ORIGINAL ARTICLE

Anti-hyperglycemic and anti-hyperlipidemic effect of poly-herbal formulation in streptozotocin-nicotinamide induced diabetic rats

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Abstract

Introduction: Diabetes mellitus is one of the important metabolic diseases, and the percentage of affected population is increasing day by day. The search for better medicament is the need of hour. **Aim:** Poly-herbal formulation (PF) containing fourteen plant materials is studied for anti-hyperglycemic and anti hyperlipidemic effect. **Material and methods:** A total of 36 rats were taken for the study. The rats of either sex randomly divided into five groups, among these five groups, four groups contained 6 animals in each, whereas one group, i.e., normal control groups contain 12 animals. Group one (control) animals were treated with carboxy methyl cellulose, whereas group two animals were treated with streptozotocin (STZ) to induce diabetes. Group three animals were treated with 300 mg dose test drug and group four treated with 600 mg of the test drug. Whereas group five was treated with a known anti-diabetic drug, i.e., glibenclamide 10 mg. **Result:** It is found that the testing drug PF is a novel medicine having significant anti hyperglycemic effect and anti hyperlipidemic effect. No significant change was found in HDL level. **Conclusion:** PF is having good antidiabetic effect against STZ-nicotinamide induced model of diabetic rats.

Key words: Diabetes, poly-herbal formulation, streptozotocin-nicotinamide

INTRODUCTION

Diabetes mellitus is one of the important metabolic diseases, and the percentage of affected population is increasing day by day. Diabetes mellitus fall into three broad categories, i.e., Type 1, Type 2, and gastrointestinal diabetes. In 2011, India had 62.4 million people with Type 2 diabetes, compared with 50.8 million the previous year, according to the International Diabetes Federation and the Madras Diabetes Research Foundation.[1] Diabetes without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, and retinal damage. Adequate treatment of diabetes is thus important. The search for better recipes is needed. Hence in the present pre-clinical study, poly-herbal formulation (PF) containing 14 plant materials were studied for anti-hyperglycemic and antihyperlipidemic effect. There are several reports about the beneficial effects of constituent plant materials of this PF in the management of diabetes. The hypoglycemic effect was observed in the plants such as *Pterocarpus marsupium, Gymnema sylvestre, Sida cordifolia, Mangifera indica, Syzygium cumini, Acacia arabica, Allium cepa, Trigonella foenum, Curcuma longa, Momordica charantia, Azadirachta indica, Emblica officinalis, Terminalia chebula,* and *Terminalia bellirica.* This study is a step made to elucidate the synergetic effect of above cited fourteen plant material containing polyherbal powder dosage form.

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MATERIALS AND METHODS

Animals

Adult Charles Foster albino rats (170 ± 10 g) of either sex were used in the study. Rats were obtained from the Central Animal House of the Institute of Medical Science, Banaras Hindu University, Varanasi. The rats were provided with commercial food pellets and water. Rats were acclimatized to laboratory condition for at least 1 week before using them for experiments. "Principles of laboratory animal care" (NIH publication number 85-23, revised 1985) guidelines were followed. Protocols of the study were approved by Animal Ethical Committee (Letter No. Dean/2011-12/386).

Drugs and Chemicals

PF prepared in Department of Rasa Shastra was used. Glibenclamide (GL) was purchased from Cipla Pharmaceutical Pvt. Ltd. India was used as the standard anti-diabetic agent. Streptozotocin (STZ) was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Nicotinamide was purchased from SD Fine Chem., Mumbai, India.

Biochemical kits used for measurement of plasma glucose, cholesterol, and triglyceride (TG) were procured from Span Diagnostics Ltd. India.

All other chemicals used were of analytical grade.

Animal Grouping

A total of 36 rats were taken. The rats of either sex were randomly divided into groups of six animals each except for control, in which 12 rats of either sex in equal ratio were taken.

Induction of Type 2 Diabetes Mellitus in Rats

Non-insulin dependent diabetes mellitus/Type 2) was induced in overnight fasted animals by a single intraperitoneal (i.p.) injection of 65 mg/kg (STZ/calbiochem), 15 min after the i.p., administration of 120 mg/kg nicotinamide.[2] Hyperglycemia was confirmed by the elevated glucose level in the blood, determined at 72 h and then on day 7 after STZ injection. Rats with fasting blood glucose level 200 ± 20 mg/dl were used for the study.

