IJMPR 2019, 3(6), 119-124

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

EFFECT OF TRIGONELLA FOENUM *GRAECUM* ON HIGH FRUCTOSE DIET INDUCED DIABETES ANDHYPERLIPIDEMIA IN ALBINO RATS

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Received on: 22/10/2019
Revised on: 12/12/2019
Accepted on: 02//01/2020

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ABSTRACT

Obesity is directly linked with cardiovascular diseases. Fenugreek significantly decreases the body weights because it's having the main chemical constituents of quercitin, 4.hydroxyisoluecine, fenugrin, trogonelline these are decreases the lipids and cardiac marker enzymes. Flavonoids, alkaloids present in the plant extract proved to have a protective effect against the fructose induced diabetic and hyperlipidemia in animals by a significant decrease in the blood glucose levels. In the present study it shows the prolonged use of fructose high diet to the animals lead to the tissue damage (pancreas), increase in the nonfunctional pancreatic cells shows that the fructose affected the pancreas from its function. Diabetes is associated with hyperlipidemia. A reduction in serum lipids, particularly of the LDL and VLDL fraction and triglycerides, should be considered as being beneficial for the long term prognosis of the patient. In the present study LDL and VLDL levels increased and the HDL levels decreased after the 28 days treatment.

KEYWORDS: Trigonella Foenum, Graecum, Fructose, Diabetes Hyperlipidemia Activity.

INTRODUTION

Plants are the foundation of existence on world and are central to people's livelihoods. India is rich in therapeutic plants. More than 2500 plant species which contain medicinal values. A huge number of medicinal plants are being exploited from the natural plants for the industrial production of drugs.[1] Our body is show a huge number of foreign chemicals every day. Usually of which are man- made, our inability to properly metabolize them negatively affects for health by the generation of free radicals. Free radicals also are generated during the normal metabolism of aerobic cells. The oxygen utilization inside in cells growth leads to the generation of series of oxygen free radicals. Extremely dynamic free radicals and their uninhibited manufacture are responsible for many pathological processes such as cell tumor (prostate and colon cancers) coronary heart diseases, bleeding wounds, constipation, dysentery, boils and mumps. Obesity is a natural consequence of over nutrition and sedentary lifestyle. Persistent obesity dysregulates metabolic processes including action of insulin on glucose-lipid- free fatty acid metabolism and severely affects processes controlling blood glucose, blood pressure, and lipids. Thus begins a cluster of conditions; dysglycemia, dyslipidemia, hypertension, and pro-coagulant state, known as the metabolic syndrome[2,3] (Grundy *et al*., 2004). Fenugreek contains different alkaloids, flavonoids and saponins (Kumar P *et al*., 2012). but out of all these, saponins are found to be in maximum concentration in the fenugreek (Singh V, Garg AN, 2006). Alkaloid is natural bases containing at least one nitrogen atom in its heterocyclic ring and is found in plants. Alkaloid and volatiles of fenugreek seed are two major constituents which causes bitter taste and bad odour due to which people try to avoid consumption of fenugreek seed and its products (Faeste CK, Namork E, Lindvik H 2009). Fenugreek contains 35% alkaloids, primarily trigonelline (Ruby BC, Gaskill SE, Slivka D, Harger SG 2005), whereas saponin was found to be 4.8% (Jani R, 2009; Rao PV *et al*., 1996), based on that phytochemicals it has several pharmacological activities such as Antidiabetic, (Kariarasan*et al*. 2009). Concluded that ethanol-induced liver cell damage can be protected by cytoprotective action of fenugreek seed polyphenolic extract possibly due to its enhancing cellular redox status.[4-6] It's contains phenolic and flavonoid compounds which help to enhance its antioxidant capacity (Dixit P, *et al*., 2005) antioxidant, hypocholesterolemic activity (Basch E *et al*.,2003) anticarcinogenic (Bhatia K,2006) anthelmintic, antiulcer activity (Pandiana RS,2002), antifertility, enzymatic pathway modifier, antihyperglycemic activity(Abdel-Barry, J.A.*et al*., 2007), Anti-inflammatory activity (Shariffara F,2009), Antineoplastic activity (Nowrasteh

G,2008),Antiplasmodial activity(Palaniswamy M,2008), Anticataract activity(VatsV, 2004), Antipyretic activity (Ahmadiani A,2001), Immunomodulatory effects (Bin-Hafeez*et al*,2003), Antiplasmodial activity (Palaniswamy*et al*.,2008) are the major medicinal properties of the fenugreek demonstrated in various studies.[7-10]

MATERIALS AND METHODS

Plant Material

Locally obtained dull yellow, smooth, hard, and elongated seeds were collected and were shade dried. The shade dried weight of the seeds was 980 gm. After the completion of drying process, it was powdered by using mixer. Fine powder was obtained by sieving. The final weight of the dried powder was noted i.e. 660 gm.

