALT was carried out according to the method of Satoh and Clinica Chemica Acta (1978). Determination of serum AST was carried out according to the method of Hafkenscheid (1979). Determination of serum ALP was carried out according to the method of Moss (1982). Urea was determination by enzymatic method according to Patton and Crouch (1977). Serum creatinine was determined according to the method described by Henry (1974). Determination of albumin in serum was carried out according to the method by Henry (1974). The principle use of glucose determination according to Trinder (1969). Insulin was estimated according to Wilson and Miles (1977).

Statistical analysis: Data were expressed as mean±SD and were compared using One-way analysis of Variance (ANOVA) and Student's t-test to detect differences between groups. The significance levels were tested at $p<0.05$.

RESULTS

Chemical composition of fenugreek seeds and coffee powder as illustrated in Table 1 indicated that protein was existed in suitable amount 26.76±0.04 g/100 g while in coffee was 12.27±0.5 g/100 g. Fenugreek had been a high content of carbohydrate 56.27 g/100 g and more higher amount of fiber 6.75±0.03, while there was limited amount of moisture and fat either in fenugreek seeds or coffee.

Also as shown in Table 2, chromium was determined by mean 38.52±0.71 in fenugreek seeds. Total phenolic compounds were higher valued in fenugreek seeds than coffee by 1421.67±10.50 and 665.88±5.38 in contrast of flavones 7.32±0.04 and 9.42±0.06, respectively.

It is clear from Table 3 that attributed to dietary supplement with fenugreek and coffee accompanied with diabetic drug, the BWG improved dramatically in GFG1 (12.64±1.35) that did not differ significantly than NCG (15.59±4.74). Also, there was did not change between the least dose of coffee and group which have administered glimepiride only by 8.05±1.35 and 7.16±1.80, respectively.

Fasting blood glucose in diabetic rats represents an important indicator of diabetic status. As illustrated in Table 4, the inter-positive effect between antidiabetic glimepiride and fenugreek or coffee drink on glycemic profiles was stated. Compared with PCG, the fasting blood glucose level was declined significantly in twice studied drinks. Non significant was obtained among GG, GFG1 and GFG2 and GCG4 after 3 weeks of experimental duration. While, GCG3 had the lowest significant improve in diabetic rats by mean (162.37±3.76 mg/dL). Accordingly, after 6 weeks of administered different drinks, it was found that the mean comparable in both GG and GFG1 had insignificant change to the NCG (104.90±11.62, 97.27±11.91 and 98.33±2.52, for FBG, respectively), whereas for other treated groups had been a significant decline.

By evaluating the effects of adding a second antihyperglycemic dietary agent to sulfonylurea therapy for patients with type 2 diabetes, it has been determined an overall improvement of IH level as illustrated in Table 5. Non clear statistically significant differences was found in the IH level among GG, GFG1 and GFG2 after 3 weeks. While at long period, GFG4 was recorded the best affect that did not differ significantly to another studied drink at different doses as compared to GG and GFG1.

Regarding to liver enzymes, the concentration of AST and ALT was revealed a significant decrease at $p<0.05$ among treated groups as compared to PCG. In response to dietary supplementation combined with antidiabetic drug, GG had the nearest insignificant level to NCG (81.23±3.33 and 84.66±4.37 U/L, respectively) for AST. Likewise, ALT level did not differ significantly among treated groups as compared to NCG except PCG. Otherwise, GCG4 had the lowest significant mean value (62.73±2.62 and 23.23±1.01 U/L) for AST and ALT, respectively. From the same table, it could be noticed that renal profiles were improved. In serum BUN, GG, GFG1 and GFG2 were significantly ($p<0.05$) diminished as compared to PCG. While, GCG3 and GCG4 had the ameliorating significant effect than other treatments, 46.57±4.21 and 44.50±5.01 mg/dL, respectively. For serum creatinine, non significant was mentioned among treated groups as compared to NCG.