Methods

1. 5 days prior to start of the experiment, six rats were housed per case at 24°C ± 1°C and 55 ± 5% relative humidity, with a 12 h light-dark cycle (light on at 8:00 and light off on at 20:00) rats were allowed free access to food and water.
2. All the rats were weighed accurately to 1 g and randomly divided into different groups including control and study groups. An equal number of rats were allocated to each group.
3. On experimental day 1, all the rats were fasted for 6-8 h prior to nicotinamide-STZ treatment. Water was provided as usual.
4. Sodium citrate buffer (50 mM; pH 4.5) was prepared and 1 ml of the buffer was transferred into each 1.5 ml microcentrifuge tube and the tube was covered with aluminum foil.
5. Immediately prior to injection, STZ was dissolved into 50 mM of sodium citrate buffer (pH 4.5) to a final concentration of 10 mg/ml. The STZ solution was freshly prepared for each rat and was injected within 5 min after being dissolved.
6. STZ solution was injected intraperitoneally using 3 ml syringe and 23 G needle, at 65 mg/kg, 15 min after the i.p., administration of 120 mg/kg nicotinamide. An equal volume of citrate buffer (pH 4.5) was injected intraperitoneally for the control group.
7. Rats were returned to their cages and were provided normal food and 10% sucrose water to minimize risk of hypoglycemic shock.
8. On experimental day 2, 10% sucrose water was replaced with normal drinking water.
9. On experimental day 7, rats were fasted for 6-8 h (between 7 a.m. and 1-3 p.m.) and blood samples were collected. Blood glucose level will be estimated. The animals with blood glucose concentration 200 ± 20 mg/dl were used for this study.

Experimental Design

The rats were allocated into different treatment groups after the induction of diabetes including control and standard groups are follows:

Group I: Normal control (vehicle-treated)
Group II: Diabetic control (vehicle-treated)
Group III: Diabetic rats + 300 mg PF
Group IV: Diabetic rats + 600 mg PF
Group V: Diabetic rats + standard (GL 10 mg/kg/day, p.o.).

Treatment was started on the 7th day after induction of diabetes (day 1 of treatment). The PF was orally administered in the form of a suspension in 0.3% carboxymethylcellulose, once daily for 15 consecutive days.

Biochemical Parameters

On day 15 after appropriate fasting, blood samples were collected from the retro-orbital venous plexus under light ether anesthesia using a glass capillary tube. Plasma was separated, and samples will be analyzed for glucose, total

cholesterol (TC), TG, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL) levels using commercially available biochemical kits.

Statistical Analysis

The data of all experiments were expressed as mean ± standard error of mean (SEM) of animals in each group. Differences among different treatment groups were determined by one-way analysis of variance followed by Tukey's multiple comparison tests. Graph Pad Prism (Version 5.03) was used for statistical analysis.

RESULTS

Effect on Fasting Blood Glucose Level

On 0th day of the treatment fasting blood glucose level of animals challenged with nicotinamide- STZ were significantly ($P < 0.05$) higher compared to the normal control animals. In the diabetic control group, blood glucose level remained elevated until the 15th day of the experiment. Repeated oral dose administration for 15 days of PF (300 and 600 mg/kg/day) and standard drug, i.e., GL (10 mg/kg/day) to diabetic rats significantly ($P < 0.05$) reduced fasting blood glucose level compared to vehicle treated diabetic rats. Furthermore, unlike the PF 600 mg/kg/day and GL 10 mg/kg/day treated diabetic rats, PF 300 mg/kg/day treated diabetic rats exhibited significantly ($P < 0.05$) higher fasting blood glucose level in comparison to the normal control rats [Figure 1].

Effect on Lipid Profile of Diabetic Rats

On the 15th day of post-treatment, diabetic control rats showed significant ($P < 0.05$) elevation in plasma TG, TC, LDL cholesterol, and VLDL cholesterol level. Whereas the plasma HDL-cholesterol level was significantly ($P < 0.05$) decreased in comparison to normal control animals. Although the repeated dose administration for 15 days of PF (300 and 600 mg/kg/day) and GL (10 mg/kg/day) in diabetic rats significantly ($P < 0.05$) reversed these change in plasma lipid profile when compared with diabetic control rats but, in comparison with normal control rats, between PF (300 and 600 mg/kg/day) and GL (10 mg/kg/day), only diabetic rats treated with GL (10 mg/kg/day) showed non-significant difference in TC and LDL levels and in PF (600 mg/kg/day) group non-significant changes were seen in HDL level [Table 1].

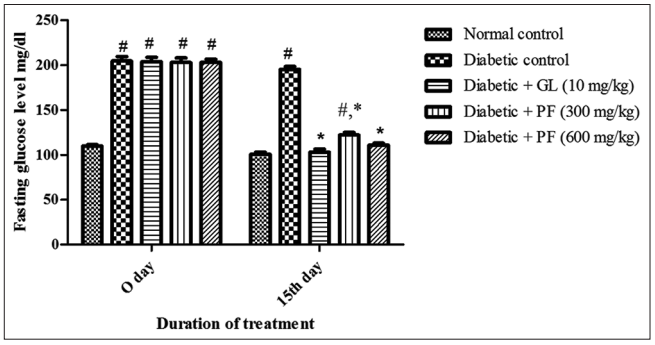

Figure 1: Effect of PF on blood sugar level in nicotinamide-streptozotocin induced diabetes in rats. GL: Glibenclamide, PF: Poly-herbal formulation. Values are mean±standard error mean, *n*=6 (except for normal control, where *n*=12). Statistical analysis performed by one-way analysis of variance followed by Tukey's multiple comparison tests; #*P*<0.05 versus normal control, **P*<0.05 versus diabetic control

DISCUSSION

In our study, rats subjected to nicotinamide-STZ challenge showed a significant increase in plasma glucose level. It is well established that GL produces hypoglycemia by increasing the secretion of insulin from the existing pancreatic beta cells.[3] Researchers involved in herbal based anti-diabetic drug development also reported that the treatment of moderate diabetic rats with medicinal plant material resulted in stimulation of beta cells of islet of Langerhans, showing an insulinotropic effect.[4] In present study likewise GL, repeated oral administration of PF significantly lowered the fasting blood glucose level in nicotinamide- STZ induced diabetic rats. This is strongly suggesting that like many therapeutically used anti-diabetic drugs, this PF has an effect on secretion of insulin release from remnant beta cells or/and have anti-hyperglycemic action through an extrapancreatic mechanism.