Animlas

Male Wister Albino rats (200-250g) were obtained from Mahaveer enterprises, Hyderabad, habituated for a week and maintained in the institutional animal house at 25 $\pm 2^{o}$Ctemperature with 12 hour light and dark cycle, fed with standard pellet of diet and water ad libitum throughout the experiment study.

Preparation of the Plant extract

The powder is extracted in a soxhlet apparatus with a solvent ethanol and it is pre-treated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pre-treated dry powder was obtained, as described previously. The pre-treated dry powder was extracted with deionised water, while the temperature of the water bath ranged from 70-100^{0}C and was kept steady. The entire mixture is stirred during the entire extraction process. The extract obtained was evaporated to get a concentrated drug.

Preliminary chemical characterization of the extract Leucas diffusa

The extracts prepared were tested for the type of chemical constituents present by known qualitative tests.

Induction of Diabetes and Hyperlipidemia

First of all obesity and diabetes was induced in all the groups except normal group of by administration of high fructose diet (66% in the diet) along with the standard diet for 6 weeks. The dietary ingredients were prepared by mixing the supplemented ingredients with previously triturated standard diet; therefore all diets provide sufficient amounts of vitamins, minerals, essential amino acids, and lipids. In the normal group the diet is same as the above but without fructose. The dietary ingredients were homogenized in distilled water at 60^{o}C and the homogenate was used to prepare the pellets. Diets were given fresh each day as dry pellets. Groups were fed daily with the standard 66gm of fructose in diet, after 24 hours, the uneaten remains were weighed. Body weights were determined once daily.

Collection of serum samples

After 28 days of treatment period, blood samples were collected from all the animals by tail vein puncture using fine gauze needles. For serum separation, collected blood was centrifuged using the table top centrifuge at 3000 rpm for about 10 min. supernatant i.e., serum was separated and estimated the biochemical parameters like glucose, triglycerides, total cholesterol, HDL cholesterol, SGPT, SGOT, G6PD, GGT serum creatinine, and serum total protein. The above biochemical estimations were done in a biochemical semi auto analyzer by standard procedures using commercial kits.

Estimation of glucose

The estimation of glucose was done by the Bayer Contour Ts glucose kit by tail vein puncture technique. The blood from the tail vein was introduced to the strip of the glucose meter which shows the glucose value respectively.

Estimation of lipid profile

The estimation of lipid profile was done by the EXCEL diagnostic Pvt. Ltd kit. The blood was collected from the retro orbital puncture and it was stored in the vaccutainer and centrifuged at 3000 rpm, then the serum was separated and the serum cholesterol, triglycerides, HDL, LDL, VLDL reagents were mixed respectively and it was aspirated by semi auto-analyzer in biochemistry laboratory. Finally the report was collected through a hard print copy.

Histopathological Studies

After sacrificing the animals, liver was carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from the harvested liver. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power- 40X).

Statistical Analysis

All the data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data was assessed by one-way ANOVA followed by Tukey'spost hoc testing using graph pad instant software. A probability level is less.

RESULTS

In the ethanolic extract the alkaloids, flavonoids, are rich whereas saponins, tannins, glycosides, carbohydrates, amino acids, triterpenoids are present at low levels. In water extract the alkaloids, flavonoids, saponins, and carbohydrates are rich and glycosides and amino acids are present at low levels and tannins and triterpenoids are absent and results shown in Tab.1

Tab.1: Phytochemical constituent studies.

Phytoconstituents	Ethanol	Aqueous
Alkaloids	+++	+++
Flavonoids	+++	+++
Saponins	++	+++
Tannins	++	--
Glycosides	+	+
Carbohydrates	-- +	+++
Amino acids	+	++
Triterpenoids	+	--

Tab.2: Phytochemical Results.