Intracellular glucose and lipid metabolic homeostasis is vital for maintaining basic life activities of a cell or an organism. So the current data demonstrated the occurrence of serum hyperlipidemia, hypertriglyceridemia and hypercholesterolemia in diabetic rats as detected in Table 6, the serum lipid profiles was improved after administered dietary supplements. The lower dose in both GFG1 and GCG3 showed a significant ($p<0.05$) decrease in serum TG and TC as compared to the positive control group but did not reach to the normal level. While, the higher dose in GFG2 and GCG4 were recorded the best effect by mean (44.21±5.04 and 57.63±3.90 mg/dL) for TG and (55.67±2.59 and 62.63±3.23 mg/dL) for TC. For HDL-c level, GFG1 and GFG2 (37.83±0.66 and 36.16±1.05 mg/dL) demonstrated a higher significant ($p<0.05$) differ than both GCG3 and GCG4 (33.23±1.05 and 34.13±1.05 mg/dL), in contrast GFG1 and GFG2 had the ameliorated effect in LDL-c (12.69±0.46, 8.80±1.01 mg/dL, respectively) and VLDL-c (17.47±1.04 and 10.70±1.43

Table 1: Proximate chemical composition (%) of fenugreek seeds and coffee seeds (on dry weight basis)

	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Fenugreek	6.56±0.04	26.76±0.04	6.33±0.03	6.75±0.03	4.00±0.03	56.27±0.49
Coffee	3.87±0.05	12.27±0.5	1.45±0.09	0.91±0.02	8.77±0.04	73.60±0.03

Table 2: Total phenolic content (g/100 g) of fenugreek and coffee

	Phenol	Flavon	Cr
Fenugreek	1421.67±10.50	9.42±0.06	38.52±0.71
Coffee	665.88±5.38	7.32±0.04	0.00

g/dL, respectively) when compared to NCG (11.46±0.14 and 11.50±4.59 mg/dL, respectively).

As shown in Table 8, MDA was diminished significantly in treated groups when compared with PCG. Interestingly, non significant was illustrated between GCG4 (31.64±0.49) and NCG (31.78±0.32), as well as between GFG1 and GCG3 at lower dose 0.5 g/kg B.W by mean 22.37±0.79 and 22.27±1.94, respectively. Regarding to GST, the best effect was shown in GFG1 and GFG2, (100.82±5.26 and 95.66±2.53, respectively), while GCG4 did not differ than GG. Moreover, catalase was increased significantly in particular GFG2 that did not differ significantly than NCG, 29.57±0.91 and 30.07±0.67, respectively.

DISCUSSION

The results obtained showed that fenugreek seeds contain similar amounts of nutrients which in agreement of Kochhar *et al.* (2006). Moreover, Esmaeili *et al.* (2012) indicated that antioxidant compounds synthesized by fenugreek as secondary products is mainly phenolics, that related to a number of different mechanisms, such as free radical scavenging, hydrogen-donation, singlet oxygen quenching and metal ion-chelation. Also fenugreek could be exploited as an important supplement as functional foods due to the widely reported antioxidant activity of flavonoids as stated by Benayad *et al.* (2014). Also, coffee is considered to be one of the richest sources of phenolics as determined by Higdon and Frei (2006). The most abundant class of phenolic compounds in coffee are chlorogenic acids (CGAs) (Ky *et al.*, 2001).

Weight gain that associated with therapies for managing T2DM is an important consideration in clinical practice and a major limitation in achieving good glycemic control. Glimepiride differs from other agents in this class, that it is associated with equivalent metabolic control with weight neutral effects on patients with T2DM (Hydrie *et al.*, 2006). Additionally, glimepiride has many extra-pancreatic glucose-lowering effects including decreased endogenous glucose production as well as improved peripheral glucose uptake (Fonseca *et al.*, 2008). These effects may explain the weight loss or weight neutrality associated with glimepiride use (Basit *et al.*, 2012).

Our findings supported by Duarte *et al.* (2012) who stated that caffeine consumption significantly reduced the weight gain and pre-prandial glycaemia in diabetic mice. In the same attitude, Shimoda *et al.* (2006) reported that caffeic acid is possibly effective against weight gain and fat accumulation by inhibition of fat absorption and activation of fat metabolism in the liver. Lipolysis is another indicator of lipid metabolism and increase thermogenesis in part by increasing fat oxidation has frequently been observed after caffeine or coffee intake by human subjects (Zheng *et al.*, 2004).

Compared with PCG, the decreased in BWG that determined in diabetic groups administered with fenugreek seeds may attribute to coumarin derivatives. Ramadan *et al.* (2011) mentioned that fenugreek seeds may be useful as a dietary adjunct for the control of the metabolic syndrome in diabetic/obese patients. Also, Ilavenil *et al.* (2014) evidenced that trigonelline is a natural alkaloid mainly found in fenugreek, may inhibit the adipogenesis. Moreover, Vijayakumar *et al.* (2010) stated that fenugreek ameliorate the abnormalities in lipid homeostasis due to its hypolipidemic properties that diminished fat accumulation.

