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Anti-Diabetic Activity of Aqueous and Methanolic Extract of *Abutilon Muticum*

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Research Article

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Abstract

Diabetes mellitus is a collection of diseases that culminate in defects in carbohydrate metabolism and result in inappropriate hyperglycemia. Since ancient times, exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45 000 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes has shown anti-diabetic property. The present paper based on antidiabetic activity that have been mentioned/used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity. We are study here of *Abutilon muticum* an Indian plants which are most effective in relation to diabetes.

Keywords: *diabetic, methanolic extract, abutilon muticum, carbohydrate metabolism.*

Introduction

Diabetes is one of the most prevalent chronic diseases in the United States. The morbidity and mortality associated with the disease is significant and derives primarily from complications of persistent hyperglycemia. Longstanding hyperglycemia has been shown to lead to vascular complications involving large and small blood vessels, such as arteriosclerosis, glomerulosclerosis, and retinopathy. Diabetic neuropathy, characterized by pain and paresthesias, is among the most frequent complications of longstanding, poorly controlled diabetes and is often associated with a reduction in physical activity and with sleep disturbances.

Diabetes mellitus affects approximately 17 million adults in the United States and has profound implications in terms of long-term microvascular and macrovascular complications and their associated costs. In type 2 diabetes, insulin resistance and a relative beta-cell defect are the underlying pathologic problems leading to hyperglycemia. Notably, insulin resistance is also associated with obesity, dyslipidemia, and hypertension. Diabetes can be defined as a disease of accelerated cardiovascular deterioration associated with elevated blood glucose levels. Glycemic control has been shown to reduce the long-term complications associated with diabetes. Although medical nutrition therapy and appropriately prescribed increased physical activity are important components of a diabetes management plan, most patients need medication to lower glucose to near-normal levels.

Material and Method

The present study is undertaken to screen the Hypoglycemic activity of drugs on alloxan induced diabetic rats.
Materials and methods for the present study will be discussed under following headings.

A. Procurement of sample
B. Preparation of extract.
C. Experimental design.
D. Induction of diabetes by Alloxan.
E. Experiment (Hypoglycemic activity)

A.Procurement of Sample

Drugs will be collected from farmlands and Hills of Mandvi and Nakhatrana areas of Kutch Region, Gujarat and also from farmers and tribal's of Madhya Pradesh and were identified and authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, APS, University, Rewa, Madhya Pradesh, India.
The leaves will be dried in shade and the powder will be used for the extraction of antidiabetic

constituents in different solvents [water, methanol, chloroform, benzene, and ethylacetate.]

B.Preparation of Extract

1. Extraction Procedure

Extraction is defined as isolation of the soluble constituents from insoluble residue either liquid or solid by treatment with suitable solvents. The extraction of the soluble constituents from solid by means of a solvent is commonly referred as leaching.

2. Methods Of Extraction

Ø Maceration
Ø Percolation
Ø Infusion
Ø Decoction
Ø Continuous extraction (soxhlet apparatus)

In the present work maceration and continuous extraction (soxhlet apparatus) will be used for preparation of extracts.

3. In vivo Models

Method of studying oral hypoglycemics includes effect on glucose levels in normal animal by the measurement of the reduction of the blood glucose levels of animals having glucose induced hyperglycemia and also with alloxan, streptozotocin or any other diabetic models.

Diabetes in experimental animals can be produced by the following methods:

· Administration of anterior pituitary extract.
· Complete or partial pancreactomy
· Chemical means (alloxan or streptozotocin)
· Administration of adrenaline.

Alloxan is an accepted model for the induction of the diabetes, M. Kaleem, Sheema.h.sarmad and B.bano (2005) have used this model for screening *piper nigram* and *vinca rosea*.
Vinuthan, M.K. Girish Kumar.v have used this model for screening ***Murraya koenigii*** leaves.
V.Babu, T. Gangadevi, A. Subramanyam, have use for screening ***Cassia keleini***.
V.Babu, T. Gangadevi, A. Subramanyam, have used streptozotocin for induction of diabetes for screening antidiabetic activity of ***Cassia keleini***

C.Experimental Design

1. Animal under study

Male wistar albino rats weighing in the range of 180-200 grams will be selected for inducing and treating diabetes. They will be fed a standard rat pellets and water and maintained under standard laboratory conditions.

D.Induction of Diabetes Using Alloxanmonohydrate

Alloxan monohydrate [SD Fine Chemicals] is one of the chemical agents used to induce diabetes mellitus in rats. It induces diabetes by partial destruction of beta cells of islets of langerhans. The partial destruction of beta cells leads to decreased levels of insulin resulting in hyperglycemia. There is a possibility for the survival of a few beta cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like Glibenclamide, olbutamide etc, in Alloxan induced diabetic rats.

