HYPOLIPIDEMIC EFFECTS OF *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK) SEED POWDER ADMINISTRATION IN RABBITS WITH EXPERIMENTAL DIETARY HYPERLIPIDEMIA

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Abstract

The present study is designed to investigate the efficacy of the most common dietary component fenugreek (seed powder) consumption on dietary dyslipidemia and its comparison with atorvastatin.

Twenty Four white rabbits of either sex were used in the study. After one week acclimatization period, animals were randomly divided into two groups. Group I animals received normal diet. Group II animals were administered with atherogenic diet for four weeks. Group II is than subdivided as *Group II-a*: 10mg of fenugreek seed powder were fed daily to these hyperlipidemic animals for next 15 days and *Group II-b*: hyperlipidemic animals receive 0.5mg/Kg BW of atorvastatin for 15 days. At the end of experimental period blood specimens were obtained and assayed for alterations in plasma lipid profile (TC, TG, HDL, LDL, VLDL), atherogenic index, glucose, antioxidant enzymes (CAT, SOD, GSH), liver enzymes (AST, ALT) and plasma levels of urea & creatinine.

The results of the present study showed that administration of atherogenic diet increase plasma lipid profile, glucose, liver enzymes, urea and creatinine while decrease antioxidant enzymes in experimental animals. Administration of fenugreek seed powder reduce plasma levels of TC ($P>0.05$), TG ($P<0.05$). LDL-C ($P>0.05$), VLDL ($P<0.05$), AIP ($P>0.05$) and increase HDL levels ($P>0.05$) hence effectively control AIP. Glucose levels were also decreased ($P>0.05$), FsP administration significantly decrease plasma AST ($P<0.005$), ALT ($P<0.01$), urea ($P<0.05$) and creatinine levels ($P<0.005$). Fenugreek consumption significantly improve body antioxidant status by increasing plasma SOD ($P<0.005$), CAT ($P>0.05$) and GSH ($P<0.05$) levels in hyperlipidemic animals. From obtained results it is suggested that daily supplementation of fenugreek seeds effectively reduces dietary hyperlipidemia and other risk factors of CAD.

Introduction

Globally, coronary artery disease (CAD) is a prevalent cause of mortality and casual element of chronic heart problems (Lichtenstein *et al.,* 2006; Amani *et al.,* 2010). According to WHO (world health organization) estimate, globally 3.8 million men and 3.4 million women die every year due to coronary heart disease (CHD) (Mackey *et al*., 2004). Indo-Asian population is more prone to developing CAD (Jafar, 2008) and past research studies, in Pakistan, reveals the high ascendancy of cardiovascular disease (CVD) with more than 20% of the nation being influenced with ratios swiftly increasing in urban population (Reddy *et al*., 2005; Saeed *et al*., 2011).

CAD is a multifactorial, pathologic, vascular phenomenon with a life time threat (Liu., 2009), remains asymptomatic in most of the cases and results in sudden cardiac death. Lipid imbalances consider as the central dogma of coronary disease. Increase blood levels of low density lipoprotein (LDL) have long been known to be associated with CAD but atherogenic lipoprotein phenotype (ALP) syndrome is the most important type of dyslipidemia identified in patients of CAD (Griffin *et al*., 1994), characterized with normal or high blood cholesterol & triglyceride levels, decreased high density lipoprotein (HDL) cholesterol concentration together with small & oxidized circulating LDL species (Witztum *et al*., 2005). To treat lipid disorders both pharmacological and non-pharmacological therapies are available with emphasis on dietary interventions for primary cases of CAD.

Trigonella foenum-graecum (fenugreek) (methi), a well-known herb and culinary spice, belonged to family *Fabaceae* has been used extensively in folk medicine of many cultures as anticold, antidiarrheal, antihyperglycemic and hepato-reno protective agent. Fenugreek seeds are rich source of dietary fibers (50%) [20% insoluble and 30% soluble fibers] with principle polysaccharide galactomannan.

Present study has been themed to investigate the efficacy of fenugreek seed consumption in the management of dietary hyperlipidemia in experimental animal models and its effects are also compared with a well-known statin (atorvastatin).

Material and Methods

Animals: New Zealand white rabbits (*Ortyctologus cunniculus*) of either sex with average body weight of 1-2 Kg and 3 months old (at the start of experiment) were selected for the study, (n=24).

