EVALUATION OF *Flemingia macrophylla L.*, A TRADITIONALLY USED PLANT OF THE NORTH EASTERN REGION OF INDIA FOR HYPOGLYCEMIC AND ANTI-HYPERGLYCEMIC EFFECT ON MICE

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Summary

Leaves of *Flemingia macrophylla* (Family-Fabaceae) traditionally used by local health practitioners as folk remedy in the North Eastern Region of India have been evaluated for its hypoglycemic and anti-diabetic activity. The effects of different doses (150-450 mg/kg b.w.) of crude aqueous methanolic (1:4) extract administered to normal and alloxan-induced diabetic mice varied with the dosage used. At 250 mg/kg body weight, the blood glucose level was reduced to 37% and 39% from that of control in normal and diabetic mice respectively, indicating a hypoglycemic and anti- hyperglycemic activity. No sign of toxicity at this dose was observed. However, dosage of 350mg/kg body weight and above was toxic to the mice. Glibenclamide, Metformin and Insulin were used as reference drugs for comparison.

Key words: Hypoglycemic, Antihyperglycemic, Alloxan, *Flemingia macrophyll,* Glibenclamide, Metformin, Insulin

Introduction

Plant infusions and decoctions have been used as medicine in developing countries for treatment of various patho-physiological conditions. One of the main reasons as pointed out by the World Health Organisation (WHO) is that the rural communities comprising upto 80% of developing nations do not have access to conventional health care relying mostly on local health practices as these are easily available, affordable and time tested. In recent years, there is a growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. Medicinal plants are sought after in view of the perceived minimal adverse effects (Rates, 2001). They are considered to be effective, non toxic and have a vast potential but are only partly explored by modern methods. The traditional knowledge on the use of plants for medicines can provide the initial information towards drug discovery (Cordell, 2000; Craker & Gardner, 2005). Natural products research most often is guided by ethno-pharmacological knowledge, and it can make substantial contribution to drug innovation (Cordell, 2000; Fabricant & Farnsworth, 2001). However, both plant derived drugs and herbal formulations have to take the same pharmaco-economic hurdle (WHO/TRM/91.4; Rates**,** 2001).

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Diabetes mellitus which is increasing in prevalence even in developing countries including India (King *et al*., 1998; Wild *et al*., 2004) is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism (Arky, 1982; Davis & Granner, 1996). Non-insulin dependent diabetes mellitus (NIDDM) common amongst diabetic subject, is characterized by reduced circulating concentration of insulin, poor insulin sensitivity or insulin resistant, poor glucose tolerance resulting in high sugar in plasma. Hyperglycemia condition *per se* impairs insulin secretion and prolonged hyperglycemia leads to other micro and macro vascular complications (Davis & Granner, 1996; Steppan *et al*., 2001).

Many plants have been reported to exhibit hypoglycemic and anti-hyperglycemic activity with or without toxic **e**ffects (Bailey & Day, 1989; Ivora *et al*., 1989; Swanston-Flatt *et al*., 1990; Handa, 1991; Marles & Farnsworth, 1995; Murthy, 1995; Day, 1998; Arai *et al*., 1999; Srinivas *et al*, 2000; Rao *et al*., 2001; Shapiro & Gong, 2002; Mukherjee *et al*., 2006). Literature survey have revealed that a large number of plants traditionally used by local communities of the North Eastern States of India have not been evaluated scientifically for their reported medicinal properties. While there are several scattered ethnobotanical reports (Jain, 1980; Jain *et al*., 1989; Kayang *et al*., 2005; Kharbuli *et al*., 2006) no serious attempts have been made to follow up on such *leads* provided by local knowledge which has both biomedical and commercial implications. We have earlier reported the hypoglycemic and anti-hyperglycemic effects of *Potentilla fulgens* and *Osbeckia chinensis* roots on mice (Syiem *et al.*, 2002; 2006). This paper describes the study of *Flemingia macrophylla* (Family-Fabaceae), a common plant of North Eastern India, widely used by indigenous tribe of Manipur for controlling diabetes mellitus.

Material and Methods

Chemicals

Alloxan was procured from Sigma Co. USA, Glibenclamide from Hoechts, Insulin from Knoll Pharmaceutical Ltd., Metformin from USV limited, Maharashtra, while other chemicals used were of analytical grade obtained from E. Merck and Hi-media, India.

Test Animals

Healthy, adult female Swiss albino mice, weighing 20-30 gms were used for the study. Mice were housed in a room kept under controlled conditions with temperature maintained at 22 ^{o}C on a twelve hour light/dark cycle and were fed with balanced mice feed obtained from Amrut Laboratory, Pune, India.

