Effect of Polyherbal Extract on Diabetic Rats

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ABSTRACT
Objective: To study and compare the effect of Poly herbal plant extract (PHPE) with Glibenclamide (GL) on various parameters in Streptozotocin (STZ) induced diabetic rats. Methods: Diabetes was induced by combining High Fat-diet and injecting low dose Streptozotocin (35 mg/kg body wt.) to Sprague-dawley rats. Diabetic rats were treated with chloroform leaf extract of Azadirachta indica, aqueous leaf extract of Bougainvillea spectabilis and ethanolic seed extract of Trigonella foenum graecum combined in ratio of 1:2:3 respectively at dose of 600 mg/kg body weight by oral gavaging for 28 days. The results were compared with standard anti-diabetic drug Glibenclamide given in dose of 500 µg/kg body weight. Results: Increase in body weight of both PHPE and GL treated diabetic rats was found to be statistically significant ($p<0.05$) compared to diabetic control rats. Decline in FBG levels of both PHPE and GL treated diabetic rats were found to be highly significant statistically ($p<0.001$) when compared to diabetic control rats at the end of study. Total Cholesterol (TC) and Triglycerides level in diabetic rats treated with PHPE were found to be highly statistically significant ($p<0.001$) compared to diabetic control rats. Pancreas of PHPE treated diabetic rats revealed partial restoration in size and number of islet of langerhans. Reduction in widening between acinar and islet cells noted. Glibenclamide treated diabetic rats showed much more improvement in pancreatic cell architecture by returning to its normal structure and size. Conclusion: In present study PHPE has shown to decrease elevated FBG level and improve in body weight at the end of study in diabetic rats which can be suggested due to modification in carbohydrate metabolic pathways, stimulation of insulin production by the pancreas, increased peripheral utilization of glucose in the cells and regeneration of β-cells of the pancreas.
Keywords: Poly herbal plant extract, Glibenclamide, Fasting blood glucose, Total Cholesterol.

INTRODUCTION

Diabetes mellitus is one of the most common endocrine diseases, associated with a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, lipids, protein metabolism, glycosuria, ketosis and acidosis resulting from defects in insulin secretion, insulin action or both. The effect of Diabetes mellitus includes long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.

Diabetes is a disease of global distribution affecting individuals of all ages with widely varying prevalence rates of 150 million in 2010 which is predicted to double by 2025 to 300 million. Oral hypoglycemic agents are useful in the treatment of diabetes mellitus but their use is restricted by their pharmacokinetic properties, secondary failure rates, and accompanying side effects and the World Health

Organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated.

In ancient literature, more than 800 plants are reported to have antidiabetic properties. Ethano pharmacological surveys indicate that more than 1200 plants are used in traditional medicine for hypoglycaemic activity. Ancient Indian medicine has mentioned numerous dravyas (things which has biological functions and properties) have been reported effective in madhumeha. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Many analysis- based studies regarding pharmacological research in India have been conducted in the past. In the traditional system of plant medicine, it is usual to use plant formulation and combined extract of plant are used as a drug of choice rather than individual ones, to get the benefit of synergism and to find suitable antidiabetic activity.

The concept of polyherbalism is peculiar to Ayurveda. Sarandghar Samhita highlights the concept of synergism behind polyherbal formulations. It is evident that there are many herbal formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that combination of these extract will produce better therapeutic response. So present study was designed to assess the combined effect of chloroform leaf extract of Azadirachta indica, aqueous leaf extract of Bougainvillea spectabilis and ethanolic seed extract of Trigonella foenum graecum on various parameters in streptozotocin induced diabetic rats.

