

Antidiabetic Activity of *Maesa indica* (Roxb.) Stem Bark in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Maesa indica belonging to family Myrsinaceae, commonly known as Atki is a large shrub. Ethnobotanical studies of Kolhapur district revealed that stem bark of *M. indica* is used in the treatment a diabetes. The literature survey revealed that there is no experimental evidence of antidiabetic effect of the plant. Therefore the present study was carried out to investigate antidiabetic effect of *M. indica* stem bark in streptozotocin induced diabetic rats. The Plant was subjected to pharmacognostic, physico-chemical and phytochemical evaluations which will assist in standardization for authenticity, quality and identification of the herbal products. Treatment with 2ml/kg stem bark distillate to diabetic rats resulted in significant reduction in blood glucose level. The preset study clearly demonstrated that the plant is having potential hypoglycemic activity which may be beneficial for the management and treatment of diabetes mellitus. It also shows good alpha glucosidase inhibition activity.

Keywords: Antidiabetic activity, *Maesa indica*, Streptozotocin, Rats, Alpha glucosidase, Traditional medicine.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both¹. Diabetes mellitus is a common disorder among the Indian population. According to WHO, it is estimated that 3% of the world's population

have diabetes and the prevalence is expected to double by the year 2025 to 6.3%.² Management of diabetes without any side effect is still a challenge to the medical community. Plant drugs are frequently considered to be less toxic and free from side effects than synthetic one. Thus searching for a new class of compounds is essential to overcome diabetic problems.

There is a continuous search for an alternative drug.³

Ethnobotanical information indicates that more than 800 plants have been used as traditional remedies for the treatment of diabetes.⁴ The anti-hypoglycemic activity of a large number of these plants have been evaluated and confirmed in different animal models.⁵ In India, a number of plants are mentioned in ancient literature (Ayurveda) for the treatment of diabetes and some of them have been tested experimentally.⁶ *Maesa indica* belonging to family Myrsinaceae, commonly known as Atki is a large shrub. Ethnobotanical studies of Kolhapur district revealed that stem bark of *M. indica* is used in the treatment a diabetes. The literature survey revealed that there is no experimental evidence of antidiabetic effect of the plant. Therefore the present study was carried out to investigate antidiabetic effect of *M. indica* stem bark in streptozotocin induced diabetic rats.

MATERIAL AND METHODS

Experiments were carried out according to the guidelines of the institute and CPCSEA (Registration no. 233/CPCSEA).

Preparation of plant extract

The plant material were air-dried at room temperature and ground to a fine powder using a laboratory grinder. Powdered materials were maintained at room temperature and protected from light until required for analysis. For the antidiabetic study the plant material (5 g) was soaked in 100 ml distilled water and distilled. The distillation was carried out at 60 °C for 3 hrs. The 50 ml distillate was collected and used for further analysis.

Experimental Animals

Male albino rats weighing between 150–200 gm were used. The rats were fed

with standard rat diet purchased from Amruth, India. Animals were provided with food and water *ad libitum* and maintained at 25–28 °C. Diabetes was induced by injecting 65 mg of Streptozotocin per kg body weight intra-peritoneally to overnight fasted animals. The diabetic status of the animals was verified by checking their blood glucose on 14th day. Animals with blood glucose above 200 mg/dl were considered diabetic and included in the experimentation.

Oral Glucose Tolerance Test (OGTT)

Glucose tolerance test was administered by feeding the rats with 3mg glucose/gm of body weight by following the method of Andrade *et al.* (2005).⁷ After glucose administration the diabetic rats (glucose level above 200mg/dl) were separated and divided into three groups of three diabetic rats each. Group I was previously selected from normal rats and served as normal control and was given 0.5 ml distilled water. Group II served as diabetic control and was given distilled 0.5ml water. Group III received standard antidiabetic drug Gliclazide at an oral dose of 1.6mg/kg body weight. Group IV was treated with 2ml/kg body weight plant distillate with introgastic tubes. The dose was selected after preliminary behavioral and acute toxicity tests. Blood sample was withdrawn from tail vein just prior to 30 min., 60 min. and 120 min. after drug administration. The blood glucose level was measured using Accu-check Glucometer purchased from Roche, India and were verified using appropriate controls. All the results presented are average values of experiments conducted on a set of three rats.