Plant material

Leaves of *Flemingia macrophylla* were collected from Bungmual village of Lamka, Churachandpur district of Manipur (Voucher No: 5452) The specimen was submitted and identified by Dr. P.B.Gurung Curator herbarium, Department of Botany, NEHU, Shillong Meghalaya.

Extraction

The leaves were separated, weighed, washed, shredded and dried in the shade. It was then powdered, homogenized and repeatedly extracted with 10 volume of aqueous-methanol solution (1:4) (Harborne, 1998). The mixture was filtered and the filtrate evaporated to dryness at 40 ^{0}C in a Buchi-rotatory vacuum evaporator. The dried mass obtained was used for the investigation. The yield of methanolic extract (w/w from dried starting material) was 6.57 %. Prior to use, weighed powder was dissolve in 2% ethanol and centrifuged at low rpm for 10 minutes. The clear supernatant was used for further study.

Normoglycemic studies

Experimental design

Following the method used in our earlier studies (Syiem *et al.*, 2002), mice were divide into four test and one control group to study the effects of varying doses of the extracts *F. macrophylla* in normal (Table-I) and diabetic mice (Table-II). Each group comprised 6 mice (n=6). Varying doses of the extract of *F. macrophylla* ranging from 150-450-mg/kg body weight (b.w) were administered to the test group intraperitoneally (i.p) and glucose level was monitored at different time intervals up to 24 hours following the extract administration. The control groups received only 2% ethanol, being the solvent used for preparation. Food, but not water was withheld during test period not exceeding 24 h. Food, fluid intake and body weights were monitored for 4 weeks after administration of the extract.

Table –I

The animals were divided into five groups. Each group comprises of six mice.

1	Control	Normal mice untreated
2	T-1	Normal mice treated with 150mg/kg b.w of plant extract
3	T-2	Normal mice treated with 250mg/kg b.w of plant extract
4	T-3	Normal mice treated with 350mg/kg b.w of plant extract
5	T-4	Normal mice treated with 450mg/kg b.w of plant extract

Antihyperglycemic studies

Induction of non insulin dependent diabetes mellitus (NIDDM)

Animals were administered alloxan monohydrate (150 mg/kg b.w. i.p) prepared in acetate buffer (0.15 M, pH-4.5) as described earlier (Syiem *et al.*, 2002). The control group received only the buffer. Prior to administration, mice were fasted over night but given water *ad-libitum.* Mice with more than 3-4 fold increase in their blood sugar levels were considered diabetic and used for further tests.

Administration of extract to alloxan-induced diabetic mice

Following the same experimental design (Table-II) as with normoglycemic studies, alloxan-induced diabetic mice were administered the test extract (i.p) at varying doses (150-450 mg/kg b.w.) and the blood glucose levels was measured at varying time intervals. All animals treated were observed for behavioral changes like polydipsia and polyphagia.

Table –II

The animals were divided into five groups. Each group comprises of six mice

1	Control	Diabetic mice untreated
2	D1	Diabetic mice treated with 150mg/kg b.w of plant extract
3	D2	Diabetic mice treated with 250mg/kg b.w of plant extract
4	D3	Diabetic mice treated with 350mg/kg b.w of plant extract
5	D4	Diabetic mice treated with 450mg/kg b.w of plant extract

Oral Glucose Tolerance Test (OGTT)

Experimental design

Mice were divided into a control and four test groups to study the glucose tolerance in normal (Table-III A) and alloxan-induced diabetic mice (Table-III B) following administration of the effective dose of extracts of *F.macrophyll.* Normal or alloxan-diabetic mice, fasted overnight but provided water *ad libitum*, were administered the test samples intraperitoneally 1.5h prior to the oral glucose load of 2gm/kg b.w. according to the method used earlier (Syiem *et al.*, 2002). Glucose concentration was measured before administration and subsequently at 30, 60, 120, 480 and 1440 minutes after the glucose load. A control group received only the glucose load, while the reference drugs metformin (Zhang & Tan, 2000a), glibenclamide (Shirwaikar *et al.*, 2004), and insulin (Srinivas *et al.*, 2000) were administered following the respective cited method. Each group comprise of 6 mice.

Table-III

(A) Glucose Tolerance Test on normal mice

The animals were divided into five groups. Each group comprises of six mice.

1	Control	mice given oral glucose load of 2gm/kg b.w
2	OT-1	mice given oral glucose load of 2gm/kg b.w + 250mg/kg b.w of plant extract
3	OT-2	mice given oral glucose load of 2gm/kg b.w + 10mg/kg b.w of glibenclamide
4	OT-3	mice given oral glucose load of 2gm/kg b.w + 500mg/kg b.w of metformin
5	OT-4	mice given oral glucose load of 2gm/kg b.w + 10U/kg b.w of insulin

(B) Glucose Tolerance Test on diabetic mice

The animals were divided into five groups. Each group comprises of six mice.