In our results, an overall reduction of FBG and IH level was detected. Glimepiride reduced blood glucose levels and increased insulin levels in blood, these results were confirmed by Muller (2000) who shown a linear relationship between serum glimepiride concentrations and insulin release during euglycemia and a nearly linear relationship under hyperglycemic conditions. Moreover, Basit *et al.* (2012) stated that glimepiride was improved both first-and second-phase insulin secretion. Glimepiride acts at ATPase-dependent potassium channels in β cells of the pancreas to stimulate insulin release.

Further homeostasis in blood glucose level was observed by auxiliary the second antihyperglycemic agent of caffeine that was confirmed by the results of Urzua *et al.* (2012). Furthermore, Zheng *et al.* (2007) investigated that caffeic acid inhibited the activities of alpha-amylase and alpha-glucosidase and reduced the postprandial blood glucose concentration. Thus, the present study demonstrated that both factors caffeine and glimepiride can in a dose-dependent manner inhibit and eventually abolish the response of diabetes. On the other hand, Mohiuddin *et al.* (2009) showed that concurrent administration of caffeine and SUs have not made noticeable changes in plasma concentration of caffeine.

On the other hand, fenugreek seeds may improve glycemic control in type 2 diabetes, in agreement of our results Baquer *et al.* (2011). The hypoglycemic effects of trigonella have been attributed to several mechanisms,

Table 3: Effect of fenugreek and coffee drinks on initial weight, final weight and body weight gain of diabetic rats

	NCG	PCG	GG	GFG1	GFG2	GCG3	GCG4	LSD
Initial weight	177±10.95^{ab}	181±2.23^{a}	179±2.23^{a}	171±2.24^{bc}	170.2±0.44^{c}	169±2.24^{c}	169±2.23^{c}	6.164
Final weight	204.2±5.26^{a}	156.2±2.38^{e}	191.8±1.09^{b}	192.6±0.55^{b}	188.2±2.38^{c}	186.4±1.34^{c}	182.6±0.89^{d}	3.302
BWG	15.59±4.74^{a}	13.69±0.62^{e}	7.16±1.80^{d}	12.64±1.35^{ab}	10.57±1.51^{bc}	10.31±1.85^{bc}	8.05±1.35^{cd}	2.996

NCG: Negative control group, PCG: Positive control group, GG: Glimepiride group, GFG1: Glimepiride+0.5 g fenugreek group, GFG2: Glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: Glymepiride+1 g coffee group, BWG: Body weight gain. $^{a-e}$Means in the same row with different letters are significantly different ($p \leq 0.05$)

Table 4: Effect of glimepiride, fenugreek and coffee on fasting blood glucose (mg/dl) of diabetic rats

	0	3 weeks	6 weeks	LSD
NCG	96.33±1.53^{cA}	97.67±2.08^{dA}	98.33±2.52^{cA}	5.449
PCG	220.00±4.36^{bB}	224.20±5.29^{aAB}	231.67±3.51^{aA}	11.149
GG	227.00±3.61^{bA}	145.30±7.09^{cB}	104.90±11.62^{cC}	22.036
GFG1	227.67±2.52^{bA}	147.83±7.73^{cB}	97.27±11.91^{cC}	15.618
GFG2	237.33±4.51^{aA}	144.70±5.94^{cB}	150.07±8.19^{bB}	9.277
GCG3	223.33±8.50^{bA}	162.37±3.76^{bB}	143.67±1.51^{bC}	9.523
GCG4	236.33±3.51^{aA}	154.13±6.98^{bcB}	143.40±7.93^{bB}	14.913
LSD	8.247	10.918	14.077	

NCG: Negative control group, PCG: Positive control group, GG: Glimepiride group, GFG1: Glimepiride+0.5 g fenugreek group, GFG2: glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: Glymepiride+1 g coffee group.
Means in the same column with different small letters are significantly different ($p \leq 0.05$)
Means in the same row with different capital letters are significantly different ($p \leq 0.05$)

Table 5: Effect of glimepiride, fenugreek and coffee on insulin hormone (µIU/ml) of diabetic rats

	0	3 weeks	6 weeks	LSD
NCG	120.66±2.08^{dA}	123.67±2.08^{dA}	123.67±2.08^{cA}	4.266
PCG	183.00±6.00^{aA}	178.66±5.86^{aA}	139.66±7.09^{bB}	16.380
GG	157.66±5.03^{cA}	147.00±3.61^{bcB}	119.67±4.73^{cC}	8.862
GFG1	181.33±3.51^{aA}	141.33±5.03^{cB}	119.67±8.39^{cC}	14.055
GFG2	170.33±4.16^{bA}	149.67±9.45^{bcB}	151.01±5.19^{aB}	9.724
GCG3	177.67±5.51^{abA}	157.67±9.07^{bB}	149.33±7.57^{aB}	16.947
GCG4	180.67±2.52^{aA}	156.67±7.09^{bB}	145.33±4.93^{abB}	11.507
LSD	7.67	10.255	7.215	

