

ANTIDIABETIC ACTIVITY OF A POLYHERBAL PREPARATION

C. Hari Kumar^{1*}, Dr. J.N. Suresh Kumar¹, B. Mohammed Ishaq², G. Usha Rani³,
Dr. K. Vanitha Prakash²

1. Dept. of Pharmacology, Deccan School of Pharmacy, Hyderabad, A.P. INDIA
2. Dept. of Pharmaceutical Analysis, SSJ College of Pharmacy, Hyderabad, A.P. INDIA
3. Dept. of Pharmaceutical Chemistry, CMR College of Pharmacy, Hyderabad, A.P. INDIA

*Corresponding author:

C. Hari Kumar

Dept. of Pharmacology,
Deccan School of Pharmacy,
Hyderabad, A.P. INDIA.

E mail: haricology@gmail.com

Phone no: +91-9160895257

Summary

The present work was executed to evaluate the anti-diabetic potency of a polyherbal preparation. The objective of this study is to induce experimental diabetes mellitus using Alloxan in normal Albino wistar rats and study the anti diabetic activity of polyherbal formulation by comparison of changes in body weight and levels of glucose between normal and diabetic rats. Hypoglycemic agents from natural and synthetic sources are available for treatment of diabetes. Indian medicinal plants have been found to be useful to successfully manage diabetes. The effect of hydroalcoholic extract of poly herbal preparation containing seeds of *Eugenia jambolana*, fruits of *Momordica charantia*, and leaves of *Ocimum sanctum* was investigated in normal, glucose load conditions and alloxan induced diabetic rats. Significant anti diabetic activity was exhibited by the poly herbal formulation. Serum glucose and serum cholesterol levels were found to be increased in diabetic animals. Treatment with the polyherbal Preparation 200 mg/kg body wt and 400 mg/kg body wt for 11 days in diabetic animals has shown significant decrease in serum glucose, AST, ALT and biochemical parameters (urea, creatinine, triglycerides and cholesterol) levels in comparison to control animals.

Key words: *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, Polyherbal preparation.

Introduction

Diabetes mellitus is a heterogeneous metabolic disorder characterised by altered carbohydrate, lipid and protein metabolism (1). The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. The modern drugs, including insulin and oral hypoglycemic agents, control the blood sugar level as long as they are regularly administered and they also produce a number of undesirable effects (2, 3). The treatment of diabetes mellitus has been attempted with different indigenous plants and polyherbal formulations (2, 4, 5).

Traditional medicines all over the world have advocated the use of herbs to treat diabetes since time immemorial. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals (6). In the Ayurvedic system of medicine, as mentioned in ancient Indian books like Charak, Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property (7). Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic activity and their possible use in the treatment of diabetes mellitus. Indian plants which are most effective and commonly studied in relation to diabetes and its associated complications are : *Allium cepa*, *Allium sativum*, *Aloe vera*, *Cajanus cajan*, *Coccinia indica*, *Caesalpinia bonduc*, *Ficus bengalensis*, *Gymnema sylvestre*, *Momordica charantia*, *Ocimum sanctum*, *Pterocarpus marsupium*, *Swertia chirayita*, *Syzgium cumini*, *Tinospora cordifolia*, *graecum* and *Trigonella foenum* (8, 9).

Keeping the above information in view, an indigenous polyherbal preparation was developed containing the extracts of *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*.

Materials & Methods

Plant material

Seeds of *Eugenia jambolana*, fruits of *Momordica charantia*, and leaves of *Ocimum sanctum* were collected from local market of Mandsaur, MP Authenticated by Dr.Gyanandra tiwari, Asst. Prof/Scientist, Dept. of Fruit Science, K.N.K. College of Horticulture, mandsaur. A voucher herbarium specimen number BRNCP/E/003/2008, BRNCP/M/005/2008, BRNCP/T/008/2008 were also preserved in the Department of Pharmacognosy, BRNCP, Mandsaur, M.P.

Extraction

Seeds of *Eugenia jambolana*, fruits of *Momordica charantia*, and leaves of *Ocimum sanctum* were coarsely powdered and extracted with hydroalcoholic mixture of 70% ethanol and 30 % water in a soxhlet apparatus exhaustively. *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum* extracts were mixed properly in 1:1:1 ratio to get the polyherbal preparation.

Animals

Albino Wistar rats weighing 130–180gms of either sex were used. Animals were obtained from BRNCP animal house, Mandsaur, M.P, India were housed under standard conditions at room temperature , $60 \pm 5\%$ RH, and 12 hrs L:D cycle and fed with standard pellet diet (and water *ad libitum*. Experimental protocols were approved by Institutional Ethics Committee.