METHODOLOGY

Authentication and Extraction of Plants Material-

Leaves of Azadirachta indica (neem) and Bougainvillea spectabilis (bougainvillea) were procured from polavaram forest, while seeds of Trigonella foenum graecum (methi) were purchased from nearby local shop. The leaves and seeds of plants were shade dried, finely powdered with the help of a grinder and stored in air- tight packets. They were identified and authenticated by Professor of Department of Botany, Andhra Loyola College, Vijayawada and the samples were preserved and deposited in repository. Extracts of individual plants samples were prepared separately. Chloroform extract of leaves of Azadirachta indica was prepared by subjecting 470 gram of neem leaf powder to soxhlet which yielded 42 gram of neem leaf chloroform extract. Aqueous extract of Bougainvillea spectabilis was prepared by soaking 400 gram of Bougainvillea leaf powder in 4 litres of Distilled water for 24 hours at room temperature. The material was then filtered next day and concentrated in water bath at 50-60^{0}C to get a yield of 35.2 gram of semi-solid aqueous extract of Bougainvillea leaf. Ethanolic extract of Trigonella foenum graecum seeds was obtained by soaking 700 grams of methi seed powder in 1400 ml of absolute ethanol for 6 days at room temperature. The material was then filtered with Watt man's filter paper no.1 and evaporated in water bath at 40-450C to get a yield of 60 grams ofethanolic extract of methi seed. The extracts so obtained were weighed.

Poly herbal plant extract used in the study at a dose of 600 mg/kg body weight, consisted of Azadirachta indica (neem), Bougainvillea spectabilis (bougainvillea) and Trigonella foenum graecum (methi) in ratio 1:2:3.

Experimental Animal

Sprague Dawley rats weighing 150-200 gm were brought from Division of Laboratory Animals, Mahaveer Agencies, Hyderabadfor the present study. They were housed in spacious, polypropylene cages in KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, which was well ventilated and maintained under standard experimental conditions (Temperature 23±2^{0}C and 12-hour light/dark cycle) throughout the

experimental protocol. All the animals were fed with Normal Pellet Diet (NPD)- Rodent, obtained from Mahaveer Agencies, Hyderabad and water ad libitum for acclimatization to the laboratory conditions for 10 days prior to experimental use. All animal experiments were carried out after approval by the Institutional Animal Ethics Committee of KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, asper the rules and regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Experimental Design

Type 2 Diabetes was induced by giving High Fat diet for 2 weeks followed by injection of low dose streptozotocin to rats. After the induction of diabetes, the rats were closely monitored for 7 days to detect appearance of diabetes. Thereafter with time when diabetes developed and got stabilized, blood sugar levels were estimated by glucometer and presence of diabetes was confirmed when blood glucose levels were above 250 mg/dl by glucometer (Accu-Chek Active, Model-GC, Roche, Germany). Rats were then divided into four groups with 6 rats in each group and were fed with High fat diet throughout the study period of 28 days.

Group-I - Normal control rats administered only distilled water (vehicle)

Group-II - Diabetic control rats administered only distilled water (vehicle)

Group-III - Diabetic rats administered Poly Herbal Plant Extract (PHPE) at a dose of 600 mg/kg

Group-IV - Diabetic rats administered with Glibenclamide at a dose of 500µg/kg

At the end of study, rats of all groups were anaesthetized and euthanized. Blood sample collected by intra cardiac puncture was centrifuged at 3000 r.p.m for 5 minutes for isolation of plasma for estimating Total cholesterol and Triglyceride levels. Pancreases were carefully dissected out for histopathological analysis.

Estimation of Blood Glucose- FBG levels (mg/dl) of rats of all groups were estimated every week after overnight fasting by glucometer (Accu-Chek® Active, Model-GC, Roche, Germany).

Estimation of Total cholesterol and Triglyceride levels- Total cholesterol and Triglyceride values of all groups were determined according to the method of Span diagnostic, Liquid Gold estimation kit at the end of study.

Histopathological Studies- For histopathological studies, pancreas was identified, isolated, weighed and sectioned at 8–10-micron thickness by Microtome Cryostat and the sections obtained were stained by Hematoxylin and Eosin (H&E) for microscopic examination by Kyow Getner microscope.

Statistical Analysis- The results were expressed as Mean ± S.D. The unpaired Student's t-test test was used for analyzing the data between two groups. Values of $p < 0.001$ were considered highly significant.

