



Anti-Diabetic Activity of a Polyherbal Formulation in Streptozotocin Induced Type 2 Diabetic Rats

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Abstract

The aim of the present study was to determine the anti-diabetic activity of a polyherbal formulation in streptozotocin induced type 2 diabetic rats. The hydroalcoholic extracts of *Eugenia jambolana*, *Gymnema sylvestre*, *Momordica charantia* and *Andrographis paniculata* and sodhana processed extract of *Myristica fragrans* were prepared. The 2 day old neonatal rats were injected with 90 mg/kg of streptozotocin intraperitoneally to induce type 2 diabetes. After six weeks of streptozotocin injection, polyherbal formulation was daily administered at a dose of 400 mg/kg body weight to diabetic rats for a period of 28 days. Blood samples were collected from the retro orbital plexus of the eye and blood glucose level was estimated by glucose oxidase-peroxidase method. The study indicated that polyherbal formulation at a dose of 400 mg/kg body weight showed significant decline ($p < 0.001$) in blood glucose level.

Keywords: OGTT, Polyherbal Formulation, Streptozotocin, Type 2 Diabetes

1. Introduction

Diabetes mellitus is a severe metabolic disorder which is indicated by hyperglycemia due to lack of insulin or the action of insulin on its target tissues or both. It is one of the major public health problem which is now becoming a global epidemic¹

The rising glucose level in blood, in type 2 diabetes, results due to combination of unhealthy diet, physical inactivity, defect in insulin secretion in response to food and reduced sensitivity of the target tissues to insulin action.²

The chronic metabolic disorder which affects about 150 million people of the world is going to increase to 300 million by the year 2025.³

The synthetic oral anti-diabetic drugs and insulin which are being currently used for the control of diabetic complications are effective in controlling the elevated blood glucose levels but they have various side effects and do not control the complications related to diabetes.⁴

Traditional medicinal plants are being used worldwide for many diabetic complications. Various herbal drugs and minerals have been described in olden traditional literature for the treatment of diabetes mellitus. Herbal drugs are considered to be safe and do not have much side effects compared to synthetic drugs.⁵ Therefore, exploring the hypoglycemic potential of medicinal plants has become very important to provide mankind with safer alternative of herbal drugs.

The present study has been carried out to determine the hypoglycemic potential of some medicinal plants and

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the anti-diabetic activity of their polyherbal formulation in type 2 diabetic rats.

2. Materials and Methods

2.1 Plant Material

The leaves of *Gymnema sylvestere* (fam. Asclepidaceae) and aerial parts of *Andrographis paniculata* (fam. Acanthaceae) were procured from Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat. Dried seeds of *Eugenia jambolana* (Myrtaceae) and fruits of *Myristica fragrans* (Myristicaceae) were procured from LVG Ayurvedic Store, Ahmedabad. Fresh fruits of *Momordica charantia* (Cucurbitaceae) were procured from local market of Ahmedabad. The plant materials were identified and authenticated by ethnobotanist Dr. B.L. Punjani, PG Centre in Botany, Smt. S.M. Panchal Science College, Talod, Gujarat.

2.2 Preparation of Extracts

All the plant materials (1 kg) were air dried and separately coarsely powdered in a mixer.

500 g of each crude drug powder was extracted by triple maceration in water and ethanol mixture (9:1 ratio). The extracts were concentrated under vacuum, dried at about 60 °C and then stored in a refrigerator.

The fruits of *Myristica fragrans* were treated for sodhana process as mentioned in Ayurvedic Formulary of India.

Myristica fragrans seeds (200 gms) were tied in a porous cloth with the help of a thread and the whole assembly was attached to a horizontal rod. The cloth bag containing the seeds was dipped in a pot containing cow's milk (1.5 L) and milk was boiled for 3 hrs. After 3 hours, seeds were taken out from the boiling milk, dried in hot air oven at about 60 °C. After drying the seeds were powdered and triturated with lime juice (300 ml) in a mortar for 72 hrs. The trituration was done by tying a pestle with wooden handle on the tissue homogenizer for 72 hours. The mixture was then dried in hot air oven at about 60 °C.⁶

2.3 Chemicals and Reagents

Streptozotocin was obtained from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. Kits for glucose estimation were procured from Labcare Diagnostic (India) Pvt. Ltd. The other chemicals and reagents were procured from CDH Pvt. Ltd. New Delhi.