Acute Toxicity Studies:

The acute toxicity study was carried out in adult female albino rats by the 'fix dose' method of OECD (Organization for Economic Co-operation and Development) Guideline No. 420. The fixed dose method as in Annex 2d, test procedure with a starting dose of 2000 mg/kg body weight, was adopted. The animals were fasted overnight and next day the poly herbal preparation (suspended in 0.5% w/v Sodium CMC) was administered orally at a dose level 2000 mg/kg body weight. Then the animals were observed continuously for 3 hours for general behavioural, neurological, and autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 14 days (10).

Selection of Doses:

For the assessment of Antidiabetic activity, two dose levels were chosen in such a way that one dose was approximately one-tenth of the maximum dose during acute toxicity studies and the other high dose was twice that of one-tenth dose (200 mg/kg, 400 mg/kg body weight)

Preparation of dosing:

The dose of 200 and 400 mg/kg of polyherbal preparation was prepared by suspending appropriate quantity (1:1:1) of extracts in 0.5 % w/v Sodium CMC).

Determination of hypoglycemic activity:

Albino rats of either sex weighing 100-150 g were taken. The rats were kept fasting overnight with free access to water. During the experiment, the animals were divided into four groups of six animals in each group. The blood sample was taken by pricking the rat's tail. Polyherbal preparation was administered with glass syringe and microsuction canula no. 18. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated 1,2, and 4 hours after the initiation of the experiment.(11,12,13)

Grouping of animals:

- | | |
|-----------|---|
| Group I | Kept as normal control, i.e., neither treated with polyherbal preparation nor with standard |
| Group II | Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (0.5 mg/kg body weight |
| Group III | Treated orally with 200 mg/kg poly herbal preparation. |
| Group IV | Treated orally with 400 mg/kg poly herbal preparation. |

Oral glucose tolerance test in normal rats animals and experimental setup:

Albino rats of either sex weighing 130 – 180 g were taken. The rats were kept fasting overnight with free access to water. During experiment the animals were divided into three groups of six animals in each group. The blood sample was taken by pricking the rat's tail. Polyherbal formulation was administered with glass syringe and microsuction canula no. 18.

Grouping of animals:

- | | |
|-----------|---|
| Group I | Kept as negative control, i.e., neither treated with Polyherbal preparation nor standard. |
| Group II | Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (0.5 mg/kg) |
| Group III | Treated Orally with polyherbal preparation (400 mg/kg) |

Determination of OGTT activity:

The blood glucose concentration of animals were measured at the beginning of the study. Then the rats were orally treated with 3 g/kg body weight glucose solution after 30 minutes of the product and drug treatment. The measurements were repeated after 30, 90 and 150 minutes after the glucose load (11,13).

Antidiabetic activity:**Induction of diabetes:**

Animals were fasted for 24 hrs then a single interperitoneal injection of freshly prepared alloxan (125 mg/kg dissolved in 0.9% saline) was injected. After the animals were left aside for 4 hrs and then 10 % glucose solution was placed in the cages for 24 hrs. The diabetes was confirmed by estimation of blood glucose level (BGL) on the third day. Rats having BGL >250 mg/dl were used for study and during the experiment the animals were divided into five groups of six animals in each group.

Grouping of animals:

- | | |
|-----------|--|
| Group I | Kept as normal control, i.e., neither treated with polyherbal preparation nor with standard |
| Group II | Kept as negative control, i.e., treated with alloxan (125 mg/kg, i.p) |
| Group III | Treated with standard oral hypoglycemic drug, i.e., Glibenclamide (0.5 mg/kg) after 3 rd day of the treatment with alloxan (125 mg/kg, i.p). |
| Group IV | Treated orally with 200 mg/kg poly herbal preparation after 3 rd day of the treatment with alloxan (125 mg/kg, i.p) |
| Group V | Treated orally with 400 mg/kg poly herbal preparation. treatment with alloxan (125 mg/kg, i.p) |

Determination of Antidiabetic activity:

The blood glucose concentrations of the animals were measured at the beginning of the study and measurements were repeated on 3rd, 7th, and 11th day of the experiment. (11,13,14)

Biochemical determinations:

After the 11th day of treatment, blood was collected from the orbital plexus of overnight fasted rats. The serum was separated and urea, creatinine, triglycerides, and cholesterol level were determined by using Beacon, urea determination kit, creatinine mono reagent test kit, triglycerides test kit, and cholesterol test kit (Span diagnostic Ltd, Surat), respectively. Serums AST, ALT were determined by Liquizyme, SGOT, SGPT determination kit. Serum proteins level was determined by Liquizyme, protein determination kit.

Statistical analysis

The data were expressed as mean \pm SEM. The data of hypoglycemic activity, oral glucose tolerance test (OGTT), and antidiabetic activity were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's t test for multiple comparisons. Values with P < 0.05 were considered significant.