RESULTS

Effect on Body Weight- Table-1 shows diabetic control rats showed 8.2% loss of weight at the end of study as compared to their initial weights while diabetic rats treated with PHPE and GL showed 1.4% and 5.5% increase in body weight as compared to their initial weights respectively at the end of study. Increase in body weight of both PHPE and GL treated diabetic rats was found to be statistically significant ($p<0.05$) when compared to diabetic control rats at the end of study. Weight gain in rats treated with PHPE was comparable to weight gain in rats treated with GL at the end of study ($p>0.05$) as there was no significant difference found.

Table 1: Effect of Poly herbal plant extract on body weight of STZ induced diabetic rats

Group (n=6)	Day 0	Day 28	%Change in Body Wt	'p' value Day 0	'p' value Day 28
NC (Group 1)	323.3± 16.02	340.0± 9.48	5.2	p<0.05	P<0.001
DC (Group 2)	285.0± 10.95	261.6± 11.25	-8.2	-	-
D+PHPE (Group 3)	281.6± 11.25	285.0± 10.95*	1.4	p>0.05	p<0.05
D+GL (Group 4)	281.0± 11.25	296.6± 18.88*	5.5	p>0.05	p<0.05

Values are mean ± SD

* p > 0.05 between group 3 and group 4 at day 28

NC- Normal control

DC- Diabetic control

D + PHPE- Diabetic treated with Poly Herbal Plant Extract

D + GL- Diabetic treated with Glibenclamide

Effect on Fasting Blood Glucose – Table 2 shows rise of 7.8% in FBG levels of diabetic control rats at the end of study when compared to their initial values. PHPE treated diabetic rats showed decline of FBG levels by 7.9% while GL treated diabetic rats showed fall of 66.7% in FBG levels at the end of study when compared to their initial values. Decline in FBG levels of both PHPE and GL treated diabetic rats were found to be highly significant statistically (p<0.001) when compared to diabetic control rats at the end of study. Reduction observed in FBG levels in GL treated diabetic rats was more compared to PHPE treated diabetic rats and this difference was highly significant statistically (p<0.001).

Table 2: Effect of Poly herbal plant extract on blood glucose of STZ induced diabetic rats

Group (n=6)	Day 0	Day 28	%Change in FBG	'p' value Day 0	'p' value Day 28
NC (Group 1)	086.0± 02.46	091.6± 03.91	6.5	p<0.001	P<0.001
DC (Group 2)	326.5± 12.80	352.1± 06.68	7.8	-	-
D+PHPE (Group 3)	323.8± 10.62	298.0± 07.42*	-7.9	p>0.05	p<0.001
D+GL (Group 4)	325.4± 07.74	108.1± 05.50*	-66.7	p>0.05	p<0.001

Values are mean ± SD; *p < 0.001 between group 3 and group 4 at day 28; NC- Normal control; DC-Diabetic control; D+PHPE- Diabetic treated with Poly Herbal Plant Extract; D+GL- Diabetic treated with Glibenclamide

Effect on Total Cholesterol and Triglycerides- Table-3 shows decline of 26% in Total Cholesterol (TC) values in diabetic rats treated with PHPE as compared to diabetic control rats at the end of study which was found to be highly statistically significant (p<0.001). Reduction in TC values of rats treated with GL was 35% as compared to diabetic control rats at the end of study which was also found to be highly statistically significant (p<0.001). Although fall of TC values was observed by both group 3 and group 4 at the end of study, reduction by GL treated diabetic rats was more highly statistically significant (p<0.001) as compared to diabetic rats treated with PHPE. Similarly, decline of 37% of Triglyceride (TG) levels in diabetic rats treated with PHPE was observed as compared to diabetic control rats at the end of study which was found to be highly statistically significant (p<0.001). Fall of 54% was observed in TG levels of diabetic rats treated with GL as compared to diabetic control rats at the end of study which was also found to be highly statistically significant (p<0.001). Although fall in TG levels was observed by both group 3 and group 4 at

the end of study, reduction by GL treated diabetic rats was more highly statistically significant ($p<0.001$) as compared to diabetic rats treated with PHPE. With continuous administration of PHPE for 28 days, TG levels were found to be decreased more than TC levels in diabetic rats. Similarly, GL has also shown more reduction in TG values at the end of study.

