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Research Article

EVALUATION OF ANTIDIABETIC ACTIVITY OF POLYHERBAL PREPARATION AGAINST ALLOXAN INDUCED DIABETES IN EXPERIMENTAL ANIMAL

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A B S T R A C T

Introduction: Incidence of diabetes is on rise worldwide as well as in India. Due to adverse drug reactions from currently available medications, herbal medications have become an emerging area of research. Thus, we aimed to study the anti-diabetic activity of polyherbal preparations of *Murraya koenigii* and *Ocimum sanctum* extracts. **Material methods:** Alloxan induced experimental animals were examined for anti-diabetic behaviour for polyherbal extracts in comparison to Glibenclamide. Acute toxicity, biochemical, physiological and histopathological parameters were studies and observations were noted and compared. **Result:** The standard group shows significant decrease in blood glucose levels, which indicates hypoglycemic effect of glibenclamide. Similar results are seen in groups Test I and Test II, after alloxan administration, suggesting hypoglycemic effect of *Ocimum sanctum* and *Murraya koenigii* methanolic extracts. Favourable effects of herbal medicines on post glucose load blood glucose level, diet and body weight were also comparable to glibenclamide. The polyherbal preparation showed hypoglycemic potential by improving blood glucose profile in diabetic rats with statistical significance $p< 0.001$. **Conclusion:** Polyherbal preparation of *Murraya koenigii* and *Ocimum sanctum* in 1:1 ratio has significant antidiabetic activity.

INTRODUCTION

Various medicinal plants across the globe are potential sources of drugs. These plants are traditionally used to treat diabetes. With lesser side effects they are available at lower or no costs. Herbal medicines thus may prove as safer and low cost alternative if proven effective. (Habib *et al.,* 2005) The world health organization (WHO) has listed 21,000 medicinal plants around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. (Maurya and Shrivastava, 2011)

Diabetes is one of the refractory diseases identified by Indian Council of Medical Research and has problem in the contemporary world. Today, India is almost set to take over China as diabetic capital of the world with over 20 millions diabetes cases and this number is likely to increase to 57 million by 2025. (King *et al.,* 1998; Sridhar, 2000)

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Diabetes mellitus occurs throughout the world, but is more prevalent in developed countries. The increased incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "western-style" diet. According to the American Diabetes Association, approximately 8.6 million of Americans age 60 and older have diabetes. (Harris *et al.,* 1998)

There is increase in the prevalence of this disease with time and the currently available medications produces adverse reactions such as liver problems, lactic acidosis, diarrhoea and hypoglycaemia at higher doses. It is believed that this pathology has a significant global impact on the basic healthcare systems, the economy and the life quality of patients. Due to the development of serious side effects, the search for more effective and safer hypoglycemic compounds has continued to be an important area of research, and after the recommendations made by WHO on diabetes mellitus, research on hypoglycemic compounds from medicinal plants has become an important aspect of this project. Medicinal plants of traditional use overcome such limitations and satisfy the patient's needs with minimum harm.

Thus, we aimed to discover new drugs from potent and non toxic indigenous plants with objectives to study their acute toxicity, evaluate anti-diabetic activity against alloxan induced

diabetes in experimental animal and to compare their hypoglycaemic activity with standard drug.

MATERIALS AND METHODS

Plant material

Leaves of *Murraya koenigii* and *Ocimum sanctum* were collected from their natural habitat Buldana, Buldana District, Maharashtra; during December 2103 - January 2014 and authenticated by the department of Dravyaguna vidnayan MUPS Ayurvedic college hospital and Research centre, Degaon, Washim, Maharashtra, India. Leaves were dried, powdered and extracted with 90% methanol using Soxhlet apparatus. The phytochemical analysis for the presence of different chemical constituents such as carbohydrates, glycosides, alkaloids, phytosterol, fixed oil, saponins, proteins, amino acids and flavonoids in these herbal drugs was carried out**.** The extracts of both the plants were mixed in 1:1 ratio to get polyherbal preparation. This was freshly prepared by making a suspension of the extract in 0.3% v/v carboxymethyl cellulose in distilled water.

