

POTENTIAL BIOLOGICAL EVALUATION OF HYPOGLYCEMIC ACTIVITY OF LEAVES OF *AGANOSMA DICHOTOMA*

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SUMMARY

The hypoglycemic effects of methanolic extract of *Aganosma dichotoma* were prepared from its leaves which were examined in Swiss-albino mice for conducting the experimental study. In doses of 200 and 400 mg/kg body weight of *Aganosma dichotoma* were used to compare with its standard Glibenclamide dose of 10 mg/kg body weight. Both doses of 200 and 400 mg/kg lowered the plasma glucose statistically significant level in 3 hours administration with its standard drug Glibenclamide. From this study it has been concluded that the methanolic extract of leaf of *Aganosma dichotoma* having good hypoglycemic effect and increase in plasma insulin concentration.

KEYWORDS: *Aganosma dichotoma*; Glibenclamide, Leaf extracts, Hypoglycemic activity.

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder in men and women and the major public health problem of epidemic proportion. More than 150 million people are suffering from it worldwide and it will be near about 300 million in the year of 2025.^[1] Diabetics accelerate the level of oxidative stress and this participates massively to most neurological, cardiovascular, retinal, renal diabetic complications.^[2] Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes

have one or more adverse effects.^[3] In view of the adverse effects related to the synthetic drugs and considering the natural medicine safer, cheaper and effective, traditional anti-diabetic plants can be explored.^[4] Traditionally plants are most frequently used in the treatment of all diseases in all over the world. For example, the plants are traditionally used in the management of diabetes in Nigeria and also considering its Pharmacological and toxicological considerations.^[5] A long listed plant are also used in the diabetic complication like *Abelmoschus moschatus* (Malvaceae), *Acacia Arabica* (Leguminoseae), *Azadirachta indica* (Meliaceae), *Acer ginnala* (Aceraceae), *Illicium religiosum* (Illiciaceae) etc.^[6] *Aganosma dichotoma* (Apocynaceae) contain Kampferol, phenolic acid.^[7] The plant *Aganosma dichotoma* is a large climber and having stout stem and milky latex. Its leaves are 10-12.5 cm long and grown all over Bangladesh. From brine shrimp lethality bioassay we can predict that *Aganosma dichotoma* has many bioactive secondary metabolites. It also provides antioxidants and thrombolytic activity besides moderate antimicrobial and thrombolytic constituents.^[8] At the present study, hypoglycemic effect of methanolic extract and its different fractionates of leaf of *Aganosma dichotoma* at 200 mg/kg doses were examined & compared with relative to that of control and standard group. Here Glibenclamide was used as a standard drug.^[1, 9, 10]

MATERIALS AND METHODS

Collection of Plant Sample

The plants of *Aganosma dichotoma* were collected in September, 2012 from Sylhet, Bangladesh. A voucher specimen was deposited in the Bangladesh National Herbarium, Mirpur, Dhaka and is tagged with the accession number of 39644.

Preparation of Plant Extract

The leaves were collected and dried at 60-70° C. in the air and crash by mechanical grinder. 100gm dried powder was soaked in 500ml methanol for 7 days on a shaker machine. The plant extract was filtered with filter paper and concentrated by evaporating the solvent using a water bath at a temperature of 40° C. A paste-like deep green colored concentrate was obtained. All extracts were partitioned by modified Kupchan method.^[5]

Drugs and Chemicals

Glibenclamide were used for the standard drug to conduct the study. Glibenclamide were collected from Bangladeshi largest company “Square Pharmaceuticals Limited”.

Experimental Animal

Swiss-albino mice were selected to conduct this study of both sex and aged from 4-5 weeks. These animals were obtained from Animal Resource Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDRDB) to continue the experiment. They were housed in standard polypropylene cages and kept under controlled room with temperature ranged from ($24 \pm 2^{\circ}\text{C}$) and relative humidity was (60-70%). The room was controlled in a 12 hours light-dark cycle and fed ICDDRDB formulated rodent food and water (ad-libitum). The animals were kept before the test for at least 3-4 days in the environment where the experiment will take place as these animals are very sensitive to environmental changes.

Experimental Design

The animals were divided into ten groups from thirty experimental animals and denoted as group-I, group-II, group-III (A-B), group-IV, group-V and group- VI consisting of 5 mice in each group groups. A particular treatment received of each of experimental group. Each mouse was weighed properly prior to any treatment and the doses of the test samples and control materials were adjusted accordingly. It was necessary to identify individual animal of a group during the treatment as it was difficult to observe the biologic response of five mice at a time receiving the same treatment. The animals were marked and individualized in the following way as M1= Mice 1, M2= Mice 2, M3= Mice 3, M4= Mice 4 and M5= Mice 5.

Preparation of Test Materials

The extract was administered at the doses of 400 mg/kg body weight and 200 mg/kg body weight of mice. In order to administration, the exactly weighed extracts were measured respectively and triturated in unidirectional way. A suspending agent (Tween-80) was used for proper mixing of extracts. Normal saline was slowly added after proper mixing of extract and suspending agent. The final volume of the suspension was made up to 3.0 ml. It was stirred well by vortex mixture to stabilize the whole suspension.