1	Control	mice given oral glucose load of 2gm/kg b.w
2	OT-D1	mice given oral glucose load of 2gm/kg b.w + 250mg/kg b.w of plant extract
3	OT-D2	mice given oral glucose load of 2gm/kg b.w + 10mg/kg b.w of glibenclamide
4	OT-D3	mice given oral glucose load of 2gm/kg b.w + 500mg/kg b.w of metformin
5	OT-D4	mice given oral glucose load of 2gm/kg b.w + 10U/kg b.w of insulin

Toxicity studies

Normoglycemic mice were administered up to a dose of 450mg/kg b.w and kept under observation up to 4 weeks for any signs of distress, convulsion, coma or death (Ghosh, 1984).

Collection of Blood and Determination of Blood Glucose level

Blood samples from the control and experimental mice were collected by orbital sinus puncture using heparinised capillary glass tubes (Ivorra *et al*., 1988). The blood samples so collected were analyzed for glucose levels employing glucostix with the glucometer (Ames).

Statistical Analysis

Student's't'-tests was used for determining the levels of significance between the control and the test values. Results are expressed as Mean ± SEM.

Results

Normal mice

The hypoglycemic effect of *Flemingia macrophylla* at different doses (150-450mg/kg-body weight) on normal mice was observed to show a time- and dose- dependent response (Fig-1). A hypoglycemic effect was observed at all doses used. At 150 mg/kg b.w the blood glucose level was observed to be 45%, 6 hours after extract administration, while at 250mg/kg b.w, the sugar level came down to 37% that of control which normalizes at 24h. The higher doses of 350 and 450mg/kg b.w resulted in pronounced hypoglycemia with the sugar level being 32% of the control for either dose. Mice did not survive beyond 24h following the hypoglycemic condition at these higher doses.

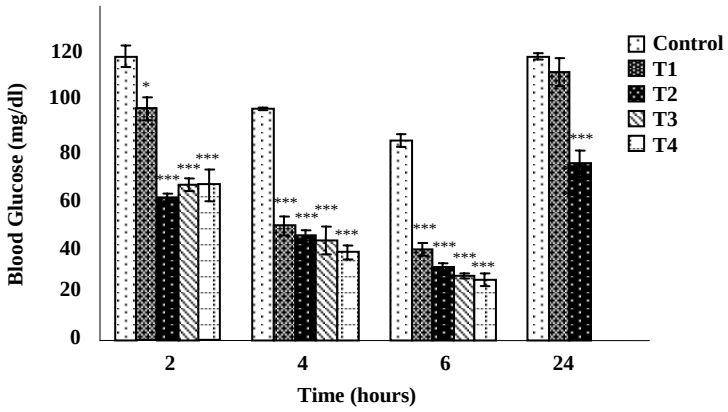

Fig-1: Effect of *Flemingia macrophylla* crude extracts (150-450 mg/kg body weight) on 18 hours fasted blood glucose level of normal mice assayed at different time intervals. Values are expressed as Mean ± SEM (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). Experimental details are described under material & methods.

Diabetic mice

The crude methanolic extract administered intraperitoneally (i.p) to diabetic mice also elicited a marked and prolonged anti-hyperglycemic action in a dose dependent manner similar to that observed in normal mice (Fig-2). The anti-hyperglycemic effect was found to be more pronounced at the 6th hour following extract administration for all the doses used. At the dose of 150 mg/kg b.w, the blood glucose levels was found to be 75%, 43% and 60% from that of control at 4, 6 and 24 hours respectively. The pattern was similar at the higher doses of 250 mg/kg b.w, with the blood glucose level being 43%, 39% and 50% from that of control at 4, 6 and 24 hours. However, in contrast to normal mice, administration of the extract at the higher doses of 350 and 450 mg/kg b.w to alloxan-induced diabetic mice resulted in marked anti-hyperglycemic effects with no accompanying contraindications. At the dose of 450 mg/kg b.w the glucose level was 36%, 22% and 32% of the control at 4, 6 and 24 hours respectively which is more marked compared to the lower dose of 350 mg/kg.