2.4 Animals

Adult healthy Wistar rats of either sex of about 6-8 weeks of age, weighing 180 - 220 g were obtained from Torrent Research Centre, Ahmedabad. Female and male animals were separately kept in polypropylene cages in groups of six. The animals were given free access to water and food and were fed with standard rat pellet diet. The protocol of the experiment IPS/PCOG/FAC10-11/003 was approved by the Institutional Animal Ethics Committee and experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.5 Development of Polyherbal Formulation

The polyherbal formulation was developed by combining the dried extracts of the plant materials based on the oral glucose tolerance test of individual plant extracts (200 mg/kg each) in normal rats. The polyherbal formulation was prepared by mixing *Gymnema sylvestre* extract, *Eugenia jambolana* extract, *Momordica charantia* extract, *Andrographis paniculata* extract and *Myristica fragrans* sodhana extract in the ratio of 1 : 0.5 : 0.75 : 0.25 : 1.5 respectively.

3. Experimental Design

3.1 Effect of Plant Extracts on Oral Glucose Tolerance Test (OGTT)

Overnight fasted normal rats were allotted into seven groups having 6 animals in each group.

Group I was kept as Normal Control and received only vehicle i.e. 0.5 % Carboxy Methyl Cellulose (CMC) solution.

Group II to VI received 200 mg /kg body weight of *Eugenia jambolana* extract, *Andrographis paniculata* extract, *Gymnema sylvestre* extract, *Momordica charantia* extract and *Myristica fragrans* shodhana extract respectively.

Group VII received standard drug Glibenclamide at 0.25 mg/kg body weight.

The single dose of each extract was administered orally in the rats. All animals received glucose (2g/kg) orally, 30 min after drug treatment. The blood samples were taken from retro orbital plexus of the animals under mild anaesthesia, at 0, 30, 60, 90 and 120 min after administration of the drug. The serum glucose was determined by the method of glucose oxidase-peroxidase using Labcare diagnostic kit.⁷

3.2 Streptozotocin Induced Neonatal Rat Model for Type 2 Diabetes

The 2 day old neonatal rats were given injection of Streptozotocin at a dose of 90 mg/kg intraperitoneally (i.p.). After six weeks of Streptozotocin injection, blood glucose levels of the animals were checked with the help of diagnostic kit and the animals which had blood glucose levels of 250 mg/dl or more were considered to be diabetic and selected for further study.⁸

The animals were allotted into four groups of 6 animals each. Group I served as normal control and was orally administered with only the vehicle, 0.5% CMC. Group II was kept as type 2 diabetic control and was

given only 0.5% CMC. Group III was type 2 diabetic and given treatment with the standard drug, Glibenclamide (0.25mg/kg). Group IV was type 2 diabetic and was administered the polyherbal formulation (400mg/kg). The treatments were given daily morning for a period of 28 days with oral feeding tube.

Blood samples were collected from the retro orbital plexus of the animals, on 0 day, 7th day, 14th day, 21st day and 28th day, under the effect of ether anaesthesia. Serum was separated by centrifuging at 10000 rpm for 25 minutes at 7°C temperature. Serum glucose levels were determined by the method of glucose oxidase-peroxidase using Labcare diagnostic kit and compared with Glibenclamide, the standard drug.

3.3 Statistical Analysis

The results were calculated as mean \pm SEM and statistically assessed by two way analysis of variance (ANOVA) followed by Bonferroni post test. The values were considered to be significant when $p < 0.05$.

4. Results and Discussion

4.1 Effect of Extracts on Blood Glucose in Oral Glucose Tolerance Test (OGTT)

The *Myristica shodhana* extract exhibited significant decrease ($p < 0.01$) in the blood glucose levels after 30 min which was comparable to Glibenclamide. (Table 1)

Table 1: Oral Glucose Tolerance Test (OGTT)