S. No	Groups	Body weights (gms)	Pancreas	
		Initial	Final	Weight (mg)
01.	I (Normal)	128.2 ± 0.7923	137.8 ± 0.7032	750 mg
02.	II (Diseased)	198.3 ± 1.9260	219.5 ± 2.9640	910 mg
03.	III (Control)	184.2 ± 8.1990	208.8 ± 5.9470	850 mg
04.	IV (TFG LD)	195.7 ± 1.5420	229.8 ± 1.5150	1210 mg
05.	V (TFG HD)	192.3 ± 0.7149	227.5 ± 0.7638	1090 mg

Body and organ weight Parameters

The results have shown the increase in body weights with fructose was shown in Tab.2.

Glucose Parameter

The results showed that at a significant ratio of ***P<0.001 diabetes was induced significantly (P<0.001) by high fructose diet when compared with normal group of animals and also showed that when treated with water extract and ethanolic extract showed significant (P<0.001) decrease in the fasting glucose and post prandial glucose levels in the high fructose diet fed rats.

Tab. 3: Effect of Fenugreek on Glucose.

S. No	GROUPS	BLOOD GLUCOSE	
		Fasting	Post prandial
01.	I (Normal)	82.33 ± 1.9440	103.00 ± 0.5774
02.	II (Diseased)	63.33 ± 5.0900***a	86.83 ± 3.6190***a
03.	III (Control)	71.50 ± 0.7638	99.17 ± 0.4773
04.	IV (TFG LD)	83.33 ± 1.5150***b	127.50 ± 1.3350***b
05.	V (TFG HD)	84.17 ± 1.4470***b	126.80 ± 1.0140***b

***p<0.001; "a" compared with normal: "b" compared with diseased control group.
Date was analyzed by one way ANOVA followed by post hoc Tukey's multiple Comparision "t" test.

GROUPED BLOOD GLUCOSE GRAPH

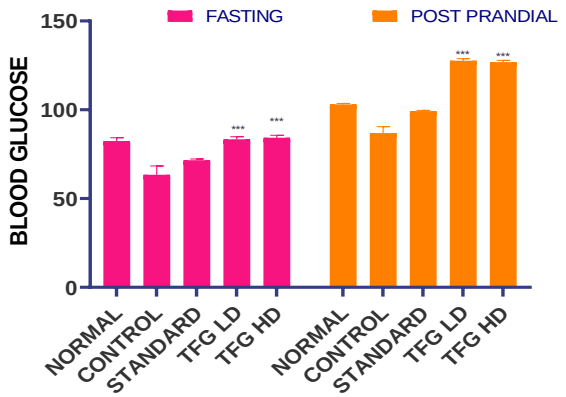


Fig.1: Effect of Fenugreek on GLUCOSE.

***p<0.001; "a" compared with Normal; "b" compared with Disease control group. Data was analyzed by one way ANOVA followed by post hoc Tukey's multiple comparision "t" tes

Lipid Profile

The results showed that at a significant ratio of ***P<0.001 hyperlipidemia was induced significantly (P<0.001) by high fructose diet when compared with normal group of animals and also showed that when treated with water extract showed significant (P<0.001) decrease in the lipid profile of the animals in the high fructose diet fed rats but in ethanolic extract treated animals the effect was not significant (P<0.01) for HDL and VLDL.

Tab.4: Effect of Fenugreek on LIPID PROFILE (mg/dl).

S .No	Groups	SC	ST	LDL	HDL	VLDL
01.	I (Normal)	26.33 ± 1.430	168 ± 4.34	38.00 ± 0.5774	40.17 ± 1.352	34.83 ± 1.515
02.	II (Diseased)	47.33 ± 6.009***a	202 ± 6.30*a	43.17 ± 0.6540***a	26.33 ± 4.477*a	39.67 ± 0.6667*a
03.	III (Control)	35.67 ± 1.145	165 ± 5.25	41.17 ± 0.3073	29.83 ± 4.665	31.50 ± 0.7638
04.	IV (TFG LD)	37.83 ± 0.3073*	177 ± 6.01*	41.50 ± 0.4282*	37.00 ± 1.155*	35.33 ± 1.116*
05.	V (TFG HD)	36.50 ± 0.7638*b	175 ± 5.72nsb	39.00 ± 0.3651nsb	36.33 ± 1.229nsb	34.50 ± 0.9916*b

All the values are expressed as Mean ± SEM of each group (n=6) and are significant when analysed by one way ANOVA with Tukey's post hoc test. 'a' P<0.05; 'b' P<0.01; 'c' P,0.001 when compared with normal control; *P<0.05 ** P< 0.01; *** P<0.001 when compared with diseased control.