1. Preparation of alloxan monohydrates

Alloxan was prepared by weighing 1 gm of alloxan and dissolving in 20ml of water for injection. Alloxan at this calculated dose is said to have a concentration of 50mg/ml. Hypoglycemic Activity: 30 animals were used for these study and divided in to five groups of each six rats. The basal concentration of blood glucose level of all the animals was recorded and 6 animals were separated to serve as normal control. The remaining animals received a single Injection of Alloxan monohydrate in water for injection at a dose of 150-mg/kg bodyweight given by intra-peritoneal route. After 4 days of Alloxan administration, the blood glucose was estimated and animals with blood glucose levels in the range 280 mg/dl and 380 mg/dl were selected and divided into groups.

The animals will be grouped as follows.
Group I :- Normal control groups [6 rats]
Group II :- Diabetic control groups [6 rats] (Alloxan treated).
Group III:- Alloxan treated + Glibenclamide standard group [6 rats], dose – 10 mg/kg B.W.
Group IV:- Alloxan treated + Aquase extract groups [6 rats], dose – 250 mg.
Group V :- Alloxan treated + Methanolic extract groups [6 rats], dose – 250 mg

E.Experiment

All the rats in the above group are weighed individually and the dose of Alloxan is calculated according to the individual weight of the rat. Alloxan is injected through the intra peritoneal route to all the groups of rats except the normal control.

Then after 4 days of treatment with alloxan, the blood glucose levels were detected by using glucometer. It has found a marked increase in the blood glucose levels, which shows the development or induction of diabetes in the rats. The diabetic rats exhibiting blood glucose levels in the range 280-380mg/dl were selected to determine the efficacy of the drug extracts.

Animals are treated with the methanolic Extract at the dose of 250mg. On the same day, the blood samples were collected from the tail of the rat and the glucose levels were estimated at 1hr, 2hr, 5hr, 7hr, and 12hr. using one touch Glucometer.

The glucose level in the diabetic control group will be observed after administration of standard and test drug. In the Alloxan treated group the glucose level will increase from 112mg/dl-320mg/dl, indicating significant Hyperglycemia.

Results and Discussion

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed.

Table 1 (Graph 1) indicates effect of administration of feeding the aqueous extract & methanolic extract of plants on body weight and Fluid intake in normal and diabetic rats. Table 2 showed effect of administration of feeding the aqueous extract & methanoilc extract of plants on Total hemoglobin and Urine sugar in normal and diabetic rats. It was observed in table 2 and graph 2 that significant lowering of sugar in methanolic extract (15.97±0.154). According to the world health organization the blood glucose level above 150 mg/dl indicates the Hyperglycaemic activity. Severe thirst, reduction in body weight were also noticed (Table-3). The administration of *A muticum,* aqueous and methanolic extract at a dose of 250 mg/kg body weight showed significant anti-hyperglycaemic effect which was evident from the 1st day on wards. The methanolic extract shoed better efficacy than the aqueous extract. The anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in table 3 and graph 3. The decreasing blood glucose levels are comparable to that of 10 mg/kg of Glibenclamide.

The present study was undertaken to evaluate the anti-diabetic activity of an aqueous and methanolic extract of *A. muticum* in alloxan induced diabetic rats. Alloxan damage to the islet tissue was confined to the insulin secreting pancreatic beta cells (*Dunn Js kekpatric*) through a direct effect (*Hellman Diderholn*). The alpha cells being resistant to Alloxan (*Dunnis Js Duffing*). Thus Alloxan proved to be a suitable compound for inducing experimental diabetes with typical symptoms such as mentioned above. Considering the problems associated with marketed hypoglycaemic, health care providers have been looking for a safe substitute for them. Currently several herbal medications with different mechanism of action have been found to have hypoglycaemic effect. The blood glucose levels of the Anti-diabetic Activity of Alloxan Induced Diabetic Rats were shown in table – IV. It represents that decrease in blood glucose levels. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 7th Day compare to the initial. The methanolic extract (250mg/kg body weight) of the shows significant (P*<0.01), effect on 7th hr of the administration of extract.

The blood glucose levels of the Anti-diabetic Activity of Alloxan Induced Diabetic Rats were shown in table – 3. It represents that decrease in blood glucose levels. The glibenclamide (10 mg/kg body weight) shows significant (P*<0.01) effect on 7th Day compare to the initial. The methanolic extract (250mg/kg body weight) shows significant (P*<0.01), activity than the aqueous extract (250mg/kg body weight). Based on the current results, it appears that both extract showed pronounced blood glucose – lowering in alloxan induced diabetic rats. Further studies are required to identity the extract mechanism of action of the anti-diabetic activity.

Table 1: Effect of administration of feeding the Aqueous extract/ methanolic extract of plants on body weight and Fluid intake in normal and diabetic rats.

GROUP	Body Weight (g)		Fluid intake g/animal/day
	Before treatment	After treatment	
Untreated control (Normal saline water)	194±1.88	220.5±1.839	22.047±0.247
Diabetic control	202.66±2.33	168.5±2.513 ###	75.288±0.223###
Diabetic+ Glibenclaminde (10mg/kg)	206.66±1.745	222.33±1.96***	53.610±0.375***
Diabetic + A. muticum aq. Extract (250mg)	209±1.932	220.16±1.078***	59.436±0.166***
Diabetic + A. muticum meth. Extract (250mg)	197±2.176	213.6±1.476***	58.43±0.357***

All values are expressed as mean ± S.E.M (n=6). ***P<0.001, **P<0.01 as compared to diabetic control, ###P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison test.

