ANTI-DIABETIC EFFECT OF *FLACOURTIA JANGOMAS* EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

Ajay Kumar Singh*[1], Jyoti Singh[1], Mathew George[1] and Lincy Joseph[2]

[1]Department of Pharmacology, School of Pharmaceutical Sciences,
Jaipur National University, Jaipur-302025 (Raj.) India
*E-mail: ajayks09451244476@gmail.com
[2]Department of P'chemistry, School of Pharmaceutical Sciences,
Jaipur National University, Jaipur-302025 (Raj.) India

Summary

The present study defines the systematic evaluation and effect of methanolic extract of *Flacourtia jangomas* (MEFJ) leaves and stem (1:1) in alloxan-induced diabetic rats. Diabetes was induced by an i.p. injection of single dose of alloxan monohydrate (120 mg/kg) in normal saline. Two days after alloxan injection, rats with blood glucose levels of >140 mg/dl were included in the study. Glibenclamide (5mg/kg) and extract (100mg/kg, 200mg/kg and 400mg/kg) were given orally. Treatment was continued for 14 consecutive days, with twice a day dose. Before the treatment (0 day) and after the treatment finally on 14th day, blood samples were collected from the tip of the tail of each rat. Fasting blood glucose (FBG) level was estimated with the help of glucometer. MEFJ at the dose 400mg/kg produced highest significant reduction (39.42 %) in FBG as compare to other doses (100mg/kg and 200mg/kg) of MEFJ. Glibenclamide produced significant reduction in FBG level (44.68 %) which was highest as compare to all doses of *F. jangomas* extract. Thus it could be concluded that methanolic extract of *F. jangomas* possess significant anti-diabetic activity

Key words Alloxan monohydrate**,** hyperglycemia, Antidiabetic action, *and Flacourtia jangomas*

***Corresponding author:**
Postal address:
Ajay Kumar Singh S/o Ram Nagina Singh
Vill. - Indrasenawa
Post.-Batrauli Bazar
Dist. - Kushinagar (U.P.)-274303
Contact No. : 09314934349; 09670658191
E-mail: ajayks09451244476@gmail.com

Introduction

Diabetes mellitus is a major disease characterized by derangement in carbohydrate, fat and protein metabolism, affecting nearly 10% of the population.[1] Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[2] Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia.[3] The -cells of islets secrete insulin directly into the blood. Insulin is a protein that is essential for proper regulation of glucose and for maintenance of proper blood glucose levels.[4] In the recent many hypoglycemic agents are introduced, still the diabetes and the related complications continue to be a major medical problem not only in developed countries but also in developing countries.[1] In traditional practice medicinal plants are used in many countries to control diabetes mellitus. Plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones.[5] Synthetic hypoglycemic agents can produce serious side effects and in addition, they are not suitable for use during pregnancy. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. *Flacourtia jangomas* (family Flacourtiaceae), is large shrubs or small trees, 5–10 m tall, deciduous; trunk and older branches usually unarmed, young branches with simple or divaricate spines; bark yellow-brown, reddish brown, or light brown, flaky; young branches smooth, glabrous or sparsely pubescent, lenticellate.[6] The fruit, stem bark and bark yielded a coumarin, ostruthin.[7] Two limnoids, i.e. limolin and jangomolide were reported from the stem and bark of *Flacourtia jangomas.*[8] The leaves and young shoots taste like rhubarb, and are supposed to possess astringent and stomachic properties, and are prescribed in diarrhoea and weakness. The leaves are said to have diaphoretic properties.[9] The ripe fruits of *F. jangomas* contain good amount of potassium, having high bioavailability and thus, may serve as a good source for sufficient potassium intake.[10]

Methods

Collection and authentication of plant:

Leaves and stem of plant were collected from local region of Kushinagar, District of Gorakhpur, India in the month of March 2009. The botanical identity was confirmed by a taxonomist Prof Kamal, Department of Botany; Gorakhpur University, Gorakhpur where voucher specimen (No. GU0309186) has been deposited for further reference.

Preparation of methanolic extract:

The leaves and stem of *Flacourtia jangomas* were washed, shade dried and powdered. The powdered material was defatted with petroleum ether (60-80 ºC) and then extracted with methanol in Soxhlet apparatus (40 cycles). The extract was concentrated for further studies at reduced pressure and temperature in a rotary evaporator. Methanolic extract of *Flacourtia jangomas* (MEFJ) was tested for presence of secondary metabolites by different phytochemical tests.

Experimental animals:

Normally healthy adult Wistar strain rats of either sex weighing of 150-200gm were used in the experiment. Animals maintained under standard environmental conditions, were fed with

a standard diet and water ad libitum. The animals were fasted for 18h prior to the experimentation, but allowed free access to water only.
The experimental protocol has been approved by the Institutional Animals Ethics committee (Reg. No. 003/2009/IAEC/jnu.)