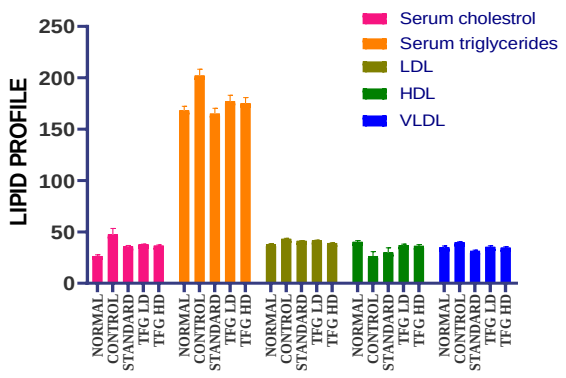
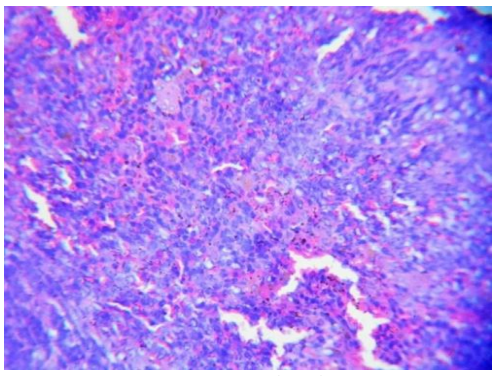


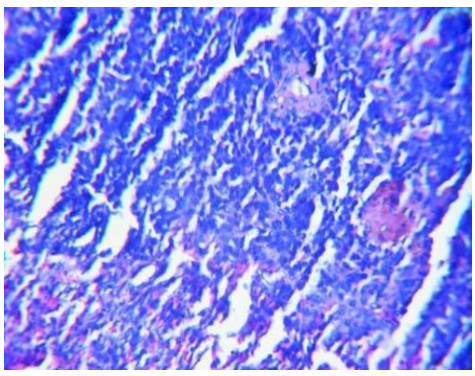
Fig. 2: Lipid profile.

Histo -Pathological Examination

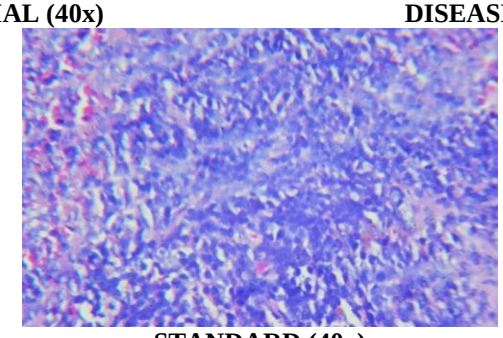
A histology of the pancreas shows acinar cells stain blue at their base because of the high content of RNA and the presence of nuclei they are pink at their apex (luminal aspects) where there is a high content of Zymogen protein (digestive enzymes). The nuclei of centroacinar cells are sometimes seen within an acinus. The presence of numerous round empty capillaries in the interstitial spaces indicates that the pancreas was perfused with fixation. (Daniel et al 2014). After 28 days of treatment when observed histologically the pancreas section of normal grouped animal was completely uniform, well organized acinar cells are present in the tissue, The diseased group animal tissue was seen broken down and nonfunctional tissue cells was found, and the control grouped animal was with blue stains of the nuclei. While in the treated animals the ethanolic and water extract show the changes in the tissue cells in both staining blue and breakage variation. Hence the treatment of the plant extract was significant.