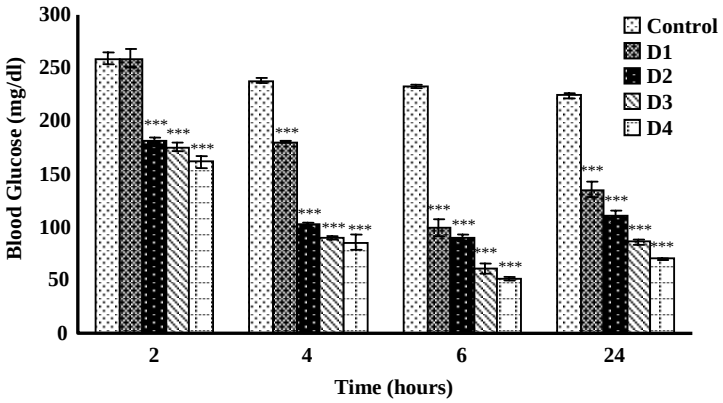

Fig-2: Effect of *Flemingia macrophylla* crude extracts (150-450 mg/kg body weight) on 18 hours fasted blood glucose level of diabetic mice assayed at different time intervals. Values are expressed as Mean ± SEM (*, p<0.05; **, p<0.01; ***, p<0.001). Experimental details are described under material & methods.

Glucose tolerance test

The extract (250 mg/kg b. w.) administered intraperitoneally (i.p) one and a half hour prior to the oral glucose load improved glucose tolerance in normal mice (Fig-3). As shown, at 120 min the glucose level was 49% (p<0.001) of the control, while it was 41% (p<0.01) at the 480 min.

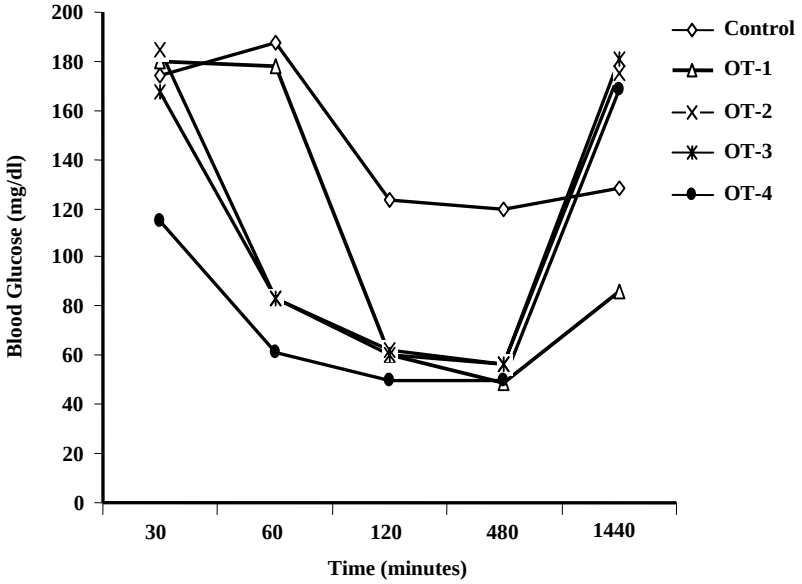

Fig-3: Glucose tolerance test in normal mice administered with crude extract (250 mg/kg), and standard drugs assayed at different time intervals. Values are expressed as Mean ± SEM. Experimental details are described under material & methods.

Glucose tolerance in alloxan-induced diabetic mice exhibited a more significant effects vis-à-vis suppression of the glucose peak at all the time intervals measured. At the dose of 250mg/kg b.w, the glucose levels were 73% ($p<0.001$), 63% ($p<0.001$), 57% ($p<0.001$) and 61% ($p<0.001$) from that of control at 30, 60, 120 and 480 minutes respectively (Fig-4). As evident from the figure, the glucose tolerance was significantly improved compared to the anti-diabetic agents metformin and glibenclamide. At the higher doses of the extract the pattern was very similar to insulin.

Toxicity studies carried out on normal mice upto a dose of 250 mg/kg b.w. did not show any adverse effects during the 4-weeks of observation. However, doses of 350 mg/kg b.w resulted in severe hypoglycemia followed by death within 24 h after administration of extract to normoglycemic mice. In contrast the higher dose (>350 mg/kg b.w.) were not fatal to diabetic mice which indicate that in normal mice hypoglycemia could be the probable cause of death.