For the preparation of standard (Glibenclamide) at the dose of 10-mg/kg body weight, 10 mg tablet was dissolved into 3.0 ml normal saline (0.9% NaCl).

Experimental Procedure

Control (1% Tween-80 solution in saline) and Glibenclamide (in standard groups) were administered orally by means of a long needle with a ball shaped end, at zero hour test

samples. The test samples were also administered orally to the test groups. All groups were treated with 10% glucose solution (2gm/kg body wt.), after 60 min. Blood samples were collected from tail vein, after 30, 90 & 150 min of glucose loading. Blood glucose level is measured by using glucometer.

Statistical Analysis

All data were expressed as mean and Standard error. T-test was used to evaluate the different sample. The statistical significance was accepted at the range of $P < 0.001$, $P < 0.01$ and $P < 0.05$.

RESULTS AND DISCUSSION

In animals treated with *Aganosma dichotoma* (MESF-1 & MESF-2) and Glibenclamide (STD) showed a significant decrease in serum glucose levels, when compared with its control group (CTL). Both dose of 200 and 400 mg/kg showed statistically significant level of reduction of plasma glucose level with P-value of (0.0183 & 0.0419) with its control (Table-1).

The effects of methanolic extract of leaf of *Aganosma dichotoma* at 200 mg/kg and 400 mg/kg dose to lower blood glucose level were observed as follows to evaluate their hypoglycemic activity (Figure-1).

Table-1: Plasma level of glucose of different groups of mice.

Code No. & Identification	Test Sample	Group & Dose (mg/kg)	t-Test Value	Standard Error	P-Value	Level of Significant
CTL (Control group)	1% Tween-80 and DMSO in normal saline	I (0.1 ml/10 gm of body wt)	-	-	-	-
STD (Standard group)	Glibenclamide	II (10 mg/kg)	2.5841	0.792	0.0415	Statistically significant
MESF-1 (Test sample-200 mg/kg)	Methanolic extract of <i>A. dichotoma</i>	III- A (200 mg/kg)	3.2119	0.4842	0.0183	Statistically significant
MESF-2 (Test sample-400 mg/kg)	Methanolic extract of <i>A. dichotoma</i>	III-B (400 mg/kg)	2.5774	0.579	0.0419	Statistically significant

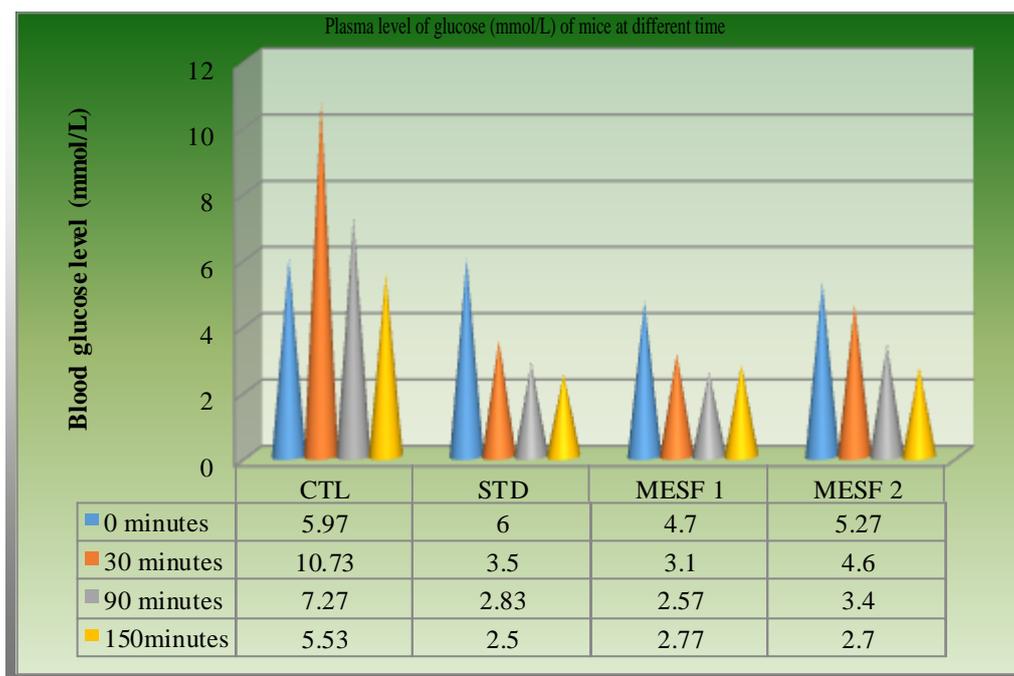


Figure 1: Plasma level of glucose of different groups of mice at different times.

CONCLUTIONS

In the present investigation, the results demonstrate that *A. dichotoma* exhibit potential hypoglycemic activity. This plant may effectively use for the treatment of diabetic patients. Further investigation will provide us more biological effectiveness of this plant extracts and may lead to develop new moieties. It may provide further hypoglycemic agent by isolation, purification as a lead compounds.

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