Preparation of Fenugreek Seed Powder (FsP): Fenugreek seeds were purchased from the local market in Karachi, Pakistan. Seeds were washed with clean water and dried. To make powder, dried seeds were crushed in electrical grinder. The powder was than stored in a clean, dried and covered plastic container at room temperature.

Experimental Protocol: Animals were initially acclimatized for seven days. Acclimatization was don*e at 25*^{0}C room temperature with a 14-h light: 10-h dark cycles in animal house of Department of Physiology, University of Karachi. Feeding periods with amount of intake, animal's body weight and other physical conditions were closely monitored throughout the study. Food and water were provided *ad libitum*. Blood was drawn, after an overnight fast from marginal ear vein and base line values for all parameters were checked.
Rabbits were randomly divided into two experimental groups:
Group I (n = 8) are control animals and were fed normal rabbit chow.
Group II (n = 16) are hyperlipidemic animals received atherogenic diet (1g butter fat / 100g of daily diet) for four weeks (modified from Moghadasian *et al*., 1999).

After four weeks
Group II-a: (n=8) Hyperlipidemic animals received 10mg of FsP per Kg of body weight (modified from Mowla *et al.,* 2009) in addition to above mentioned atherogenic diet for 15 days.
Group II-b: (n=8) Animals receive 0.5 mg atorvastatin per Kg of body weight per day (Sharma and Choudary, 2014) for next 15 days with atherogenic high fat diet.
Blood samples were collected after every dietary modification. Body weight, plasma lipid profile, atherogenic index of plasma (AIP) and plasma levels of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), were measured.

Biochemical Analysis: Plasma total cholesterol (TC) concentration was determined by CHOD-PAP enzymatic endpoint method using enzymatic kit (Global, UK). Plasma triglyceride (TG) concentration was determined by GPO-PAP enzymatic endpoint method using enzymatic kit (Global, UK). Plasma HDL concentration was determined by Phosphotungstic precipitation method using enzymatic kit (Global, UK). Plasma LDL concentration was determined by using Friedewald's formula (Friedewald *et al*., 1972). Plasma very low density lipoprotein (VLDL) levels will be calculated by another formula as mentioned by Bairaktari *et al*, (2005). Atherogenic index of plasma will be estimated by formula of Umeshchandra *et al*, (2012). Plasma glucose concentration was determined by Enzymatic-Colorimetric, GOD-PAP in vitro method using enzymatic kit (Global, UK). Estimation of AST and ALT were measured by using enzymatic kit (Randox, UK). Plasma urea levels will be assessed by enzymatic method kit (Erba Diagnostic, Germany). Estimation of creatinine levels were measured by Jaffe's method using enzymatic kit (Biogene Diagnostics, USA). Recommended procedures will be used to assess plasma catalase (CAT) (Sinha, 1972), superoxide dismutase (SOD) (Kono, 1978) & glutathione (GSH) (Carlberg and Mannervik, 1985).

Statistical Analysis: The data expressed as mean ± S.E.M. and were analyzed by t-test. A value of $P < 0.05$ was chosen as the criteria of statistical significance.

Results

Animals from all experimental groups were remain normal throughout the period of study and had no sign of decreased physical activity or altered behavior.

Effect of Atherogenic Diet Administration: Atherogenic diet (1g butter fat /100g of daily diet) administration for four weeks increase the body weight ($P>0.05$), plasma total cholesterol ($P=0.005$), triglyceride ($P<0.05$), LDL ($P<0.05$), VLDL ($P<0.05$) and AIP levels ($P<0.05$) whereas plasma HDL concentration decrease non-significantly ($P>0.05$). Plasma antioxidant enzymes (CAT-- $P>0.05$; SOD-- $P<0.01$; GSH—$P<0.01$) decreased in these animals with experimental hyperlipidemia. Plasma glucose ($P<0.05$), AST ($P<0.005$), ALT ($P>0.05$), urea ($P<0.005$) and creatinine ($P<0.005$) levels were increase in these experimental animals compared to controls (Table I).