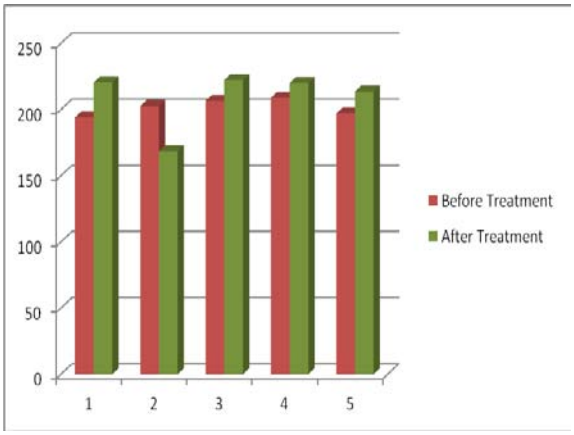
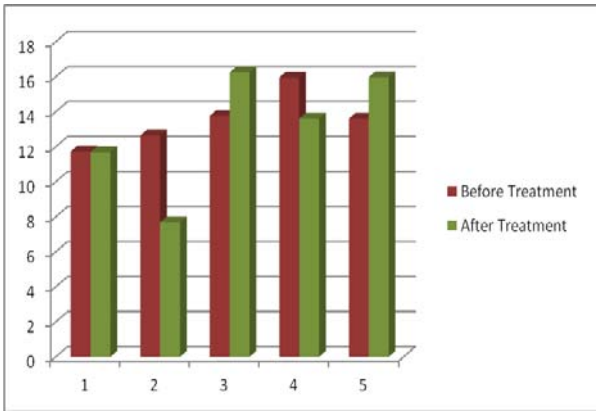


Table 2: Effect of administration of feeding the Aqueous extract/ methanolic extract of plants on

Total hemoglobin and Urine sugar in normal and diabetic rats.

	Total hemoglobin (%)		Urine sugar	
GROUP	**Before treatment**	**After treatment**	**Before treatment**	**After treatment**
Untreated control (Normal saline water)	11.73±0.448	11.7±0.481	Nil	Nil
Diabetic control	12.64±0.162	7.69±0.744$^{###}$	+4	+4
Diabetic+ Glibenclamide (10mg/kg)	13.77±0.283	16.25±0.399***	+4	+1
Diabetic + A. muticum aq. Extract (250mg)	15.96±0.242	13.61±0.297***	+4	+2
Diabetic + A. muticum meth. Extract (250mg)	13.61±0.297	15.97±0.154***	+4	+1

All value are expressed as mean ± SEM (n=6). ***P<0.001 as compared to diabetic control, $^{###}$P<0.001 as compared to untreated control .One-way ANOVA followed by Bonferroni multiple comparison test.



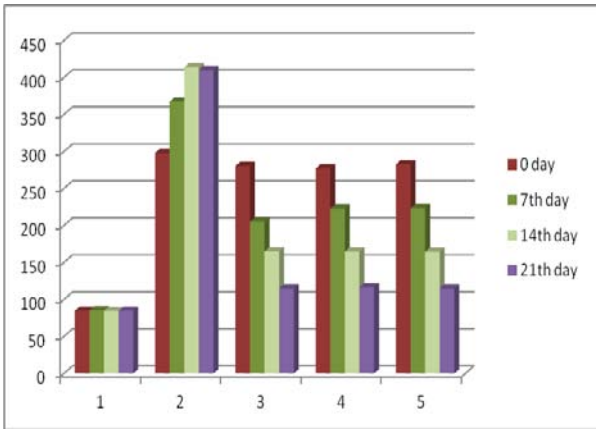


Table 3: Effect of administration of feeding the Aqueous extract/ methanolic extract of plants on serum glucose estimation in normal and diabetic rats

GROUP	Serum glucose (mg/dL)			
	0 day	**7th day**	**14th day**	**21th day**
Untreated control (Normal saline water)	84.83±5.41	85.33±5.87	84.66±5.77	84.83±5.09
Diabetic control	298.16±17.20	367.33±4.7$^{##}$	413.83±16.61$^{###}$	410±2.045$^{###}$
Diabetic+ Glibenclaminde (10mg/kg)	280.33±2.44	205.33±1.145**	165±1.29***	114.83±1.302***
Diabetic + A. muticum aq. Extract (250mg)	277±7.69	223±4.195**	164.66±1.406***	116.5±1.232***
Diabetic + A. muticum meth. Extract (250mg)	282.66±4.49	223.33±1.94***	164.16±1.406***	115±0.966***

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01 as compared to diabetic control. $^{##}$P<0.01, $^{###}$P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison test.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.