Drugs and Chemicals used:
Alloxan monohydrate was purchased from Central Drug House (P) Ltd. New Delhi, India and used as a diabetogenic agent. Glibenclamide, a standard antidiabetic agent was obtained from Aventis Pharma. Ltd., Goa,India. Other chemicals used for extraction purpose and phytochemical tests were of laboratory grade.

Phytochemical screening:
Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, carbohydrate, phenolic compounds, flavonoids, saponins and tannins by using standard procedures.[11, 12]

Acute toxicity test:
Acute oral toxicity study for the test extract of the plant was carried out using OECD/OCED guideline.
Limit Test at 2000 mg/kg: Dose 2000mg/kg body weight was administered orally to one animal. This first test animal survived. Since, four other animals were dosed (orally) sequentially, so that a total of five animals were tested. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. No animals were died. So the LD50 is greater than 2000 mg/kg.[13]

Antidiabetic activity:
The rats were made diabetic by an i.p. injection of single dose of alloxan monohydrate (120 mg/kg) in normal saline.[14] Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study.[15]

After confirmation of diabetes, rats were classified into 6 groups of six rats in each group.
Group I – Normal Control received water only.
Group II – Diabetic control received water only.
Group III – Diabetic treated with MEVV (100mg/kg).
Group IV – Diabetic treated with MEVV (200mg/kg).
Group V – Diabetic treated with MEVV (400mg/kg).
Group VI – Diabetic treated with Glibenclamide (5mg/kg)

Glibenclamide (5mg/kg) standard drug used for treatment and methanolic test extract (100mg/kg and 400mg/kg) were given orally. Treatment was continued for 14 consecutive days, with twice a day dose (morning and evening).[16]
Before the treatment (0 day) and after the treatment finally on 14^{th} day, blood samples were collected from the tip of the tail of each rat. Fasting blood glucose (FBG) level was estimated with the help of glucometer (one touch ultra, Johnson & Johnson Ltd.) using strip method. The percentage reduction in fasting blood glucose level was calculated by the following formula.

$$\% \text{ reduction in FBG} = \frac{[\text{FBG level before treatment - FBG level after treatment}]}{[\text{FBG level before treatment}]} \times 100$$

Statistical Analysis:
The data obtained in present investigation was subjected to statistical analysis. All results are expressed as Mean ± S.E.M. The data was analyzed using Analysis of variance (ANOVA) and the group means were compared by Dunnet test. Values were considered statistically significant when $P<0.05$ and non-significant when $P>0.05$. Graph Pad Instat was used for the analysis of data.

Results

Phytochemical screening: Phytochemical screening revealed the presence of flavonoids, saponins and carbohydrate, Tannins and Phenolic Compounds in methanolic extract.

Acute toxicity test:
Acute toxicity studies revealed that *F. jangomas* extract did not produce any toxic symptoms when administered (2000mg/kg) orally to rats.

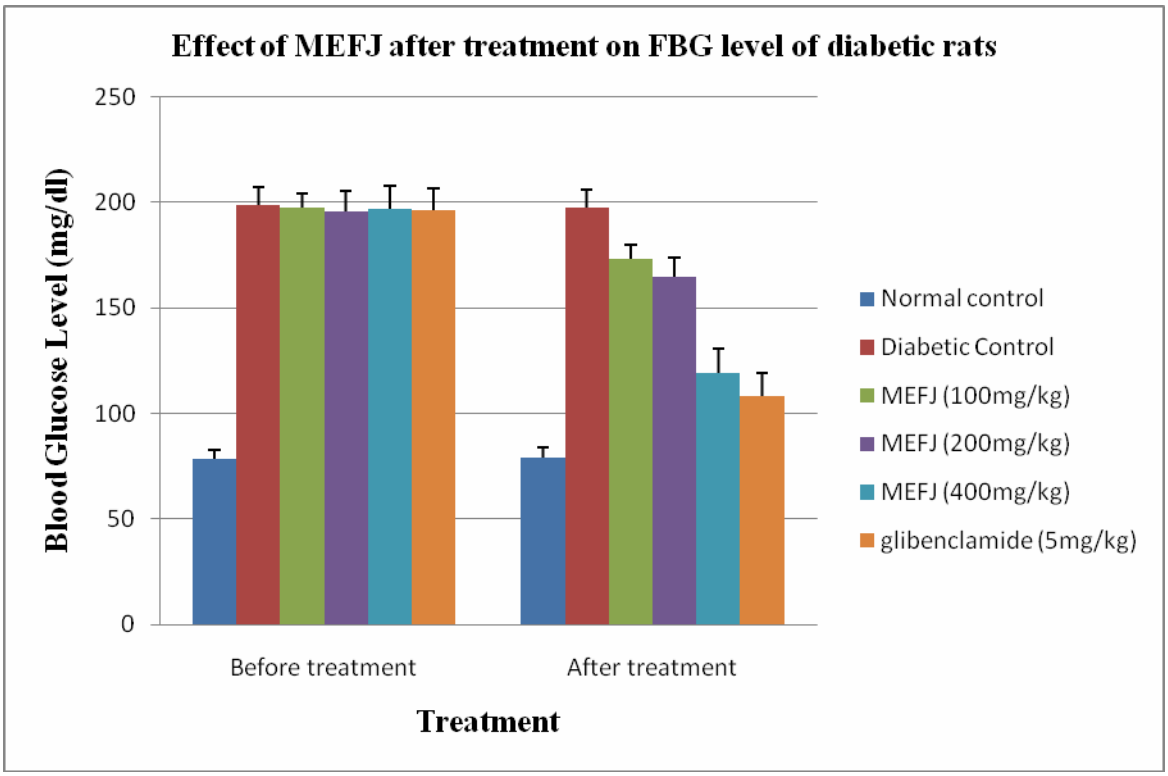
Antidiabetic activity:
MEFJ at the dose 400mg/kg produced highest significant reduction (39.42 %) in FBG as compare to other doses (100mg/kg and 200mg/kg) of MEFJ [Table 1]. Glibenclamide produced significant reduction in FBG level (44.68 %) which was highest as compare to all doses of *F. jangomas* extract [Table 1]. Before treatment and after treatment data has also shown in figure 1.