Effect of FsP and Atorvastatin on different Blood Parameters

Changes in Plasma Lipid Profile Levels among Different Experimental Groups: As compared to control group, TC and TG levels in hyperlipidemic animals were increased significantly (TC—$P=0.005$; TG--$P<0.05$). Fenugreek administration for 15 days non-significantly decreased ($P>0.05$) TC levels but that decrease was significant as compared to control ($P<0.05$) group. Triglyceride levels were significantly decreased with 15

day's fenugreek administration in hyperlipidemic animals (P<0.05). Atorvastatin treatment of hyperlipidemic animals decrease plasma TC (P>0.05) and TG levels in these animals (P<0.005) after 15 days of administration. Plasma LDL and VLDL levels were significantly (LDL--P<0.05; VLDL--P<0.05) increase in hyperlipidemic animals as compared to control. Both fenugreek and atorvastatin non-significantly (LDL & VLDL--P>0.05) reduce plasma LDL levels in hyperlipidemic animals. Plasma VLDL were decrease in FsP administered group (P<0.05). 15 days administration of atorvastatin also significantly decrease VLDL levels in hyperlipidemic animal models (P<0.005). Non-significant changes were observed in plasma HDL levels of hyperlipidemic animals as compare to control (P>0.05). Fenugreek seed powder increases HDL level (P>0.05). Atorvastatin non-significantly increase HDL levels but this increase is non-significant (P>0.05).
As compared to control, AIP levels were significantly increase in hyperlipidemic animals (P<0.05). Both fenugreek and atorvastatin decrease plasma AIP levels in hyperlipidemic animal models. (Fenugreek-- P>0.05; Atorvastatin--P<0.005).

Changes in Plasma CAT, SOD & GSH Levels among Different Experimental Groups: In comparison with control, plasma CAT and SOD levels were decrease in hyperlipidemic animals (CAT-- P>0.05; SOD-- P<0.01). Fenugreek administration for 15 days increased CAT and SOD levels in hyperlipidemic animals (CAT-- P>0.05; SOD-- P<0.005). Whereas atorvastatin non-significantly increase plasma CAT and SOD levels in hyperlipidemic animals (CAT-- P>0.05; SOD-- P>0.05).

GSH levels were significantly decreased in hyperlipidemic animal models as compared to the control group (P=0.005). Fifteen days treatment with 10mg FsP significantly increased the GSH levels in hyperlipidemic animals (P=0.01) similarly 15 days treatment with atorvastatin also significantly increase plasma levels of GSH in hyperlipidemic group (P<0.05). However, when compared with FsP treated group GSH levels were decreased in atorvastatin group but non significantly (P>0.05)

Changes in Plasma Glucose Levels among Different Experimental Groups: Plasma glucose levels were decreased in hyperlipidemic animals as compared to the control (P<0.05). After 15 days consumption of fenugreek plasma glucose levels were decrease in hyperlipidemic animals P>0.05 whereas 15 days treatment of atorvastatin significantly reduce plasma glucose levels in hyperlipidemic animals (P=0.01) as compared to control.

Changes in Plasma Urea and Creatinine Levels among Different Experimental Groups: As compared to control group, high fat diet increases plasma levels of urea and creatinine in hyperlipidemic animals (P>0.05 for both urea & creatinine). Plasma urea and creatinine levels decrease with atorvastatin treatment (urea-- P<0.005; creatinine—P<0.005) in these animals whereas fenugreek treatment reduce urea (P<0.05) and creatinine levels (P<0.005) in hyperlipidemic animals.

Discussion

Although cholesterol is an essential type of fat for body and is derived from many natural sources as well as from liver biosynthesis but raised blood levels of certain circulating lipids (cholesterol, triglycerides, LDL) also is an sturdy risk element for the development of CHD (Roeters *et al.*, 2002, Toft-peterson., 2011). This increased blood lipid profile level has many genetic as well as non-genetic links but decreased body's physical activity & dietary imbalances usually appear as the base line etiology for most of the cases.

To treat hyperlipidemia, dietary modifications (daily use of herbs, culinary spices, vegetables & fruits) are considered as important for both types of patients (primary & secondary) of CHD but physicians usually found it difficult to restrict patients to recommended diets and therefore gave medicines to control blood lipid profile levels in high risk patients (Jafri *et al.*, 2010). Almost all of the antihyperlipidemic pharmacological agents such as statins have side effects and therapeutic tribulations therefore non-pharmacological therapies are the only choice of treatment remained for primary cases of CHD therefore non-pharmacological therapies are the only choice of treatment for primary cases of coronary artery disease (Natarajan *et al*., 2010).