Animals

Male-Female Wister Rats (180–230 g) were procured from the Anuradha College of Pharmacy, Chikhli and used in the experiments. The experimental design was approved by Institutional Animal Ethical Committee and the study was performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of animals. The animals were randomly distributed and housed in polypropylene cages, 4 in each. All the animals were subjected to a period of observation and acclimatization of at least two weeks between the date of issue and the start of treatment. The rats were individually identified by marking done on the body.

Induction of diabetes and treatments on experimental animal

Experimental diabetes was induced by single intraperitoneal injection of 150 mg/kg of alloxan, solubilised with 0.5 ml of normal saline. This was done in all the rats except the normal control (group I). The Wilstar rats were divided into five groups and treatments were carried out after 48 hours of alloxan administration and continued for 21 days.

Groups	Treatments (per oral)
Group I (Normal control	0.3% CMC
Group II (Diabetic control)	0.3% CMC
Group III (Standard)	Glibenclamide (5mg/kg)
Group IV (Test- I)	Methanolic extract of *Murraya koenigii* (100mg/kg) and *Ocimum sanctum* (100mg/kg)
Group V (Test- II)	Methanolic extract of *Murraya koenigii* (200mg/kg) and *Ocimum sanctum* (200mg/kg)

Acute toxicity studies

Acute toxicity evaluation of the polyherbal formulation as per the test guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft 423. (OECD guidelines, 2001) The principle of the test is based on a stepwise procedure with the use of a minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Following the period of overnight fasting, the animals were weighed and the test substance was delivered (3 animals/dose). Different doses of the formulation (1000mg/kg and 2000mg/kg body weight) were administered to different groups and the animals were observed periodically during the first 24 hours, with special attention to first 4 hours and daily thereafter for next 14 days.

Biochemical and physiological analysis

Blood was collected after 48 hours of alloxan administration by retro- orbital puncture method and the blood glucose level was determined before and after induction of diabetes by GOD- POD method. After 30 minutes of the alloxan treatment to the groups, 2gm/kg body weight glucose was given orally and blood samples were collected just prior to the glucose administration and at 1 hr, 2 hr and 3 hr after glucose loading to study oral glucose tolerance. (Fatima *et al.,* 2013) The studies were carried after 21 days. The food consumption and water intake of all groups of animals were monitored on a daily basis for 21 days at regular time. The body weights were measured on 1st and 21st day by using animal weighing balance.

Histopathological studies

After observation of all the parameters, the rats were subjected to histopathological study. Their pancreas were removed, sliced and fixed in paraffin for 12 hours. 5µm sections of these pancreatic tissues were grained with alum haematoxylin and eosin, and observed microscopically for histopathological changes i.e. normal, damaged or recovered forms.

Statistical analysis

Data was expressed as mean ± SEM. Statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post-test using Graph Pad software. $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemical studies

The yield of methanolic extracts of *M. koenigii* and *O. sanctum was* 18% and 10% w/w respectively. The phytochemical studies of the methanolic extract of *M. koenigii* showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, proteins, amino acids, saponins and flavoinds and of *O. sanctum* showed the presence of same constituents except carbohydrates. All of these chemical constituents mainly flavonoids, saponins, alkaloids, glycosides in *M. koenigii and O. sanctum* are responsible for hypoglycemic activity.

Acute oral toxicity and dose selection for herbal treatment

No mortality and signs of any toxicity seen after the administration of a limited dose of 1000 mg/kg of *Murraya koenigii* and *Ocimum sanctum* methanolic extracts, each in acute oral toxicity test. Hence, 200 mg/kg and 400 mg/kg of doses were selected for oral administration respectively.