NCG: Negative control group, PCG: Positive control group, GG: Glimepiride group, GFG1: Glimepiride+0.5 g fenugreek group, GFG2: glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: Glymepiride+1 g coffee group.
Means in the same column with different small letters are significantly different ($p \leq 0.05$)
Means in the same row with different capital letters are significantly different ($p \leq 0.05$)

in recent studies of Puri *et al*. (2002) who had isolated 4-hydroxyisoleucine an active compound from fenugreek that showed hypoglycemic properties in diabetic patients by increased glucose-induced insulin release in human pancreatic islet cells of Langerhans (Baquer *et al*., 2009).

Furthermore, the antihyperglycemic effect of fenugreek may be hypothesized to the presence of a significant quantity of bioactive compounds saponins, alkaloids and polyphenol that may be inhibit intestinal glucose uptake (Kochhar *et al*., 2006). A secondary mechanism for the hypoglycaemic effect attributed to dietary fibres present in the fenugreek seeds which help in the management of metabolic abnormalities associated with diabetes such as peripheral insulin resistance and lipid peroxidation abnormalities. In combination, Haritha *et al*. (2013) proved that fenugreek and glimepiride treatment reduced the blood glucose levels. On the other hand, the impaired amelioration of glycemic parameters at higher dose of fenugreek supplement may attenuate to the rich fiber in fenugreek seeds that can interfere with the absorption of oral medications because its fiber is mucilaginous and has high viscosity in the gut (Basch *et al*., 2003).

The present study illustrated a significant reduction of biomarker enzymes AST and ALT among treated groups as compared to PCG and GG. This reduction may attribute to administrating fenugreek seeds that restore the activities of the aforementioned enzymes to near their normal level. The same tendency was noticed by Haritha *et al*. (2013) who revealed that added fenugreek to glimepiride drug had positive interaction in improving the liver parameters in diabetic rats, as compared to individual agent-treated groups, which could be due to reactivation of glycogen synthase system as a result of increased insulin secretion as mentioned by Collier and Scott (2004). A hepatoprotective association of coffee drinking and the risk of liver-associated enzyme elevations have been confirmed as illustrated in our results. In parallel, many studies have reported an inverse relationship between them as reported by Machadoa *et al*. (2014) who stated that coffee have an attractive explanation for anti-fibrogenic effects.

Table 6: Effect of glimepiride, fenugreek and coffee on liver and kidney functions in diabetic rats

	AST (***)	ALT (***)	BUN (***)	Creatinine (***)
NCG	84.66±4.37^{b}	28.75±2.38^{b}	57.23±1.80^{b}	0.68±0.03^{b}
PCG	135.26±1.75^{a}	60.23±21.68^{a}	104.20±2.19^{a}	0.83±0.02^{a}
GG	81.23±3.33^{bc}	29.57±0.98^{b}	52.23±0.93^{b}	0.78±0.07^{ab}
GFG1	76.46±1.28^{cd}	26.23±0.98^{b}	54.60±3.86^{b}	0.72±0.11^{ab}
GFG2	73.45±1.13^{d}	33.52±2.17^{b}	57.50±2.58^{b}	0.75±0.04^{ab}
GCG3	72.36±1.75^{d}	28.48±4.04^{b}	46.57±4.21^{c}	0.79±0.03^{ab}
GCG4	62.73±2.62^{e}	23.23±1.01^{b}	44.50±5.01^{c}	0.77±0.06^{ab}
LSD	4.85	15.67	5.410	0.120

AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase BUN: Blood Urea Nitrogen, NCG: Negative control group, PCG: Positive control group, GG: Glimepiride group, GFG1: Glimepiride+0.5 g fenugreek group, GFG2: Glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: Glymepiride+1 g coffee group.
Means in the same column with different letters are significantly different ($p \leq 0.05$)

Table 7: Effect of glimepiride, fenugreek and coffee on triglyceride and cholesterol profiles in diabetic rats

