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Research Article

COMBINED EFFECT OF HORSE GRAM AND FENUGREEK SEED POWDERS IN HIGH FAT-HIGH SUCROSE DIET-INDUCED PRE-DIABETES IN RATS

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ABSTRACT

Objective: The objective of the study was to investigate the combined effect of horse gram (HG) and fenugreek (FG) seed powders in high fat-high sucrose (HFHS) diet-induced pre-diabetes in rats.

Methods: Wistar rats (140–200 g) of either sex were randomly divided into four groups (n=6). Group 1, normal control, received normal pellet diet; Group 2, pre-diabetic control, received HFHS diet; Group 3, prophylactic group, received HFHS diet along with HG (200 mg/kg) and FG (200 mg/kg) combination; and Group 4, therapeutic group, received HFHS diet for 9 weeks followed by HG (200 mg/kg) and FG (200 mg/kg) combination for 28 days. Fasting blood glucose, lipid profiles, and OGTT were carried out on the 9th week in Group 1, Group 2, and Group 3, and at the end of the study in Group 1, Group 2, and Group 4.

Results: Treatment with HFHS diet for 9 weeks has significantly increased fasting blood glucose and lipid profiles in the pre-diabetic group when compared to the normal control group indicating induction of pre-diabetes in the pre-diabetic group. In the prophylactic group, horse gram and fenugreek are given simultaneously with HFHS has significantly decreased fasting blood glucose, lipid profiles when compared to the pre-diabetic group. In the therapeutic group, treatment was given after induction of pre-diabetes for 28 days. In the therapeutic group, there was a significant decrease in fasting blood glucose, lipid profiles compared to the pre-diabetic group.

Conclusion: HFHS diet-induced pre-diabetes or insulin resistance is an effective model to study and evaluate various new therapeutic modalities. The findings of the present study suggest that consumption of FG and HG as food supplements could reduce the risk of getting diabetes and progression of pre-diabetes to diabetes.

Keywords: Pre-diabetes, OGTT, Insulin resistance, Horse gram, Fenugreek.

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INTRODUCTION

Pre-diabetes is a metabolic disorder with glycemic parameters slightly above normal but not reaching up to the level of diabetes threshold [1]. It is a state of intermediate hyperglycemia, characterized by impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 6.1–6.9 mmol/L (110–125 mg/dL), impaired glucose tolerance (IGT) set as 2 h plasma glucose of 7.8–11.0 mmol/L (140–200 mg/dL) after ingestion of 75 g of an oral glucose load [2]. It is estimated that worldwide 6.7% of the adult population have intermediate hyperglycemia. The prevalence of intermediate hyperglycemia is increasing worldwide, and it is expected that more than 470 million people will have intermediate hyperglycemia in 2030 [3]. It has been estimated that around 70% of persons with prediabetic states gradually develop diabetes. Individuals with both IFG and IGT have approximately double the rate of developing diabetes compared with those with just one of them [4,5].

In multiple studies, prediabetes has been demonstrated as a toxic environment for the initiation of microvascular and macrovascular complications and has also been shown to have a cause-effect relationship to cardiovascular disease and all-cause mortality. A cohort meta-analysis by Cai *et al*. reported that "prediabetes was associated with an increased risk of all-cause mortality and cardiovascular disease in the general population and patients with atherosclerotic cardiovascular disease" [6,7].

Insulin production is boosted by β-cells in the prediabetic state to compensate for peripheral insulin tolerance, which leads to incremental disruption to insulin-producing pancreatic β-cells. Early-phase insulin secretion is reduced in both IFG and IGT, and late-phase insulin secretion is also compromised in subjects with IGT. Detecting and treating prediabetes early improves insulin sensitivity and restores insulin-producing β-cells [8]. A study conducted in prediabetic patients reported a reduction in the incidence of Type 2 diabetes with lifestyle interventions and metformin treatment. Treating prediabetes improves insulin resistance and decreases the incidence of diabetes and associated secondary complication [9]. Hence, screening and appropriate management of prediabetes might contribute to primary and secondary prevention of cardiovascular diseases.