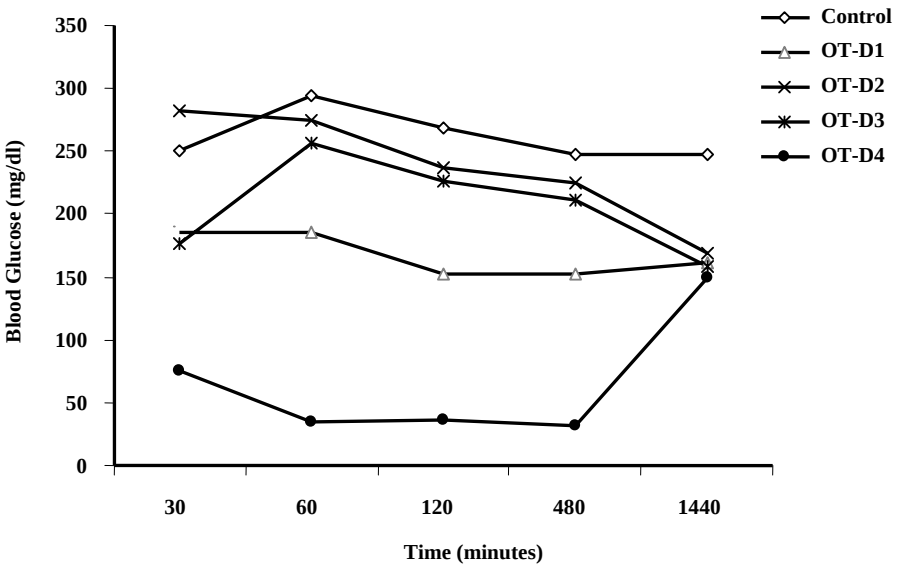

Fig-4: Glucose tolerance test in alloxan diabetic mice administered with crude extract (250 mg/kg), and standard drugs assayed at different time intervals. Values are expressed as Mean ± SEM. Experimental details are described under material & methods

Discussion

In the present study, the results indicate that the aqueous-methanolic extract of *Flemingia macrophylla* exerts hypoglycemic and anti-hyperglycemic effects on mice. The magnitude of effect was found to be dose- and time–dependent.

The extract exhibited a marked effect on normal mice vis-à-vis its glucose lowering property which was also observed in diabetic mice. In normal mice the extract exerted hypoglycemic effect which resulted in death at the higher doses (>350mg/kg b.w.). The hypoglycemic effect implies a mechanism different from metformin which is known to be anti-hyperglycemic but not

hypoglycemic in action. Metformin, a biguanide is not an insulin secretagogue and does not cause hypoglycemia even in large dose (Davis & Granner, 1996; De Fronzo & Goodman, 1995; Bailey, 1992). Its glucose reducing property is due to effect in enhancing insulin action in the peripheral tissues and inhibiting gluconeogenesis and absorption of glucose. In alloxan-induced diabetic mice, where β-cells are partly compromised (Yang Xin Bo *et al*., 2000) an anti-hyperglycemic activity was observed even at dose toxic to normal mice. It is possible that *flemingia macrophylla* also act as an insulin secretagogue similar to the well known sulphonylurea-glibenclamide. It is well known that sulfonylureas and biguanides are the major oral hypoglycemic agents used worldwide. Glibenclamide, a sulfonylurea derivative, causes hypoglycemia by stimulating pancreatic β- cells to release more insulin, and inhibiting glucagon secretion. As these effects require a functional pancreas, it can lower blood sugar levels in non-diabetic subject (Zhang & Tan, 2000b). Significantly, in diabetic mice the blood sugar level was brought down to near normal levels following extract administration.

Glucose tolerance was similarly improved in both normal and alloxan-induced diabetic condition. The OGTT is indicative of the extent of intestinal glucose absorption and hepatic glucose metabolism (Kessler *et al*., 1975; Wilcock, *et al*., 1990). The suppression of the peak as shown in Fig-3 & 4 when compared to the oral anti-diabetic drugs, metformin and glibenclamide indicate close similarity to glibenclamide in pattern and magnitude of effect. Therefore, it is possible that the active principle(s) present in *F. macrophylla* is similar to glibenclamide, which are known to act by directly stimulating β-cells of Langerhans to release more insulin and are active in mild alloxan-induced diabetes but inactive in intense alloxan diabetes (nearly all β-cells have been destroyed (Davis & Granner, 1996). However, one cannot rule out extrpancreatic effect as plants are known to contain more than one active principle (Karam, 1982) or a more direct insulin like effects as reported for *Cuminum nigrum* (Akhtar *et al*., 1985) and *Momordica charantia* (Day *et al*., 1990) or even as a β-receptor antagonist (Kimura *et al*., 1988).

In conclusion, the present study shows that the methanolic extract of *Flemingia macrophylla* has hypoglycemic as well as anti-hyperglycemic activity in diabetic mice. Its toxic effect needs to be understood within the pharmacological framework and viewed also from the perspective that this plant is locally consumed without any report of adverse effects in humans. This study also calls attention to the needs of further biochemical investigation of the plants constituents.

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