Preparation of rat intestinal alpha glucosidase

All procedure for preparation of rat intestinal alpha glucosidase was performed at 4°C. The small intestinal mucosal tissue

was collected by scraping the luminal surface firmly with a spatula. The mucosal scrapings were homogenized with 0.2 M sodium phosphate buffer pH 7.0 containing 1 % triton X 100 and then centrifuged at 12000 rpm for 15 min. The supernatant fraction contained rat small intestinal alpha glucosidase. Butanol was added to the supernatant fraction in 1: 1 proportion and centrifuged at 15000 rpm for 15 min. The aqueous layer was dialyzed overnight against the same buffer. After dialysis, the concentrated enzyme was stored at 4⁰C. This crude enzyme was used in the study to observe inhibition by different concentrations of plant distillate.

In vitro alpha glucosidase inhibition

The activity of the rat small intestinal alpha glucosidase was determined by measuring the formation of glucose. The standard incubation mixture contained 0.2 M sodium phosphate buffer (pH 7.0), 25mM maltose and 0.2 mL enzyme. The plant distillate was added in mixture as 100µl, 200µl, 300µl, 400 µl, 500 µl and acarbose used as positive control. After incubation at 37⁰C for 30 min, the liberated glucose was measured by GOD / POD kit. One unit of enzyme activity was defined as the quantity of enzyme producing 1mmol glucose per min at 37⁰C at pH 7.0. In all the inhibitor studies, enzyme was pre-incubated with the inhibitor for 3 min before addition of the substrate. Assays were performed in quadruplet.

RESULT AND DISCUSSION

OGTT (Oral Glucose Tolerance Test)

The hypoglycemic activity of stem bark of *Maesa indica* plant species was analysed by OGTT (Oral Glucose Tolerance Test) and was recorded in table 1. In the OGTT test, the glucose concentration showed the maximum level of glucose at 30 min. and the decreased at particular level

with particular time. From the fig.1 and table 1 it was clear that in the untreated animals (control animals), blood glucose level did not change significantly but diabetic animals treated with plant distillate shows statistically significant and considerable fall in blood glucose level in two hours with compare to that of standard antidiabetic drug Gliclazide. There was no any previous report on antidiabetic activity of stem bark of *Maesa indica* plant species under study.

In vitro alpha glucosidase inhibition

Alpha glucosidase inhibition activity of stem bark of *Maesa indica* was presented in table 2. As shown in fig. 2 and table 2 standard alpha glucosidase inhibitor Acarbose (200 µl) shows 90% inhibition activity while 100 µl plant distillate shows 20% inhibition of alpha glucosidase, 200 µl plant distillate shows 26% inhibition activity, 300 µl shows 30%, 400 µl exhibit 45% and 500 µl plant distillate shows 56% inhibition of alpha glucosidase activity. Plant distillate shows 56% inhibition at 500 µl as compare to standard inhibitor Acarbose which shows the 90% inhibition. From the above result it was revealed that alpha glucosidase inhibition activity of plant distillate of *M. indica* increases with increase in the concentration of plant distillate.

Mukhtar *et al.* (2004)⁸ worked on hypoglycemic activity of *Psidium guajava* ethanol leaf extract. The plant belongs to family Myrtaceae and leaves were useful in wounds, ulcer, cholera, diarrhea, vomiting and ulcerated mouth. It has antidiarrhoeal, anticough, analgesic, anti-inflammatory, antipyretic and antidiabetic activities. The plant material was collected from Bullandshehar district of Uttar Pradesh, India. Male Wistar rats weighing 160-200gm were used in the experiment. Alloxan monohydrate was used to induce

diabetes in normal rats. From the result it was revealed that diabetic rat treated with standard antidiabetic drug Gliclazide reduces blood glucose level from 462.32 mg/dl to 242.66 mg/dl in two hours and diabetic rat when treated with *P. guajava* leaf extract reduces blood glucose level from 345.20 mg/dl to 220.00mg/dl. The result of the present work shows that diabetic rat treated with standard antidiabetic drug Gliclazide reduces blood glucose level from 462.32 mg/dl to 242.66mg/dl in two hours and diabetic rat treated with stem bark distillate of *M. indica* plant species, the blood glucose level reduces from 422.87mg/dl to 148.34 mg/dl. This shows that plant distillate of *M. indica* stem bark possesses very good antidiabetic activity with compare to standard antidiabetic drug Gliclazide. Previous authors use alloxan monohydrate instead of streptozotocin to induce diabetes in experimental rats. Stem bark distillate of *Maesa indica* also shows alpha glucosidase inhibition activity.