Anti-diabetic activity

Table 1 indicated no significant difference in blood glucose levels among different levels of normal control. There is an increased blood glucose level in diabetic control groups indicating hyperglycaemic i.e. diabetic effect of alloxan. The standard group shows significant decrease in blood glucose

levels, which indicates hypoglycemic effect of glibenclamide. Similar results are seen in groups Test I and Test II, after alloxan administration, suggesting hypoglycemic effect of *Ocimum sanctum* and *Murraya koenigii* methanolic extracts.

Table 1 Effect of blood glucose levels (BGL)

Sr No	Groups	Blood glucose (mg/dl)				
		After 24 hrs fasting	48 hrs after alloxan administration	7 Days	14 Days	21 Days
1.	Normal control	87.7±0.24	87.81±0.25*	87.83±0.25*	87.85±0.31*	87.91±0.25*
2.	Diabetic control	87.2±0.14	312.41±0.57	330.26±0.29	335.4±0.38	377.41±0.35
3.	Standard	87.3±0.14	312.03±0.32	240.49±0.43*	180.58±0.34*	120.70±0.25*
4.	Test-I	87.2±0.15	311.68±0.37	271.37±0.32*	206.61±0.31*	131.03±0.19*
5.	Test-II	87.27±0.23	311.79±0.23	256.41±0.37*	183.57±0.31*	124.87±0.20*

Note: Within each dependent measure, means differ significantly. * represents p<0.001

Table 2 shows there was no significant difference in OGT of normal controls. High attenuation of plasma sugar levels of alloxan was observed in diabetic controls, indicating diabetic effect of alloxan. In standard group, blood glucose levels increased till an hour and then significantly decreased, indicating anti-diabetic effect of glibenclamide. Similar observations were drawn from groups Test I and Test II, signifying anti-diabetic effect of *Ocimum sanctum* and *Murraya koenigii*.

Table 2 Effect on oral glucose tolerance test

Sr.No	Groups	Oral glucose tolerance test (mg/dl)			
		0 hr.	1 hr.	2 hr.	3 hr.
1.	Normal control	95.46±0.10*	95.50±0.20*	95.85±0.21*	95.87±0.33*
2.	Diabetic control	113.13±0.63	145.89±0.32	120.67±0.33	111.52±0.33
3.	Standard	102.17±0.37*	124.26±0.20*	111.02±0.18*	100.24±0.01*
4.	Test-I	108.90±0.19*	127.89±0.30*	117.4±0.29*	107.73±0.21*
5.	Test-II	107.83±0.46*	126.64±0.27*	114.02±0.23*	105.14±0.17*

Note: Within each dependent measure, means differ significantly. * represents p<0.001

Table 3 records the effects of food and water intake in experimental groups. In normal control significant increase in food and water consumption is seen. Diabetic controls showed increased intake of food and water, indicating diabetic effect of alloxan. The standard group showed decreased intake in food when compared with the diabetic controls, suggesting anti-diabetic effect of glibenclamide. Similarly, Test group I and II suggested anti-diabetic effects of *Ocimum sanctum* and *Murraya koenigii* methanolic extracts.

Table 3 Effects of food and water intake

Sr. No	Groups	Food consumption (gm/day)		Water intake (ml/day)	
		Initial	Final	Initial	Final
1.	Normal control	50.78±0.35*	51.16±0.37*	190.98±0.24*	192.11±0.30*
2.	Diabetic control	55.40±0.19	72.88±0.34	207.38±0.33	342.71±0.40
3.	Standard	52.88±0.22*	61.43±0.33*	197.28±0.23*	288.33±0.38*
4.	Test-I	53.83±0.22*	67.73±0.34*	199.00±0.23*	292.86±0.29*
5.	Test-II	53.91±0.22*	65.21±0.46*	196.98±0.31*	290.85±0.31*

Note: Within each dependent measure, means differ significantly. * represents p<0.001

Figure 1 demonstrates the effect on body weight. Increase in body weight is noted in normal controls. Diabetic control group showed decrease in weight referring to the anti diabetic effect of alloxan. The animals of standard group showed significant increase in body weight indicating anti-diabetic effect of glibenclamide. Similar observations were made from Test groups I and II signifying the anti-diabetic properties of *Ocimum sanctum* and *Murraya koenigii* methanolic extracts.