Table 3: Effect Poly herbal plant extract on lipid profile of STZ induced diabetic rat

Group (n=6)	TC	%Reduction	p Value	TG	% Reduction	p Value
NC (Group 1)	131.5± 1.57	-	p<0.001	084.9±2.06	-	p<0.001
DC (Group 2)	223.6± 2.22	-	-	223.6±2.17	-	-
D+PH(Group 3)	164.6± 1.25*	-26	p<0.001	139.6±2.05**	-37	p<0.001
D+GL (Group 4)	144.0± 1.25*	-35	p<0.001	102.3±1.33**	-54	p<0.001

Values are mean ± SD; *p < 0.001 for TC and **p < 0.001 for TG between group 3 and group 4 at day 28; NC- Normal control; DC- Diabetic control; D+PH-Diabetic treated with Poly Herbal Plant Extract; D+GL-Diabetic treated with Glibenclamide

Histopathological studies - The histological study of normal control group rats on High fat diet revealed minimal pathological changes in acini and islets of Langerhans. Pancreatic islets of diabetic control group rats revealed significant architectural disarray which extended into surrounding exocrine tissue following streptozotocin induction. Peripheral widening between pancreatic acinar and islet cells was noted with shrunken pancreatic islet of langerhans.

Pancreas of PHPE treated diabetic rats revealed partial restoration in size and number of islet of langerhans. Reduction in widening between acinar and islet cells was noted. Glibenclamide treated diabetic rats showed much more improvement in pancreatic cell architecture by returning back to its normal structure and size.

DISCUSSION

Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such a deficiency results in increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nerves.

The extracts of the chosen plants have been shown to exert antihyperglycemic and hypoglycemic effects by various mechanisms, some of them being uniquely present in the extract. Therefore, in the present study the combined antihyperglycemic and hypoglycaemic activity of these plant extracts was seen with the premise that the various components will complement each other and produce more potent effect and also become most of the herbal preparations for antidiabetic use which are polyherbal in content. The results were compared with standard oral hypoglycemic drug Glibenclamide.

Streptozotocin is a well-known agent used to induce diabetes in animal model. The mechanism by which STZ brings about a diabetic state includes selective destruction of pancreatic beta cells, leading to hypoinsulinemia which as a result decreased glucose uptake and hyperglycaemia which is the characteristic feature of diabetes mellitus. In present study High fat diet (HFD) was administered for 28 days along with low dose of STZ which has been considered as one of alternative model by Srinivasan et al. For pharmacological screening of drugs for Type 2 diabetes mellitus since it resembles the clinical manifestation of Type 2 diabetes in humans. STZ partially destroys the pancreatic β-cells resulting in deficiency of insulin and administration of HFD for 28 days along with it induces insulin resistance in rats.

The present study evaluated the effect of Poly Herbal Plant Extract (PHPE) consisting of Azadirachta indica, Bougainvillea spectabilis and Trigonella foenum graecum at a dose of 600 mg/kg for 28 days on Body Weight Fasting Blood Glucose levels, Total Cholesterol and Triglyceride levels in STZ induced diabetic rats. Histopathological findings of pancreas were also studied.

One of the symptoms of diabetes mellitus is weight loss. Induction of diabetes with STZ showed significant loss of body weight which may be due to increased muscle wasting. STZ by producing hyperglycemia and hypoinsulinemia causes decrease in body weight of diabetic rats as reported by Swanston et al. Insulin being an anabolic hormone promotes lipogenesis and inhibits protein catabolism promoting weight gain or maintaining the weight preventing further loss.