Results and discussion

Acute toxicity studies on female rats showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days. During the study, no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe. In the hypoglycemic activity studied, polyherbal preparation did not show any significant activity at dose levels of 200 and 400 mg/kg, whereas standard drug glibenclamide produced significant activity [Table 1]. In the OGTT, polyherbal preparation at a dose of 400 mg/kg significantly reduced the blood glucose level at 30 minutes after glucose administration. Standard drug glibenclamide produced activity at all the time interval tested [Table 2].

Polyherbal preparation showed significant antidiabetic activity at 7th and 11th days at both 200 and 400 mg/kg dose levels [Table 3]. In diabetic rats' urea, creatinine, cholesterol, and triglycerides level increased. After treatment with polyherbal preparation, it significantly reduced the biochemical parameters [Table 4]. Other biochemical parameters like AST, ALT and total proteins reduced significantly with the treatment of polyherbal preparation [table 5].

Table 1: Effect of polyherbal preparation on the blood glucose level (mg/dl) of normal rats

Group	Blood Glucose Level (mg/dl)			
	0 hour	1 hour	2 hours	4 hours
Normal	89.00 ± 1.46	91.56 ± 1.60	90.36 ± 2.18	85.24 ± 1.92
Positive control (Glibenclamide 0.5 mg/kg)	96.24 ± 2.61	48.50 ± 2.00**	46.33 ± 1.14**	45.67 ± 0.71**
Polyherbal preparation (200 mg/kg)	78.7 ± 1.83	85.70 ± 1.56	98.50 ± 2.48	81.50 ± 2.10
Polyherbal preparation (400 mg/kg)	82.7 ± 1.65	97.55 ± 4.80	95.42 ± 5.32	88.16 ± 4.21

n = 6, **P<0.01 vs Negative control (ANOVA followed by dunnet's test).

Values are expressed in mean ± SEM.

Table 2 Oral glucose tolerance test of polyherbal preparation on blood glucose level (mg/dl) of normal rats

Group	Blood Glucose Level (mg/dl)				
	0 min	30 min	60 min	120 min	180 min
Normal control	89.00 ± 1.46	129.00 ± 1.60	117.30 ± 2.18	102.70 ± 1.92	94.50 ± 1.38
Positive control(Glibenclamide 0.5 mg/kg)	88.33 ± 2.61	77.50 ± 2.00**	70.33 ± 1.14**	62.67 ± 0.71**	59.17 ± 1.13**
Polyherbal preparation (400 mg/kg)	90.7 ± 1.83	129.70 ± 1.56	108.50 ± 2.48*	96.50 ± 2.10*	88.83 ± 1.74 *

n=6 * p < 0.05, **p < 0.01 vs. negative control(ANOVA followed by Dunnet's test)

Value expressed in mean ± SEM

Table 3 Antidiabetic activity of extracts on BGL (mg/dl)

Group	Regimen	0 DAY	3 DAY	7 DAY	11 DAY
		BGL	BGL	BGL	BGL
G-I	Normal control	88.66 ±1.28	88.80 ±0.94	86.8 ±2.24	92.00±1.15
G-II	Negative control (alloxan)	87.33 ±1.40	354.00 ± 18.84	406.83 ±14.37	438.67±9.21
G-III	Positive control (glibenclamide)	87.50 ±2.71	339.67 ±16.48	134.67 ±3.13**	89.66±1.97**
G-IV	Drug Treated Polyherbal preparation 200	87.66 ±2.26	327.67 ±16.11	300±11.93**	171.33± 2.99**
G-V	Drug Treated Polyherbal preparation. 400	90.50 ±1.389	325.33 ±12.48	277.6 ±11.13**	100.67±1.60**

n=6 *p<0.05 **p<0.01 vs Negative control (ANOVA followed by Dunnet's test) Value expressed in mean ± SEM

Table 4 Antidiabetic activity of extracts on basis of biochemical parameter (mg/100ml)

Group	Treated	Urea	Creatinine	Triglycerides	Cholesterol
G-I	Normal control	42.83 ± 1.40	0.7 ± .07	86.83 ± 2.08	84 .33 ± 2.20
G-II	Negative control	66.33 ± 2.60	1.75 ± .16	159.30 ± 1.90	122.50 ± 1.80
G-III	Positive control	25.67 ± 1.50**	0.65 ± .07**	73.17 ± 2.24**	68.50 ± 1.60**
G-IV	Drug Treated Polyherbal preparation. 200	39.83 ± 1.81*	1.15 ± .12**	98.50 ± 2.29**	89.67 ± 2.00**
G-V	Drug Treated Polyherbal preparation. 400	31.67 ± 1.14**	0.71 ± .06**	87.67 ± 1.72**	71.67 ± 2.29**

n=6 *p<0.05 **p<0.01 vs Negative control (ANOVA followed by Dunnet's test) Value expressed in mean ± SEM