Patients with poor glucose control could benefit from a low-cost, low-risk food-based intervention aimed at restoring normalcy to their metabolic environment. Fenugreek and horse gram seed powders are two examples of traditional culinary remedies for diabetes reduction. Fenugreek (Trigonella foenum-graecum) seeds contain 4-hydroxy isoleucine, fenugreekine, trigonelline, trigoneoside, and galactomannan, which have hypoglycemic properties that delay gastric emptying, slow carbohydrate absorption, and inhibit glucose transport, as well as increase erythrocyte insulin receptors and peripheral glucose utilization, induce insulin secretion, and enhance glucose absorption into cells [10]. In underdeveloped nations, Macrotyloma uniflorum (horse gram) is one of the legumes with excellent nutritional and ethnomedicinal benefits. Bioactive compounds found in M. Uniflorum seeds include phytic acid, phenolic acid, fiber, and enzymatic/proteinase inhibitors, all of which have significant metabolic and physiological effects [11]. They lower blood glucose levels by inhibiting - amylase and also have a hypolipidemic effect [12]. Several studies have reported the hypoglycemic and hypolipidemic effects of fenugreek and horse gram seeds in animal and human models of type 1 and type 2 diabetes, there have been no research on prediabetes. The current study examines

the ability of a mixture of fenugreek and horse gram seed powders to protect rats from developing prediabetes as a result of a high fat-high fructose diet.

METHODS

Marketed powdered seeds of *Macrotyloma uniflorum* (Horse gram) and *Trigonella foenum graecum* (Fenugreek) were procured from the local Ayurvedic store. Sucrose was purchased from the local Market. The assay kits for the estimation of glucose, triglycerides, high-density lipoproteins, and total cholesterol were purchased from Span diagnostic Pvt. Ltd.

Experimental animals

Wistar rats (180–250 g) of either sex were obtained from the National Institute of Nutrition, Hyderabad, and housed in a temperature and relative humidity regulated environment for 1 week in a 12:12 h light and dark cycle. The animals were given unrestricted access to food and water. Animals were included in the research after a week of acclimatization. Experiments were carried out in compliance with the protocols approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (320/CPCSCEA). The Institutional Animal Ethics Committee (GPRCP/IAEC/07/17/01/PCL/AE-5- RATS-M/F-30), G. Pulla Reddy College of Pharmacy, Hyderabad, India, reviewed and accepted the protocols.

Experimental design

Animals were divided into four groups (n=6). Group1, normal control, received normal diet; Group 2, pre-diabetic control, received HFHS diet; Group 3, Prophylactic group, received HFHS diet along with HG (200 mg/kg) and FG (200 mg/kg) combination; Group 4, Therapeutic group, received HFHS diet for 9weeks followed by HG (200 mg/kg) and FG (200 mg/kg) combination for 28 days.

Pre-diabetes was induced by oral feeding of HFHS diet for 9 weeks. High-fat diet was given by daily oral feeding of vanaspati (3 ml/kg) and coconut oil (1 ml/kg). High sucrose diet consists of 35% w/v sucrose in drinking water was continuously fed to rats for 9 weeks.

Biochemical analysis

Biochemical parameters were estimated in overnight fasted rats and blood was withdrawn from retro-orbital plexus after the research period. After 30 min, blood samples were centrifuged at 6000 rpm for 10 min and serum was separated. Serum samples were used for biochemical analysis. Body weights were estimated weekly. Blood glucose, triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and alanine transaminase (ALT) were estimated according to the procedure of the kits supplied by Span diagnostic, at the end of treatment intervention. An automated bioanalyzer having a 490–550 nm filter was used for the estimation of all biochemical parameters. LDL and VLDL cholesterols were calculated using Friedewald equation [13]: LDL-cholesterol (mg/dl) = Total Cholesterol – Triglyceride/5 – HDL VLDL Cholesterol (mg/dl) = Triglycerides/5.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) is a well-established indicator of glucose sensitivity [14]. Blood samples were obtained using retro-orbital puncture at 0, 30, 60, and 120 min after an oral glucose load of 2 g/kg p.o was given to 6-h fasted rats. The AUTO SPAN glucose assay kit was used to determine the glucose content in samples. GraphPad Prism Software (7.0 version) was used to map serum glucose levels against time and measure the region under the curve (AUC/mg-hr/L) and rate of glucose disappearance (KOGTT/min-1).

Statistical analysis

Data expressed as mean±SEM were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism Software (7.0 version). $p<0.05$ were considered statistically significant.

RESULTS

Fasting blood glucose

In the present study, 9 weeks of treatment with HFHS diet has significantly increased FBG in the pre-diabetic group when compared to the normal control group indicating induction of pre-diabetes in the pre-diabetic group. Prophylactic treatment with the combination of HG and FG seed powder for 9 weeks has significantly decreased FBG when compared to the pre-diabetic group. Similarly in the therapeutic group, 28 days of treatment with HG and FG seed powder combination has significantly decreased FBG when compared to the pre-diabetic group (Tables 1 and 2).

Lipid profile

In the present study, 9 weeks of treatment with an HFHS diet have significantly increased plasma lipid levels as measured by triglycerides, HDL, LDL, VLDL, and, total cholesterol in the pre-diabetic group when compared to the normal control group indicating induction of pre-diabetes in the pre-diabetic group. Prophylactic treatment with the combination of HG and FG seed powder for 9 weeks has significantly decreased all lipid parameters when compared to the pre-diabetic group. Similarly in the therapeutic group, 28 days of treatment with HG and FG seed powder combination have significantly decreased all lipid parameters when compared to the pre-diabetic group (Table 3 and 4).