	TG (***)	TC (***)	HDL-c (***)	LDL-c (***)	VLDL-c (***)
NCG	57.33±0.73^{d}	61.93±2.86^{c}	38.96±1.70^{a}	11.46±0.14^{d}	11.50±4.59^{d}
PCG	91.69±2.51^{a}	90.8±0.87^{a}	31.41±1.11^{e}	18.38±0.52^{a}	41.02±1.17^{a}
GG	75.16±2.43^{b}	87.33±1.85^{a}	34.66±0.95^{cd}	15.03±0.48^{b}	37.63±1.96^{a}
GFG1	63.46±2.31^{c}	68.01±1.47^{b}	37.83±0.66^{ab}	12.69±0.46^{c}	17.47±1.04^{c}
GFG2	44.21±5.04^{e}	55.67±2.59^{d}	36.16±1.05^{bc}	8.80±1.01^{e}	10.70±1.43^{d}
GCG3	67.23±2.24^{c}	72.33±3.43^{b}	33.23±1.05^{de}	13.44±0.44^{c}	25.65±4.01^{b}
GCG4	57.63±3.90^{d}	62.63±3.23^{c}	34.13±1.05^{cd}	11.52±0.78^{d}	16.97±2.75^{c}
LSD	5.74	4.69	2.07	1.15	5.21

TG: Serum triglycerides, TC: Serum total cholesterol, HDL-c: High density lipoprotein, LDL-c: Low density lipoprotein cholesterol, VLDL-c: Very low density lipoprotein cholesterol, NCG: negative control group, PCG: positive control group, GG: glimepiride group, GFG1: glimepiride+0.5 g fenugreek group, GFG2: Glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: glymepiride+1 g coffee group. Means in the same column with different letters are significantly different ($p \leq 0.05$)

Table 8: Interactive effect of glimepiride and each of fenugreek and coffee drink on antioxidant profile in type 2 diabetic rats

	MDA (***)	GST (***)	Catalase (***)
NCG	31.78±0.32^{c}	134.44±13.69^{a}	30.07±0.67^{a}
PCG	42.40±0.99^{a}	64.14±3.96^{d}	19.50±0.61^{de}
GG	36.14±0.78^{b}	71.63±2.38^{cd}	18.63±0.45^{e}
GFG1	22.37±0.79^{d}	100.82±5.26^{b}	23.03±0.75^{c}
GFG2	36.53±0.48^{b}	95.66±2.53^{b}	29.57±0.91^{a}
GCG3	22.27±1.94^{d}	62.02±0.17^{d}	20.50±1.15^{d}
GCG4	31.64±0.49^{c}	79.59±0.46^{c}	25.10±0.30^{b}
LSD	1.730	10.346	1.334

MDA: ****, GST: *****, NCG: Negative control group, PCG: Positive control group, GG: Glimepiride group, GFG1: Glimepiride+0.5 g fenugreek group, GFG2: glimepiride+1 g fenugreek group, GCG3: Glymepiride+0.5 g coffee group, GCG4: Glymepiride+1 g coffee group

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) which is responsible for disabilities and high mortality rates in patients with diabetes. Jin *et al*., (2014) suggested that the potential protective effect of fenugreek on kidney might reduce DN risk through alleviating renal oxidative stress and inhibiting TGF-β1/CTGF signaling pathway in glomeruli of diabetic rats. Also, our results considered that coffee drink might be a promising new therapeutic agent for nephrotoxicity and oxidative renal damage as mentioned in the results of Akyol *et al*. (2014). Several plausible explanations for our results are as follows. First, the various components of coffee may protect the glomerular endothelium from oxidative stress (Kim *et al*., 2013). Second, the protective effect of caffeic acid might be due to the inhibition of leukocyte accumulation in the kidney (Akyol *et al*., 2014).

The levels of serum lipids are usually elevated in diabetes mellitus, these findings agreed with the findings of Veerapur *et al*. (2010). There is significant decrease in serum lipid profile as tabulated in our results in fenugreek treated diabetic rats. VLDL-c and LDL-c levels were lower than values in non-treated diabetic group and slight increase in HDL levels were also noted. This result suggested that fenugreek seeds would be helpful in the prevention of diabetic complications through improving dyslipidemia.

Also, chlorogenic acid inhibit the oxidation of LDL. The mechanism of caffeic acid in reducing blood lipids was most likely associated with the inhibition of absorption and transformation of lipids and with the inhibition of intestinal absorption and hepatic biosynthesis of cholesterol (Karthikesan *et al*., 2010).