Table 5 Antidiabetic activity of extracts on basis of biochemical parameter (mg / 100ml)

Group	Treated	AST	ALT	Total Proteins
G-I	Normal control	138.30 ± 2.45	54.83 ± 2.12	5.80 ± 1.20
G-II	Negative control	341.80 ± 6.46	114.20 ± 2.97	3.71 ± 0.19
G-III	Positive control	115.30 ± 2.82**	48.67 ± 1.99**	7.26 ± 0.15**
G-IV	Drug Treated Polyherbal preparation. 200	157.00 ± 3.39*	69.33 ± .076**	5.90 ± 0.17**
G-V	Drug Treated Polyherbal preparation. 400	124.00 ± 1.39**	53.67 ± 1.20**	6.39 ± 0.12**

n=6 *p<0.05 **p<0.01 vs Negative control (ANOVA followed by Dunnet's test)
Value expressed in mean ± SEM

The levels of serum lipids are usually elevated in DM and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in a diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Insulin deficiency is associated with hypercholesterolemia. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hyper triglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially. In study also, the diabetic rats showed hypercholesterolemia and hypertriglyceridemia and the treatment with polyherbal preparation ($P < 0.01$) decreased both cholesterol and significantly triglyceride levels. This implies that the polyherbal preparation can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often.

The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction.(15) The results in Table 4 showed a significant increase in the level of plasma urea and creatinine in the diabetic groups compared to control level. These results indicated that diabetes might lead to renal dysfunction. While, after treatment of alloxan-diabetic rats with polyherbal preparation, the level of urea and creatinine were significantly ($P<0.05$) ($P<0.01$) decreased compared to the mean value of the diabetic group.

This further confirms the utility of this polyherbal preparation in diabetes-associated complications. To conclude, the polyherbal preparation, was proven to have antidiabetic effect and it could be used as an alternative remedy for the treatment of diabetes.

Acknowledgements

The author is grateful to the Prof. J. N. Suresh Kumar, Principal, Deccan School of Pharmacy, Hyderabad, A.P, for his encouragement in carrying out this work and for providing research facilities.

References

1. Das AV, Padayutti PS, Paulose CS. Ind J Exp Biol, 34, 1996: 341-45
2. Upadhyay OP, Singh RM, Dutta K. Aryavaidyan, 9(3), 1996:159-67
3. Reynolds JEF, editor. Martindale-The Extra Pharmacopoeia, 30th ed., 1997, The Pharmaceutical Press, London.
4. Chaurasia AK, Dubey SD, Ojha JK. Aryavaidyan, 7(3), 1994:147-52
5. Joy KL, Kuttan R. Amala Research Bulletin, 18, 1998: 109-114
6. Deb L, Dutta A. Diabetes mellitus its possible pharmacological evaluation techniques and naturopathy. Int J Green Pharmacy 2006;1:7-28.
7. Murray, M.T.: (1995). Healing power of Herbs. 2nd edition, Gramercy Books NY, pp: 357.
8. Grover J K, Yadav S, Vats V, Medicinal plants of India with anti-diabetic potential, *J Ethnopharmacol*, 81 (2002) 81.
9. Shukla R, Sharma S B, Puri D, Prabhu K M & Murthy P S , Medicinal plants for treatment of diabetes mellitus, *Indian Journal of Clinical Biochemistry*, 15 (Suppl.) (2000) 169.
10. Ghosh MN. Fundamentals of experimental Pharmacology. 3rd ed. Hilton and company; 2005. P. 190 – 7.
11. Naik SR, mandlik RV, Desai Sk. Antidiabetic activity of a polyherbal formulation (DRF/AY/5001). Indian J Exp Biol 2008; 46:599-606.
12. Chattopadhyay RR. A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol 1999; 67:367-72.
13. Kuttan R, Joy KL, Anti-diabetic activity of Picrorrhiza kurroa extract, J Ethnopharmacol 1999; 167: 143-8.
14. Pushaparaj PN, Low HK, Manikandan J, Tan BK, Tan Ch. Antidiabetic effect of Cinchorium intybus in streptozotocin-induced diabetic rats. J Ethnopharmacol 2007; 111:430-4.
15. Alarcon AFJ, Calzada BF, Hernandez GE, Ruiz AC, Roman RR Acute and chronic hypoglycaemic effect of Ibervillea sonorae root extracts-II. J Ethnopharmacol 2005;97:447-52.