Oral glucose tolerance test

Oral feeding with 2 g of glucose in normal control, blood glucose levels have been restored to normal levels by 2 h whereas in the pre-diabetic control group and blood glucose levels have not been restored to the normal level. However, in treatment with HG and FG seed powder combination, blood glucose was restored to normal level in both the prophylactic and therapeutic groups (Figs. 1 and 2).

DISCUSSION

The primary goal in the management of pre-diabetes is to normalize glucose levels and prevent progression to diabetes and associated secondary complications [15].

Many interventions have been investigated and suggest that early identification and treatment of pre-diabetes could reduce the risk of progression to diabetes and cardiovascular complications.

Herbal medicines have been considered as a safe alternative for therapeutic usage. A significant number of studies reported that treatment with HG and FG seed powder effectively controls diabetes [16]. Findings of the previous studies reported that treatment

Table 1: Prophylactic effect of HG and FG seed powder combination on FBG in HFHS diet-induced pre-diabetes in rats

Groups (n=6)	Fasting blood glucose (mg/dL)
Normal control	69.3±4.626
Pre-diabetic control	118.5±7.453$^{\alpha}$
Prophylactic group	78.68±5.405^{b}

Data expressed as mean±SEM (n=6). The data were analyzed by ANOVA followed by Tukey's multiple comparisons test. $^{\alpha}p<0.0001$ compare with normal control, $^{b}p<0.001$ compare with pre-diabetic control

Table 2: Therapeutic effect of HG and FG seed powder combination on FBG in HFHS diet-induced pre-diabetes in rats

Groups (n=6)	Fasting blood glucose (mg/dL)
Normal control	77.95±5.273
Pre-diabetic control	131.4±3.375$^{\alpha}$
Therapeutic group	62.92±7.149^{a}

Data expressed as mean±SEM (n=6). The data were analyzed by ANOVA followed by Tukey's multiple comparisons test. $^{\alpha}p<0.0001$ compare with normal control, $^{a}p<0.0001$ compare with pre-diabetic control

Table 3: Prophylactic effect of HG and FG seed powder combination on lipid profile in HFHS diet-induced pre-diabetes in rats

Groups (n=6)	Triglycerides (mg/dL)	High density lipoprotein (mg/dL)	Low density lipoprotein (mg/dL)	Very Low density lipoprotein (mg/dL)	Total cholesterol (mg/dL)
Normal control	102.5±1.03	47.92±1.565	15.8±0.8261	20.5±0.20	84.22±1.843
Pre-diabetic control	146.1±1.12[α]	67.16±2.616[α]	38.08±1.64[α]	29.22±0.224[α]	134.5±3.886[α]
Prophylactic group	94.2±0.607[a]	51.87±1.951[a]	12.62±0.951[a]	18.84±0.121[a]	83.34±1.507[a]

[α]p<0.0001 compare with normal control; [a]p<0.0001 compare with pre-diabetic control

Table 4: Therapeutic effect of HG and FG seed powder combination on lipid profile in HFHS diet-induced pre-diabetes in rats

Groups (n=6)	Triglycerides (mg/dL)	HDL (mg/dL)	Low Density Lipoprotien (mg/dL)	Very Low Density Lipoprotein (mg/dL)	Total Cholesterol (mg/dL)
Normal control	111.9±10.56	43.01±1.729	15.89±0.8186	22.38±2.113	81.27±3.797
Pre-diabetic control	150.5±5.984[α]	55.46±0.7382[α]	34.3±2.37[α]	29.77±1.069[α]	119.5±3.487[α]
Therapeutic group	112.2±5.698[a]	48.4±1.22[a]	14.19±1.96[a]	22.43±1.14[a]	85.03±2.09[a]

[α]p<0.0001 compare with normal control: [a]p<0.0001 compare with pre-diabetic control

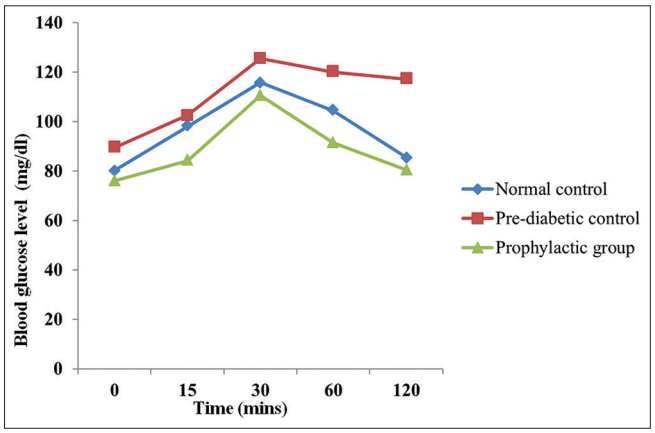

Fig. 1: Prophylactic effect of HG and FG seed powder combination on OGTT in HFHS diet-induced pre-diabetes in rats