The findings of current study showed a significant increase in the activity of GST and CAT in treated groups, Tripathi and Chandra (2010) demonstrated that fenugreek are not only useful in controlling the blood glucose levels, but also have antioxidant potential to protect vital organs. Concerning catalase, administration of fenugreek, singly, was the most effective in normalization of alterations caused by diabetes (Marzouk *et al*., 2013). On the other hand, Minamisawa *et al*. (2004) concluded that coffee is a rich source of antioxidants.

REFERENCES

AIN, (American Institute of Nutrition), 1993. Purified diet for laboratory Rodent, Final report., J. Nutrition. 123:1939-1951.,and O. compactum Benth. J. Essential Oil Res., 8: 657-664.

Akyol, S., V. Ugurcu, A. Altuntas, R. Hasgul, O. Cakmak and O. Akyol, 2014. Caffeic Acid Phenethyl Ester as a Protective Agent against Nephrotoxicity and/or Oxidative Kidney Damage: A Detailed Systematic Review. J. Scientific World. Volume 2014, Article ID 561971,16 pages.

Rai, A., E. Cicy and V.G. Prasanth, 2012. Interaction of Herbs and Glibenclamide: A Review. ISRN Pharmacology. Volume 2012, Article ID 659478, 5 pages.

Baquer, N.Z., P. Kumar, A. Taha, R.K. Kale, S.M. Cowsik and P. McLean, 2011. Metabolic and molecular action of Trigonellafoenum-graecum (fenugreek) and trace metals in experimental diabetic tissues. J. Biosci., 36: 383-396.

Baquer, N.Z., A. Taha, P. Kumar, P. McLean, S.M. Cowsik, R.K. Kale, R. Singh and D. Sharma, 2009. A metabolic and functionaloverview of brain aging linked to neurological disorders. J. Biogerontol., 10: 377-413.

Basch, E., C. Ulbricht, G. Kuo, P. Szapary and M. Smith, 2003. Therapeutic Applications of Fenugreek. J. Altern. Med. Rev., 8: 20-27.

Basit, A., M. Riaz and A. Fawwad, 2012. Glimepiride: evidence-based facts, trends and observations. Vascular Health and Risk Manag., 8: 463-472.

Benayad, Z., C. Gomez-Cordoves and N. Es-Safi, 2014. Characterization of Flavonoid Glycosides from Fenugreek (Trigonellafoenum-graecum) Crude Seeds by HPLC-DAD-ESI/MS Analysis. Int. J. Mol. Sci., 15: 20668-20685.

Collier, J.J. and D.K. Scott, 2004. Sweet changes: Glucose homeostasis can be altered by manipulating genes controlling hepatic glucose metabolism. Mol. Endocrinol., 18: 1051-1063.

Duarte, J.M.N., P.M. Agostinho, R.A. Carvalho and R.A. Cunha, 2012. Caffeine Consumption Prevents Diabetes-Induced Memory Impairment and Synaptotoxicity in the Hippocampus of NONcZNO10/LTJ Mice. J. PLoS ONE, 7: e21899.

Endocrinologic and Metabolic Drugs Advisory Committee, 2013. Canagliflozin a s an Adjunctive Treatment to Diet and Exercise Alone or Co-administered with Other Antihyperglycemic Agents to Improve Glycemic Control in Adults with Type 2 Diabetes Mellitus. JNJ-28431754 (Canagliflozin) NDA 204042.

Esmaeili, A., B. Rashidia and S. Rezazadehb 2012. Biological activities of various extracts and chemical composition of trigonellamonantha C.A. Mey. subsp. monantha Grown in Iran. Iranian J. Pharmaceutical Res., 11: 1127-1136.

Fonseca, V., M. Baron, Q. Shao and S. Dejager, 2008. Sustained efficacy and reducedhypoglycemia during one year of treatment with vildagliptin added to insulin in patients with type 2 diabetes mellitus. J. Horm Metab. Res., 40: 427-430.

Fossati, P., 1982. Pricipe I. Clin. Chem., 28: 2077 (Chemical Kits).

Fujimoto, W.Y., 2000. The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. Am. J. Med., 108: 9S-14S.

Hafkenscheid, J.C., 1979. Determination of GOT. Clin. Chem., 25: 155.

Hamden, K., H. Masmoudi, S. Carreau and A. Elfeki, 2010. Immunomodulatory, beta-cell and neuroprotective actions of fenugreek oil from alloxan-induced diabetes. Immunopharmacology and Immunotoxicology, 32: 437-445.