In present study weight loss of 8.2% in STZ induced diabetic control rats was observed. Continuous treatment with PHPE for 28 days showed 1.4% gain in body weight. Glibenclamide treated rats showed 5.5% increase in body weight at the end of the study. Glibenclamide has been known to induce weight gain as a result of its effect to increase insulin levels and cause increased utilization of glucose. Gain in body weight by PHPE could be due to appetite stimulating effect of saponins present in Trigonella foenum graecum seeds causing better utilization of nutrients in diet as reported by Blumenthal et al. Quercetin in Azadirachta indica has been reported to have β-cell regenerating capacity by Aguirre et al and D- pinitol in Bougainvillea spectabilis has been reported to have insulinomimetic activity by Botham et al.

Effect of Glibenclamide and PHPE on fasting blood glucose was evaluated after the study of 28 days' study period. Both the treatments showed ($P<0.001$) hypoglycemic activity compared to diabetic control. PHPE treated diabetic rats showed decline of FBG levels by 7.9% while GL treated diabetic rats showed fall of 66.7% in FBG levels at the end of study when compared to their initial values which was quite similar with study performed by Srivastava et al and Rafiq et al 16.0 % reduction in fasting blood glucose was found with administration of polyherbal preparation at a dose of 1 ml/kg for 30 days in the study of Srivastava et al while Rafiq et al reported 8 % reduction in fasting blood glucose level in stevia-methi extract treated diabetic rats at a dose of 500mg /kg for 28 days.

In present study after administration of PHPE to diabetic rats for 28 days' reduction of 26.0% for Total Cholesterol and 37.0% for Triglycerides was observed when compared to diabetic control group while Glibenclamide treated group showed reduction of 37.0% and 54.0% in TC and TG levels respectively at the end of study. Hypolipidemic effect of PHPE in our study was much similar with the study performed by Kumar et al and Deep et al.

Under normal conditions insulin inhibits HMG-CoA reductase which produces cholesterol and activates lipoprotein lipase which hydrolyses triglycerides. In diabetes due to deficiency of insulin reverse occurs, leading to hypercholesterolemia and hypertriglyceridemia. Thus treatment with polyherbal formulation may be responsible for decrease in lipid levels by either insulin releasing or insulin sensitizing activity.

In present study histological study of normal control group rats on High fat diet revealed pancreatic cells in their normal proportions and islet cells embedded within the acinar cells surrounded by fine capsule. Pancreatic cells of diabetic control rats of group 2 following Streptozotocin induction showed distortion in architecture of pancreas with peripheral widening between pancreatic acinar and islet cells. Shrunken size of pancreatic islet of Langerhans was noted. Similar findings were reported by Akbarzadeh et al. On the other hand, pancreas of PHPE treated diabetic rats revealed some improvement in islet of Langerhans. There was an increase in the islet cell size and peripheral widening between acinar and islet cells were reduced as islet

returned back to its normal structure. These findings are supported by studies of Oluwole et al and Ahmed et al.

CONCLUSION

Based on our present experimental data, it can be suggested that PHPE may prevent hyperglycaemia and hyperlipidaemia in STZ induced diabetic rats by its blood glucose lowering effect which can be due to various mechanisms like insulin mimetic property, β-cell regeneration, increased insulin secretion, increased peripheral glucose utilization and decreased intestinal glucose absorption.

The actual mechanism by which the combination therapy protects and regenerates the damaged pancreatic cells is still unclear. The antidiabetic activity of PHPE can further be tested in vitro and in other diabetic animal models to know the exact and detailed mechanisms of its antihyperglycemic effect.