Diabetes is associated with a marked imbalance in lipid metabolism.[5] Diabetic dyslipidemia is characterized by low level of HDL as well as the elevated level of TG, TC, LDL and VLDL particles.[6] A significant increase in plasma cholesterol, TG, LDL and VLDL particle along with significant decrease in HDL, was observed in diabetic rats in the present study, are consonant with the pathogenesis of diabetes. Decreased insulin output in diabetic rats is reported to impair adipocyte metabolism, resulting in increased lipolysis and elevated fatty acid level.[7] In our study, treatment with PF and GL significantly lowered plasma cholesterol, TG, LDL and VLDL level while HDL-cholesterol was significantly increased, which seems potentially beneficial in dyslipidemia associated with diabetes.

Moreover, STZ is associated with the generation or reactive oxygen species causing oxidative damage that culminates in beta cell destruction through the induction of apoptosis and suppression of insulin biosynthesis.[8] Induction of diabetes in rats with STZ results in an increase of lipid peroxidation[9] and decreased the activity of superoxide dismutase and catalase which subsequently lead to a number of deleterious effects.[10] It is well-known that antioxidant treatment can reduce the

Table 1: Effect of PF on lipid profile of diabetic rats

Groups	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	50.5±1.38	88.75±1.26	43.58±0.90	35.07±1.74	10.1±0.28
Diabetic control	131.17±2.09$^{\#}$	148.83±1.19$^{\#}$	25.33±0.80$^{\#}$	97.27±1.20$^{\#}$	26.23±0.42$^{\#}$
Diabetic+GL (10 mg/kg)	76.67±1.87$^{\#,*}$	83.67±2.06*	34.67±1.05$^{\#,*}$	33.67±2.71*	15.33±0.37$^{\#,*}$
Diabetic+PF (300 mg/kg)	105±2.02$^{\#,*}$	136.67±1.82$^{\#,*}$	39.33±0.76$^{\#,*}$	76.33±1.44$^{\#,*}$	21±0.40$^{\#,*}$
Diabetic+PF (600 mg/kg)	74.33±1.36$^{\#,*}$	124.50±1.84$^{\#,*}$	44.50±0.76*	63.97±1.82$^{\#,*}$	14.87±0.27$^{\#,*}$

GL: Glibenclamide, PF: Polyherbal formulation, values are mean±standard error mean, n=6 (except for normal control, where n=12). Statistical analysis performed by one-way analysis of variance followed by Tukey's multiple comparison tests; $^{\#}P$<0.05 versus normal control, $^{*}P$<0.05 versus diabetic control, LDL: Low-density lipoprotein, TG: Triglyceride, TC: Total cholesterol, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

oxidative load and lipid peroxidation in diabetic subjects as well as in animals. Chemical with anti-oxidant properties and free radical scavenging activity have been shown to prevent pancreatic islets against cytotoxic effects of STZ.[11] Increasing numbers of studies have shown a beneficial effect of various phytochemical in STZ-induced diabetes owing to their anti-oxidant properties.[10] All the ingredients of this PF have been reported as they possess hypoglycemic and hypolipidemic activity.

CONCLUSION

In the view of findings of the pre-clinical experiments, it is concluded that this PF is a novel formulation effective against nicotinamide-STZ induced diabetes in rats. The 1st time, we reported that this PF possess potential hypoglycemic and antihyperlipidemic effect against diabetic rats.

REFERENCES

1. India's Diabetes Time Bomb. Epigenetics and lifestyle are conspiring to inflict a massive epidemic of type 2 diabetes in the subcontinent. Nature 2012;485:S14-6.
2. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, *et al*. Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998;47:224-9.
3. Proks P, Reimann F, Green N, Gribble F, Ashcroft F. Sulfonylurea stimulation of insulin secretion. Diabetes 2002;51:S368-76.
4. Atta-Ur-Rahman, Zaman K. Medicinal plants with hypoglycemic activity. J Ethnopharmacol 1989;26:1-55.
5. Gadi R, Samaha FF. Dyslipidemia in type 2 diabetes mellitus. Curr Diab Rep 2007;7:228-34.
6. Cefalu WT. Diabetic dislipidimia and the metabolic syndrome. Diabetic and the metabolic syndrome. Clin Res Rev 2008;2:212-22.
7. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840-6.
8. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.
9. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003;17:24-38.
10. Punithavathi VR, Prince PS, Kumar R, Selvakumari J. Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats. Eur J Pharmacol 2011;650:465-71.
11. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res 2005;51:117-23.

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