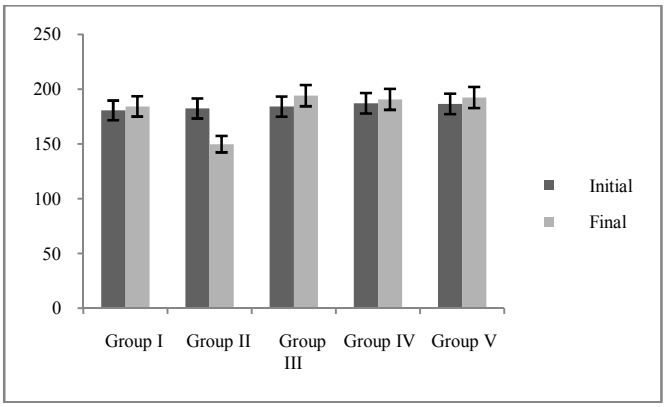

Fig 1 Effect on body weight

Histopathological studies

Histopathological examination of pancreas of normal control group showed normal architecture and no abnormalities. Necrosis, damaged mitochondria, dialated rough endoplasmic reticulum, cytoplasmic vacuolation were observed in diabetic control group. However, histopathological examination of pancreas of animals treated with standard and polyherbal preparation showed decrease in necrosis, dialated rough endoplasmic reticulum, damaged mitochondria and cytoplasmic vacuolation suggesting the anti-diabetic effects.

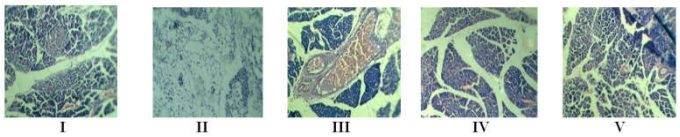

Fig 1 Histopathological studies; I- normal control, II- Diabetic control, III- Standard control, IV- Test I, V- Test II

DISCUSSION AND CONCLUSION

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with aberration in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. It has been reported that the chronic hyperglycemia of diabetes is associated with complications like renal failure, coronary artery disorder, neurological complications, cerebro-vascular disease, blindness, long term dysfunctions and failure of various organs and eventually premature death. (Lanjhiyana *et al.,* 2011)

Chemically, alloxan (2,4,5,6 tetraoxypyrimidine) is an oxygenated pyrimidine derivative and a cyclic urea analog which has been reported to produce diabetes in experimental animals such as rats. It is a well known diabetogenic agent

used to induce type- 2 diabetes. (Viana *et al.,* 2004; Gupta, 2009) Alloxan does not inhibit the function of the transporter and can therefore selectively enter beta cells in an unrestricted manner. (Vogel and Vogel, 2008)

Significant anti-diabetic activity by polyherbal preparation was observed in Test II i.e. methanolic extract of *Ocimum sanctum* (200 mg/kg p.o.) and *Murraya koenigii* (200 mg/kg p.o.) as compared to Test I i.e. methanolic extract of *Ocimum sanctum* (100 mg/kg p.o.) and *Murraya koenigii* (100 mg/kg p.o.). Oral administration of methanolic extract of *Murraya Koenigii* and *Ocimum Sanctum* improved the blood glucose profile in diabetic rats. It also showed reduced complications associated with the diabetic conditions. Hence, *Murraya Koenigii* and *Ocimum Sanctum* have a hypoglycemic potential. Similar results were reported by Jyothi *et al.,* Raja *et al.*, Ahmed *et al.* and Somasundaram *et al.* A study revealed that the O. Sanctum leaf extracts have stimulatory effects on physiological pathways of insulin secretion which may underlie its reported anti-diabetic action. (Kokate and Gokhale, 2005) Finally it was concluded that the polyherbal preparation has significant anti-diabetic activity.

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