Table 1: Effect of different doses of MEFJ on fasting blood glucose level of diabetic rats

Different groups and treatment (p.o.)	fasting blood glucose (mg/dl)		
	Before treatment	After treatment	% reduction
Normal control (---)	78.33±4.01	79.17±5	---
Diabetic control (---)	198.67±7.53	197.5±9.46	---
Diabetic + MEFJ (100mg/kg)	197.5±8.73	173.5±4.68^{NS}	12.15
Diabetic + MEFJ (200mg/kg)	195.83±10.2	164.66±8.54*****	15.91
Diabetic + MEFJ (400mg/kg)	197.0±12.45	119.33±9.76******	39.42
Diabetic + Glibenclamide (5mg/kg)	196.16±13.76	108.5±7.7******	44.68

Values are mean ± SEM; N=6 in each group. $^{NS}P>0.05$ (non-significant); *P>0.05 (significant); **P<0.01 (highly significant) when compare to diabetic control rats

Figure 1: Effect of MEFJ after treatment on fasting blood glucose level of diabetic rats



Discussion

Alloxan, a beta cytotoxin, induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cell resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues.[1] Alloxan-administered rats become hyperglycaemic in a short period of time, followed by hepatic glucose overproduction.[17] Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both type 1 and type 2 diabetic patients.[18] Antidiabetic potency of the MEFJ in diabetic rats has been indicated here by the study of FBG level as it is an important basal parameter for monitoring of diabetes. The result demonstrated that methanolic extract of *F. jangomas* induces significant decrease of blood glucose level in diabetic rats and this effect was more potent after repeated dose (200mg/kg and 400mg/kg) administration, a marked reduction of blood glucose level in these rats was achieved after 14 days of treatment. But on low dose 100mg/kg reduction in blood glucose level was not significant. Therefore, the effectiveness of the extract depends on the dose and probably on the accumulative effect of active principles. The possible mechanism includes the stimulation of β-cells and/or subsequent release of insulin and/or activation of the insulin receptors[19] and may be due to increase peripheral utilization of glucose.[20] Further investigation using biochemical tracing technique are sought to identify the exact mechanism of action of MEFJ.

Conclusion

This observation confirms the use of MEFJ in ethnomedical practice for diabetes management. Although the exact chemical compound/s responsible for the hypoglycaemic effects of *F. jangomas* extract still remain speculative, experimental evidence obtained in the present laboratory animal study indicates that MEFJ possesses antidiabetic property. More detailed studies on *F. jangomas* using different doses and covering longer periods of observation are needed before reaching a clear-cut conclusion. Further investigation required to isolate and identify the hypoglycaemic principles in this plant so as to elucidate it's mechanism of action.

Acknowledgement

Authors are thankful to the Prof. Kamal, Department of Botany; Gorakhpur University, Gorakhpur, U.P. for identification and authentication of plant and also grateful to IAEC for providing approval (registration No. 003/2009/IAEC/jnu.) to present research work. Authors are thankful to Department of Pharmacology, School of Pharmaceutical Sciences,
JNU, Jaipur (Raj.) for providing good support.

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