REFERENCES

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus Diabetes Care. 2011; 34(1):62-69.
2. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications Part 1- diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15(7):539-53.
3. King H, Aubert RE, Herman WH. Global burden of diabetes 1995–2025: prevalence, numerical estimates, and projection. Diabetes Care. 1998; 21:1414–1431.
4. Naggar E M, Antidiabetic effect of Cleome droserifolia Areial parts: Lipid peroxidation-induced oxidative stress in diabetic rats Acta Vet. Brno. 2004;74: 347.
5. Eddouks M, Maghrani M. Phlorizin-like effect of Fraxinus excelsior in normal and diabetic rats. J Ethnopharmacol. 2004; 9:149-54.
6. Kesari AN, Kesari S, Santosh KS, Rajesh KG, Geeta W. Studies on the glycemic and lipidemic effect of Murraya koenigii in experimental animals. J Ethnopharmacol. 2007;112(2):305-311.
7. Sabu MC, Subburaju T. Effect of Cassia auriculata Linn. On serum glucose level, glucose utilization by isolated rat hemidiaphragm. J Ethnopharmacol. 2002;80(2-3):203-206.
8. Dandiya PC, Bapna JS. Pharmacological research in India. Ann Rev Pharmacol. 1974; 14:115- 126.
9. Adithan C. Pharmacological research in India, 1972- 1995 - An analysis based on IPS conferences. Indian J Pharmacol. 1996; 28:125- 128.
10. Singh H. Steady decline in clinical pharmacology research in India - A decade trend- analysis of IJP research publications (1990- 1999). Abstracts of XXXIII annual conference of IPS 2000. Indian J Pharmacol. 2001; 33:51- -70.
11. Kumar J. Herbal medicine for Type 2 diabetes. International Journal of Diabetes Developing Countries, 2010; 30: 111-112.
12. Matsui T, Tanaka T, Tamura S, Toshima A, Miyata Y, Tanaka K, et al. Alpha-glucosidase inhibitory profile of catechins and theaflavins. Journal of Agricultural and Food Chemistry. 2007;55:99-105.
13. Kuriyan R, Rajendran R, Bantwal G, Kurpad AV. Effect of supplementation of Coccinia cordifolia extract on newly detected diabetic patients. Diabetes Care. 2008; 31:216-220.
14. Marles, RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine. 1995; 2: 137–189.
15. Srinivasan K. Combination of high-fat diet-fed and low-dose streptozotocin treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research. 2005; 52:313– 320.

16. Swanston Flatt SK, Day C, Bailey CJ. Traditional plant treatments for diabetes.in normal and streptozotocin diabetic mice. Diabetologia. 1990; 33(8):462-464.
17. S Ramu, Y Ashok Kumar, D Srinivasa Rao, G Ramakrishna. Formulation and evaluation of Valsartan oral dispersible tablets by direct compression method. American Journal of Advanced Drug Delivery. 2014; 2(6): 719-733
18. Leixuri A and Noemi A. Beneficial Effects of Quercetin on Obesity and Diabetes. The Open Nutraceuticals Journal. 2011; 4:189-198.
19. K Saravanakumar, Ashok Thulluru, Ramu Samineni, M Ishwarya, Pommala Nagaveni, Nawaz Mahammed. Effect of sodium alginate in combination with natural and synthetic polymers on the release of verapamil HCl from its floating microspheres. Journal of Pharmaceutical Sciences and Research. 2019; 11(5): 2028-2035
20. Srivastava N, Tiwari G, Tiwari G. Polyherbal preparation for antidiabetic activity. Indian Journal of Medical science. 2010;64(4):163-176.
21. Kazi R, Shamshad JS. Comparative efficacy of stevia leaf (Stevia rebaudiana bertoni), methi seeds (Trigonella foenum-graecum) and glimepiride in streptozotocin induced rats. International Journal of Phytopharmacology. 2011; 2(1):9-14.
22. Kumar HC, Kumar JN. Antidiabetic activity of a polyherbal Preparation. Pharmacologyonline. 2010; 2:780-787.
23. Prakash D, Murugananthan G. Herbal formulation and its evaluation for antidiabetic activity. Pharmacologyonline. 2011; 3:1134-1144.
24. Narahari Narayan Palei, S. Ramu, V. Vijaya, K. Thamizhvanan, Anna Balaji, Green synthesis of silver nanoparticles using leaf extract of Lantana camara and its antimicrobial activity. International Journal of Green Pharmacy. 2020; 14(1):1-7
25. Oluwole BA, Laura Z. Ameliorative effects of ethanolic leaf extract of Azadirachta indica on renal histologic alterations in streptozotocininduced diabetic rats. Am J Chin Med. 2011; 39(5):903-16.