In the present study we have investigated the effectiveness of cardio and atheroprotective actions of fenugreek seed powder consumption in experimental animal models of dietary hyperlipidemia. Results of present research work revealed that per day administration of 10mg fenugreek seed powder exhibit hypolipidemic effects in experimental animals with marked 28% reduction in plasma TG (P<0.05), TC by 2% (P>0.05), plasma LDL levels decreased by 9% (P>0.05 and 31% reduction has been observed in VLDL levels. 10mg/day oral consumption of fenugreek seed powder increase (42%) plasma HDL levels but that increment was non-significant as compared to hyperlipidemic group. Fenugreek consumption effectively control AIP levels. Antihypercholesterolemic and hypolipidemic effects of fenugreek are evident from several studies (Belguith-Hadriche *et al*., 2010).

Table 1. Effects of fenugreek seed powder (FsP) & Atorvastatin administration on Plasma Lipid Profile, Liver Enzymes, Glucose, Urea, Creatinine & Antioxidants Levels in Experimental Animal Models

	TC	TG	HDL	LDL	VLDL	AIP	ALT	AST	Glucose	Urea	Creatinine	CAT	SOD	GSH
Control Group	127.03	143.97	49.828	61.143	28.793	0.477	27.12	54.311	161.215	5.066	47.01	15.876	60.698	5.591
	±11.069	±38.312	±9.56	±6.568	±18.768	±0.163	±1.383	±10.711	±29.872	±0.389	±0.808	±3.005	±4.894	±1.19
Hyperlipidemic Group (compared to control)	213.03	240.96	50.275	114.57	48.192	0.703	54.626	77.448	256.703	7.285	54.001	13.31	46.36	3.516
	±23.083	±15.406	±8.055	±18.376	±7.547	±0.157	±2.358	±4.604	±25.399	±0.367	±0.738	±3.203	±10.326	±1.077
FsP treated Group (compared to hyperlipidemic)	209.75	167.625	71.683*	104.541	33.525*	0.39	46.67**	61.253***	154.076	6.443*	42.01***	16.408	74.541***	6.233*
	±30.962	±20.723	±11.988	±34.22	±10.152	±0.332	±4.939	±8.545	±64.434	±0.614	±1.73	±2.314	±6.563	±1.879
Atorvastatin Treated Group (compared to hyperlipidemic)	165.9	113.74	52.498	90.668	22.747	0.335	44.313	60.253	162.466	4.323	45.086	14.883	55.705	5.05
	±4.302	±6.526	±1.98	±3.683	±1.305	±0.038	±2.984	±4.727	±10.069	±0.433	±1.426	±1.397	±13.896	±0.84

Experimental studies have shown that fenugreek accelerates the fecal elimination of neutral sterols and bile acids thereby reducing the body's cholesterol deposits (Moosa *et al*., 2006). The steroid saponin which is an active component of fenugreek, bind cholesterol and form insoluble cholesterol complexes in digestive tract, leads to decrease blood cholesterol levels. Disogenin, an steroidal saponin, as discussed by Al-Matubsi *et al*, decrease plasma LDL and TC (total cholesterol) concentration when administered in hyperlipidemic quails (Belaid-Nouira *et al.*,2012). Plasma cholesterol levels are also affected, usually decreased, by dietary proteins (Moosa *et al*.,2006) & 26% of fenugreek plant protein may also contribute to its hypolipidemic effects in experimental animal models (Moosa *et al*.,2006) by decreasing TG and cholesterol synthesis through liver and fat cells and by increasing cellular LDL uptake (Belaid-Nouira *et al*., 2012). Results of our present study are consistent with these previous studies however changes in plasma HDL and LDL although are non-significant but needs further investigation with similar dose of FsP in experimental animals for longer duration of time.

Fenugreek seeds also contain an alkaloid trigonelline which is known to activate lipogenic enzymes of liver thereby stabilizing the adipogenesis rate in experimental (streptozotocin treated hyperglycemic) animals (Belaid-Nouira *et al.*, 2012). Body weight changes in fenugreek treated animals (67% reduction) in our study compared to the hyperlipidemic group (62% increase) are substantiating this effect of fenugreek seed powder consumption very prominently.

Plasma ALT and AST are markers of liver damage. Hepatic damage has been marked with increased serum/plasma liver enzymes. In present study administration of hyperlipidemic diet increase plasma ALT and AST levels whereas treatment of hyperlipidemic animals with fenugreek seed powder significantly decrease plasma ALT& AST levels ($P<0.01$ for both AST & ALT). Consistent to our result a study of Rao *et al.*, also shown that administration of fenugreek seeds 5, 10 and 20g% of diet exhibit non-significant effect on plasma ALT and AST enzymes in rats (Sebeae *et al*.,2006). These results showed that fenugreek administration possess significant hepatoprotective effects therefore it can maintain the plasma levels of liver enzymes.