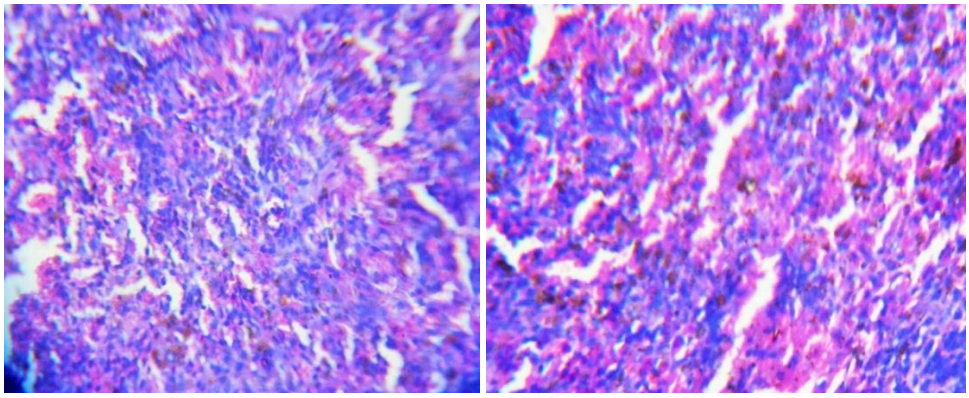
NORMAL (40x)



DISEASED (40x)



STANDARD (40x)



TFG LOW DOSE (40x) **TGF HIGH DOSE (40x)**

Fig 3: Histopathological observation.

DISCUSSION

It is quite evident that diabesity has already become a worldwide epidemic with a significant health and economic burden. Diabesity aims to identify new genes implicated in obesity and type2 diabetes and to develop strategies for validating these genes as targets for future pharmacological employment. Fructose has been regarded as an acceptable caloric sweetener for diabetic subjects for two decades, small catalytic amounts of fructose seem to improve glucose tolerance in healthy and especially in diabetic patients but high fructose diets have been postulated to cause hypertension, insulin resistance, hyperlipidemia and hyper insuliaemia. Hoebel in 2010 suggests that these latest findings contribute to the theory that high fructose corn syrup may be a significant contributing factor to the obesity epidemic. While in the experiment, found that fructose raises the body weights in the animals which are dieted with high fructose when compared to the normal group of animals. In the present study it shows the prolonged use of fructose high diet to the animals lead to the tissue damage (pancreas), increase in the nonfunctional pancreatic cells shows that the fructose affected the pancreas from its function. When the animals were sacrificed at the last day and weighed the pancreas in the wet condition, mass of the pancreatic gland was observed increased and when histopathologically observed stains of blood cells were observed in the tissues.

Regulation of blood glucose level in diabetes can prevent the various complications associated with the disease. Medicinal plants are used in several countries to manage diabetes mellitus which are thought to be less toxic than allopathic hypoglycemic drugs.

The administration of fructose at a dose of 60gm/kg body weight to the rats of group II,III,IV,V,VI,VII, produced stable diabetic condition within 6 weeks in the most of the experimental rats. Administration of the extract resulted in the decrease in the hyperglycemia in treated animals and it is significantly proved. It is suggestive that regeneration of the βcells since the pancreas has been reported to contain stable cells that have the capacity of regeneration to replace the lost cells. By which the treated animals show the positive effect with extract. Flavonoids, alkaloids, tannins as polyphenolic compounds, have been associated with hypoglycemic activity. Diabetes is associated with hyperlipidemia. A reduction in serum lipids, particularly of the LDL and VLDL fraction and triglycerides, should be considered as being beneficial for the long term prognosis of these patients. In the present study LDL and VLDL levels increased and the HDL levels decreased after the 28 days treatment.

CONCLUSION

The animals treated with the high fructose diet was resulted in the raise in their body weights which indicates the fructose in low dose will help in metabolism of the carbohydrates but in the high amounts it causes insulin resistance which results in the accumulation of the net energy which causes obesity followed by diabetes which is now known as diabesity. Flavonoids, alkaloids present in the plant extract proved to have a protective effect against the fructose induced diabetic and hyperlipidemia in animals by a significant decrease in the blood glucose levels.Based on the earlier reports and the results of the present study; it was concluded that the presence of above compounds in the plant might be responsible for its protective role in the fructose induced animals.From the results of present study, it was concluded that the Trigonella foenum-groecum having the antidiabetic and antihyper lipidemic activity in the high fructose diet fed animals.

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