Ahmed et al. (2005)⁹ carried out antidiabetic activity of *Terminalia catappa* Linn. leaf extract in Alloxan induced diabetic rats. *T. catappa* belongs to family Combretaceae. The leaves and bark of the plant have been reported to be anticancer, antioxidant, anti-HIV, hepatoprotective and antidiabetic. The plant material was collected from Mysore Botanical Garden, Karnataka. The leaf powder was extracted with distilled water. Male Wistar albino rats were used for experiments. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate. Results revealed that diabetic rats treated with standard antidiabetic drug Gliclazide reduces blood glucose level from 206.00mg/dl to 192.00 mg/dl and diabetic rat when treated with *T. catappa* leaf aqueous extract reduces blood glucose level from 207.00gm/dl to 181.22 mg/dl. The result of the present study shows that diabetic rat treated with standard

antidiabetic drug Gliclazide reduces blood glucose level from 462.32 mg/dl to 242.66 mg/dl and diabetic rat treated with *Maesa indica* stem bark distillate reduces blood glucose level from 422.87 mg/dl to 148.34 mg/dl. This shows that *Maesa indica* stem bark shows good antidiabetic activity.

HPTLC analysis and GCMS analysis of *Maesa indica* stem bark shows presence of antidiabetic compound Lupeol. Presence of lupeol in the bark of *Maesa indica* justifies their traditional use in the treatment of diabetes.

CONCLUSION

The results indicate that *Maesa indica* stem bark possesses significant hypoglycemic (antidiabetic) activity. It also shows good alpha glucosidase inhibition activity as concentration increases. It is generally accepted that streptozotocin causes permanent destruction of β cells.¹⁰ Therefore it is conceivable that the glucose lowering (hypoglycemic) activity of stem bark distillate of *M. indica* may be due to the insulin mimetic or insulin secretagogues components present in the plant distillate. It is essential to isolate and to identify bio constituents in plants so as to generate new lead molecules for drug development in treatment of diabetes.

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Table 1. Effect of *M. indica* bark distillate on blood glucose level of diabetic rats in OGTT

Group	Treatment	Blood glucose (mg/dl)			
		Basal value	30 min.	60 min. (1h.)	120 min. (2h.)
I	Normal control	86.60	124.39	108.48	86.34
II	Diabetic control	169.88	532.67	476.12	408.96
III	Standard drug (Gliclazide)	222.57	462.32	362.24	242.66
IV	Plant distillate	168.12	314.87	422.72	148.34

Table 2. Alpha glucosidase inhibition

S. no.	Treatment (plant distillate in μ l)	% inhibition of α - glucosidase
1.	Control (Acarbose - 200 μ g)	90
2.	100 μ l	20
3.	200 μ l	26
4.	300 μ l	30
5.	400 μ l	45
6.	500 μ l	56