Haritha, C., A.G. Reddy, Y.R. Reddy, Y. Anjaneyulu, T.M. Rao, B.A. Kumar and M.U. Kumar, 2013. Evaluation of protective action offenugreek, insulin and glimepiride and their combination indiabetic Sprague Dawley rats. J. Nat. Sci. Biol. Med., 4: 207-212.

Henry, R.J., 1974. Clinical Chemistry Principles and Techniques. 2nd Ed., Harper and ublishers, New York. Philadelphia.

Higdon, J.V. and B. Frei, 2006. Coffee and health: A review of recent human research. Crit. Rev. J. Food Sci. Nutr., 46: 101-123.

Hydrie, M.Z.I., A. Gul, R. Hakeem, M.Y. Ahmadani and A. Basit, 2006. Glimepiride study on type-2 diabetic subjects. Pak. J. Med. Sci., 22: 132-135.

Ilavenil, S., M.V. Arasu, J.C. Lee, da. H. Kim, S.G. Roh, H.S. Park, G.J. Choi, V. Mayakrishnan and K.C. Choi, 2014. Trigonelline attenuates the adipocyte differentiation and lipid accumulation in 3T3-L1 cells. Phytomed., 21: 758-65.

Jin, Y., Y. Shi, Y. Zou, C. Miao, B. Sun and C. Li, 2014. Fenugreek Prevents the Development of STZ-Induced Diabetic Nephropathy in a Rat Model of Diabetes. J. Evidence-Based Complementary and Alternative Medicine. Volume 2014, Article ID 259368, 11 pages.

Jorhem, L., 2000. Determination of metals in foods by atomic absorpition spectrometry after dry ashing. NMKL collaborative study. J. AOAC. Int., 83: 1204-1211.

Karthikesan, K., L. Pari and V.P. Menon, 2010. Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts potential antihyperglycemic effect on streptozotocin-nicotinamideinduced diabetic rats. J. Gen. Physiol. Biophys., 29: 23-30.

Kim, B.H., Y.S. Park, H.M. Noh, J.S. Sung and J.K. Lee, 2013. Association between Coffee Consumption and Renal Impairment in Korean Women with and without Diabetes: Analysis of the Fourth Korea National Health and Nutrition Examination Survey in 2008. Kor. J. Fam. Med., 34: 265-271.

Kochhar, A., M. Nagi and A. Sachdevar, 2006. Prooximatecomposition, available carbohydrates, dietary fibre and anti-nutritional factors of selected traditional medicinal plants. J. Hum. Ecol., 19: 195-199.

Kumar, P., R.K. Kale and N.Z. Baquer, 2012. Anti-hyperglycemic and protective effects of *Trigonella foenum graecum* seed powder on biochemical alterations in alloxan diabetic rats. Eur. Rev. Med. and Pharmacol. Sci., 16: 18-27.

Ky, C.L., J. Louarn, S. Dussert, B. Guyot, S. Hamon and M. Noirot, 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild Coffea arabicaL. and C. canephora P. accessions. J. Food Chem., 75: 223-230.

Lee, R. and D. Nieman, 1996. Nutritional Assessment. 2nd Ed., Mosby, Missouri, USA.

Lopez, M.F., 1977. HDL-cholesterol colorimetric method. J. Clin. Chem., 23: 282.

Machadoa, S.R., E.R. Parise and L.D. Carvalho, 2014. Coffee has hepatoprotective benefits in Brazilian patients with chronic hepatitis C even in lower daily consumption than in American and European populations. Brazilian J. Infectious Dis., 8: 170-176.

Marzouk, M., A.M. Soliman and T.Y. Omar, 2013. Uropean Review for Medical and Pharmacological Sciences Hypoglycemic and antioxidativeeffects of fenugreek and termis seeds powder in streptozotocin-diabetic rats, 17: 559-565.

Massi-Benedetti, M., 2003. Glimepiride in type 2 diabetes mellitus: a review of the worldwide therapeutic experience. Clin. Ther., 25: 799-816.

Mathers, C.D. and D. Loncar, 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med., 3: e442.

Meda, A., C.E. Lamien, M. Romito, J. Millogo and O.G. Nacoulma, 2005. Determination of the total phenolic, flavonoid and praline contents in burkina fasan honey, as well as their radical scavenging activity. Food Chem., 91: 571-577.

Minamisawa, M., S. Yoshida and N. Takai, 2004. Determination of biologically active substances in roasted coffees using a diode-array HPLC system. Anal. Sci., 20: 325-328.

Mohiuddin, M., A.T. Azam, M.S. Amran and M.A. Hossain, 2009. *In vivo* effects of gliclazide and metformin on the plasma concentrationof caffeine in healthy rats. Pak. J. Biol. Sci., 12: 734-737.