Groups	Treatments	Blood Glucose Levels (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
Group 1	Normal Control	91.28 \pm 2.87	145.23 \pm 2.45	132.42 \pm 3.56	118.56 \pm 3.80	92.45 \pm 2.85
Group 2	<i>Eugenia</i> extract (200 mg/kg)	92.76 \pm 4.56	123.76 \pm 2.42	112.56 \pm 5.56	98.34 \pm 3.50*	93.67 \pm 4.56
Group 3	<i>Myristica</i> extract (200 mg/kg)	88.35 \pm 5.52	115.46 \pm 3.06**	104.32 \pm 4.56**	95.32 \pm 5.22*	88.24 \pm 4.88
Group 4	<i>Andrographis</i> extract (200 mg/kg)	95.54 \pm 2.74	127.52 \pm 4.58	115.58 \pm 5.16	101.78 \pm 3.56	95.23 \pm 5.16
Group 5	<i>Momordica</i> extract (200 mg/kg)	94.23 \pm 3.35	120.45 \pm 5.18*	108.89 \pm 4.92*	97.15 \pm 2.85	92.03 \pm 5.42
Group 6	<i>Gymnema</i> extract (200 mg/kg)	94.98 \pm 4.50	118.65 \pm 4.24*	105.76 \pm 5.68*	94.78 \pm 3.47*	93.43 \pm 1.26
Group 7	Glibenclamide (0.25mg/kg)	90.58 \pm 3.48	112.22 \pm 2.72**	100.87 \pm 3.84**	91.54 \pm 3.22**	90.21 \pm 4.85

Values are mean \pm SEM, n = 6, * $p < 0.05$, ** $p < 0.01$ compared to normal control group

Table 2: Anti-diabetic activity in streptozotocin induced type 2 diabetic rats

Groups	Blood Glucose Levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal Control	92.02 ± 3.24	95.56 ± 2.80	94.23 ± 2.18	92.58 ± 3.75	95.35 ± 3.18
Diabetic Control	265.34 ± 4.72	278.84 ± 3.85 ^a	286.68 ± 5.62 ^a	295.32 ± 4.25 ^a	302.12 ± 5.92 ^a
Glibenclamide (0.25 mg/kg)	258.72 ± 2.46	210.56 ± 4.68 ^b	172.32 ± 3.92 ^b	140.58 ± 4.86 ^b	115.45 ± 2.72 ^b
Polyherbal extract (400 mg/kg)	260.92 ± 4.55	232.94 ± 5.78 ^b	200.39 ± 4.84 ^b	158.81 ± 5.32 ^b	122.54 ± 3.54 ^b

Values are mean ± SEM, n = 6, ^ap < 0.001 compared to normal control group, ^bp < 0.001 compared to diabetic control group

Gymnema extract and Momordica extract also showed significant decline in blood glucose levels at 30 min and 60 min (p < 0.05) in comparison to the normal control group. Eugenia extract exhibited decline in the blood glucose level at 90 min (p < 0.05) in comparison to normal control group. Andrographis extract also reduced the blood glucose level at each time period but the reduction was not so significant. There was not any significant decline in the blood glucose level of the control group during the observation period.

4.2 Anti Diabetic Activity as per Type 2 Model

The diabetic control group exhibited significant increase (p < 0.001) in blood glucose levels at all time periods in comparison to the normal control group. (Table 2) The polyherbal formulation indicated significant decrease (p < 0.001) in blood glucose level at all time intervals of day 7, day 14, day 21 and day 28 against the diabetic control group and the reduction in blood glucose level was comparable to Glibenclamide at 28 day.

Eugenia jambolana extract acts by increasing the production of insulin whereas the *Gymnema sylvestre* extract helps in regeneration and restoration of β cells of the pancreas. *Momordica charantia* is reported to have insulin like action.⁹ One study reports that *Myristica fragrans* produces hypoglycemic activity due to PPAR γ/α agonist mechanism thus improving insulin resistance condition.¹⁰

5. Conclusion

The study indicates that the developed polyherbal formulation at a dose of 400 mg/kg body weight is effective in significantly reducing blood glucose levels in type 2

diabetic rats and its anti-diabetic activity is comparable to Glibenclamide. The significant hypoglycemic activity of the polyherbal formulation might be due to the varied mechanism of action of each of the herbal drug present in the formulation. Hence, the developed polyherbal formulation might prove to be a safe alternative for the existing anti-diabetic synthetic drugs.

However further studies need to be carried out to explore the mechanism of action of each plant and to define the active phytochemicals present in each plant extract.

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7. References

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