Atherosclerosis also is accelerated by prolonged hyperglycemia because it provokes pathological changes in diabetic vasculature at cellular level. Hyperglycemia induce oxidative stress, it activates protein kinase C (PKC) following with altered expression of growth factor. Hyperglycemia also potentiates vascular abnormalities by promoting non-enzymatic glycation of lipids and proteins thereby increasing their atherogenicity. (Aronson *et al*., 2002). Results of present work also showed 40% reduction in blood glucose level of hyperlipidemic animals when treated with 10mg fenugreek seed powder/day for 15 days but this decrease was non-significant ($P<0.05$). In accordance to our results study of Phadnis *et al*, (2011) also showed similar hypoglycemic effects of 5g, 10g and 15g fenugreek seed powder consumption for 30 days in diabetic patients. But small amount of FsP (5g) is not effectual in lowering blood glucose significantly (Phadnis *et al.*, 2011). Several mechanisms have been identified to describe the antihyperglycemic effects of fenugreek. 4-hydroxyisoleucine (4-OH-lle), an amino acid found in fenugreek seeds, has insulin secretagogue activity (Wursch., 1997; Phadnis *et al*., 2011).

Various experimental and epidemiological studies demonstrate strong co-relation between oxidative stress and vascular disease in particular with atherosclerosis. Present study also found that atherogenic diet decrease plasma levels of SOD and catalase by (23% and 16% respectively) in hyperlipidemic group as compared to control group. Our data indicates that 10 days treatment with fenugreek significantly restore antioxidant enzymes i.e. SOD and CAT in hyperlipidemic animals. Consistent to this data several other studies also explore the antioxidant properties of fenugreek. Fenugreek seeds are rich in polyphenols which phenomenally increase antioxidant enzymes levels in hyperlipidemic animals thereby improving and regulating the antioxidant system via lowering lipid peroxidation reducing the risk of vascular diseases in these animals. Phenols activelydecrease lipid peroxidation hence regulating and improving the body's antioxidant status (Szeto *et al*., 2002; Vinson *et al*., 2002; Belguith-Hadriche *et al*., 2013). Glutathione (GSH) is an important hepatic antioxidant enzyme, GSH protect body cells from free radical damage and from other toxins. Hyperlipidemia related hepatic damage and decrease plasma GSH levels is an established phenomenon. In agreement, present study also shown hyperlipidemia related decrease in GSH levels however 15 days treatment with fenugreek seed powder significantly increase the plasma GSH levels showing hepato-protective effects of fenugreek on hyperlipidemia induced liver damage. In notion to this, study of Kumar and Bhandari, also demonstrate the hepatoprotective effects of fenugreek by increasing plasma levels of GSH, CAT and SOD thereby restoring the body antioxidant system (Harlan *et al.,* 1984; Hiraishi *et al.,* 1994; Kumar and Bhandari, 2013).

Several clinical and experimental studies revealed strong association between dyslipidemia and altered renal functioning. According to these studies in humans increase blood lipoprotein levels are autonomous risk factor for renal dysfunctions and its progression. Dyslipidemia induced renal impairments are possibly because of high oxidative stress and insulin resistant (Trevisan *et al.,* 2006). In parallel, plasma profile of hyperlipidemic animals showed marked increase in plasma urea and creatinine levels when compared with control group. However 15 days treatment of these hyperlipidemic animals with fenugreek seed powder showed decrease in urea and creatinine

levels, demonstrating renal protective effects of fenugreek. Previous studies on animal models has been demonstrated that nephroprotective effect of fenugreek is because of its antioxidant (particularly free radicals scavenging abilities) (Kaviarasan *et al*., 2007; Devi *et al*., 1999; Belaïd-Nouira *et al*., 2013) and hypoglycemic (Xue *et al.*, 2011; Belaïd-Nouira *et al.,* 2013) properties and its constructive effects on collagen which prevent ROS-induced destruction of filtration and basement membrane of kidneys (Kaviarasan *et al*., 2007; Belaïd-Nouira *et al.,* 2013).

Conclusion

The results of the present study indicate that daily administration of fenugreek seed powder can be used to control primary cases of CAD but further detailed experimental studies are recommended for its use in diagnosed patients.

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