Moss, D.W., 1982. Alkaline phosphatase isoenzymes. Clin. Chem., 28: 2007-2016.

Müller, G., 2000. The molecular mechanism of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug Amaryl. Mol. Med., 6: 907-933.

Overkamp, D., A. Volk and E. Maerker 2002. Acute effect of glimepiride on insulin-stimulated glucose metabolism in glucose-tolerant insulin resistant offspring of patients with type 2 diabetes. Diabetes Care, 25: 2065-2073.

Patton, C.J. and S.R. Crouch, 1977. Enzymatic determinationof urea. J. Anal. Chem., 49: 464-469.

Puri, D., K.M. Prabhu and P.S. Murthy, 2002. Mechanism of action of ahypoglycemic principle isolated from fenugreek seeds. Ind. J. Physiol. Pharmacol., 46: 457-462.

Ramadan, G., N.M. El-Beih and H.F. Abd El-Kareem, 2011. Anti-metabolic syndrome and immunostimulant activities of Egyptian fenugreek seeds in diabetic/obese and immunosuppressive rat models. Br. J. Nutr., 105: 995-1004.

Satoh and Clinica Chemica Acta, 1978. 90, 37. (Chemical Kits).

Shimoda, H., E. Seki and M. Aitani, 2006. Inhibitory effect of greencoffee bean extract on fat accumulation and body weight gain in mice. BMC Complement. Altern. Med., 6: 9.

Shukla, U.A., E.M. Chi and K.H. Lehr, 2004. Glimepiride pharmacokinetics in obese versus non-obese diabetic patients. Ann. Pharmacother, 38: 30-35.

Sridevi, B.V., P. Giridhar and G.A. Ravishankar, 2011. Evaluation of roasting and brewing effect on antinutritional diterpenes-cafestol and kahweol in coffee. Glob. J. Med. Res., 11: 17-22.

Thomas, L., 1992. Labor and diagnose, 4th Ed. (Chemical Kits).

Trinder, P., 1969. Glucose enzymatic colorimetric method. J. Ann. Clin. Biochem., 6: 24.

Tripathi, U.N. and D. Chandra, 2010. Anti-hyperglycemic and anti-oxidative effect of aqueous extract of Momordicacharantia pulp and Trigonellafoenum-graecum seed in alloxan-induced diabetic rats. Ind. J. Biochem. Biophys., 47: 227-233.

Urzua, Z., X. Trujillo, M. Huerta, B. Trujillo-Hernandez, M. Rios-Silva, C. Onetti, M. Ortiz-Mesina and E. Sanchez-Pastor, 2012. Effects of chronic caffeine administration on blood glucose levels and on glucose tolerance in healthy and diabetic rats. J. Int. Med. Res., 40: 2220-2230.

Vats, V., J.K. Grover and S.S. Rathi, 2002. Evaluation of Antihyperglycemic and hypoglycemic effect of Trigonella foenumgraecum Linn, Ocimum sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanized diabetic rats. J. Ethnopharmacol., 79: 95-100.

Veerapur, V.P., K.R. Prabhakar, V.K. Parihar, P. Bansal, K.K. Srinivasan, K.I. Priyadarsini and M.K. Unnikrishnan, 2010. Antidiabetic, hypolipide-micandantioxidant activity of Dodonaeavis-cosaaerial partsinstreptozotocin-induced-diabeticrats. Int. J. Phytomed., 2: 59-70.

Vijayakumar, M.V., V. Pandey, G.C. Mishra and M.K. Bhat, 2010. Hypolipidemic effect of fenugreek seeds is mediated through inhibition of fat accumulation and upregulation of LDL receptor. J. Obesity (Silver Spring), 18: 667-674.

Wilson, M.A. and L.E.M. Miles, 1977. Radio Immuno Assay of Insulin-in Hand Book of Radioimmunoassay. G.E. Abraham. E.d. Inc. New York, pp: 257.

World Health Organization, 2012. Global status report on noncommunicable diseases 2014, Geneva.

Zheng, G., K. Sayam, T. Okubo, L.R. Juneja and I. Oguni, 2004. Anti-obesity effectsof three major components of green tea, catechins, caffeine and theanine, in mice. *In vivo*, 18: 55-62.

Zheng, Y.N., K. Liu and G.Y. Jia, 2007. Effect of hot-water extractof coffee seeds on postprandial blood glucose concentration inrats. Chinese Pharmaceutical J., 42: 32-35.