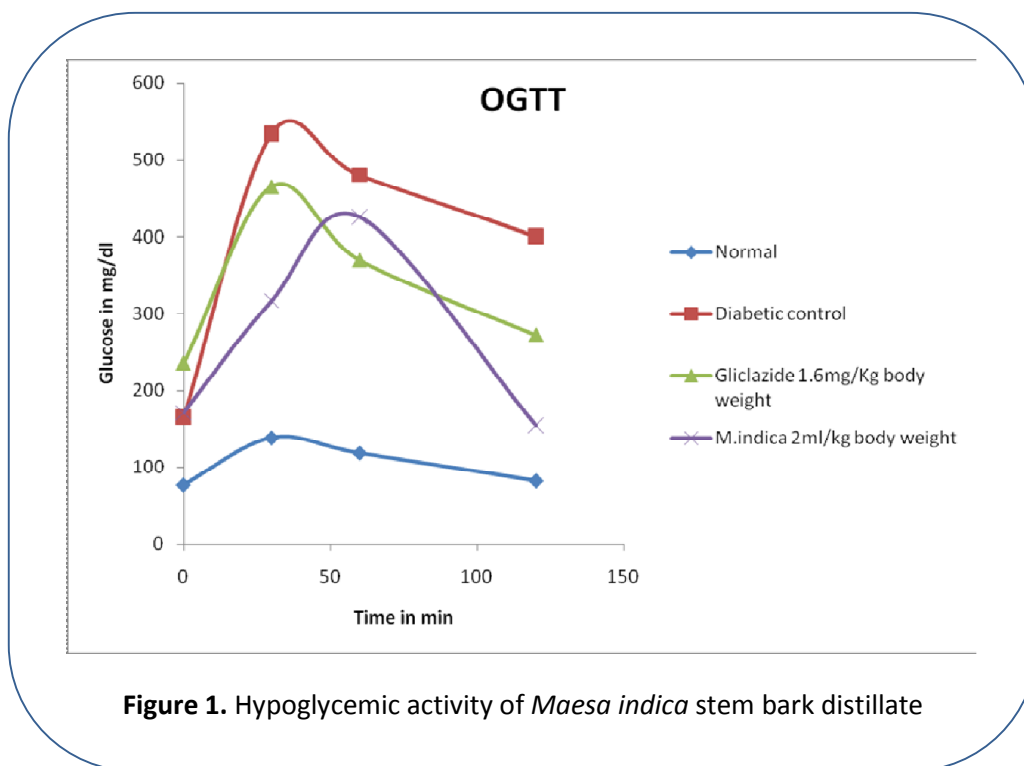


Figure 1. Hypoglycemic activity of *Maesa indica* stem bark distillate

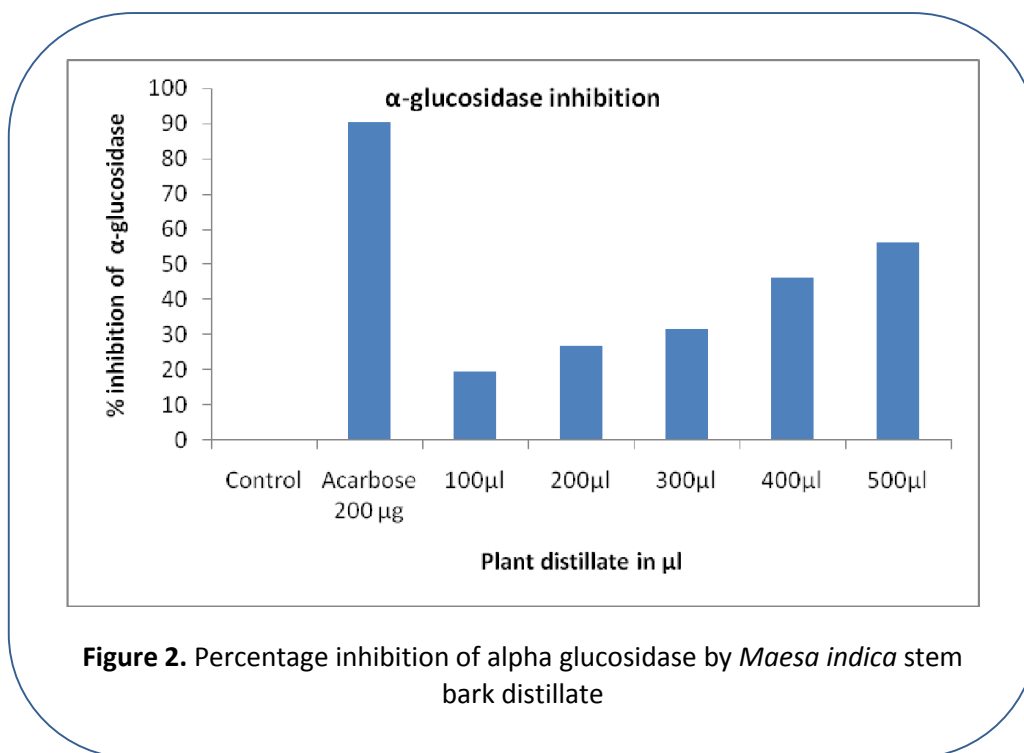


Figure 2. Percentage inhibition of alpha glucosidase by *Maesa indica* stem bark distillate