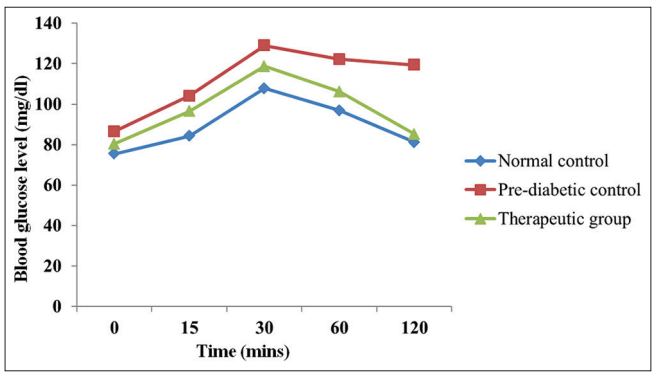

Fig. 2: Therapeutic effect of HG and FG seed powder combination on OGTT in HFHS diet-induced pre-diabetes in rats

with HG seed powder decreases lipid profiles and FG seeds stimulate insulin secretion and enhances glucose uptake into muscle cells [17,18]. Hence, the present study was aimed to investigate the combined effect of FG and HG seed powders in pre-diabetes in rats.

HFHS diet for 9 weeks has been shown to induce pre-diabetes characterized by an increase in FBG and lipid profile. This lowers insulin sensitivity and increases plasma triglyceride levels (hypertriglyceridemia) by increasing the formation of glycerol-3phosphate, a precursor of lipid synthesis [16,19]. In the present study, HFHS diet for 9 weeks was used to induce pre-diabetes in rats.

Many studies have demonstrated that consumption of HFHS diet for 9 weeks in rats induces a pre-diabetic state characterized by impaired glucose tolerance, FBG, and increased lipid profiles [20,21]. In the present study, pre-diabetes was induced by an HFHS diet for 9 weeks in rats. Induction of pre-diabetes was characterized by measuring OGTT (Indicator for IGT), FBG level, and lipid profiles.

According to the literature, oral fenugreek seed powder has hypoglycemic and anti-hyperlipidemic activity [22]. According to the literature, HG seed powder can reduce postprandial hyperglycemia by slowing down carbohydrate digestion and reducing insulin resistance by inhibiting protein-tyrosine phosphatase 1 beta enzyme [23,24].

In the present study, treatment with HG and FG seed powder showed a significant decrease in elevated FBG levels. In support of that report, Eidi *et al*., 2007, had reported that treatment with FG and HG has shown a significant decrease in elevated FBG levels [25].

In the present study, blood glucose level was not restored to normal level in pre-diabetic control but with the treatment with HG and FG combination restored to the normal level. In support of that report, Chaturvedi *et al*., 2004, had reported that improved OGTT curve after prolonged treatment [26].

According to the literature, insulin resistance is accompanied by lipid metabolism disorders. Therefore, the parameters that are widely used for diagnosis are the total content of TG, TC, LDL, VLDL, and HDL. Elevated serum TG, TC, LDL, VLDL, and HDL cholesterol contents were observed in HFHS treated rats consistent with other reports [27,28].

In the present study, treatment with HG and FG seed powder showed a significant decrease in TC, TG, LDL, VLDL, and HDL levels. In support of that report, Kumar *et al*., 2013, and Gaddam *et al*., 2015, had reported that treatment with FG and HG has shown a significant decrease in elevated TC, TG, LDL, VLDL, and increase in HDL levels [10,29]. Further studies are required to investigate the reason behind the increase in HDL levels in the present study.

CONCLUSION

HFHS diet-induced pre-diabetes or insulin resistance is an effective model to study and evaluate various new therapeutic modalities. Pre-diabetes in Wistar rats are characterized by FBG, lipid profiles, and glucose intolerance during OGTT which represent clinical and pathophysiology of pre-diabetes.

The findings of the present study suggest that consuming HG and FG seeds as food supplements could reduce the risk of getting diabetes and progression of pre-diabetes to diabetes.

AUTHOR CONTRIBUTION

R. Padmavathi designed the work and made necessary corrections and, revisions in the manuscript. G. Soumya collected the content and performed the literature review and also contributed to writing the manuscript. Both the authors drafted the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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