ANTIDIABETIC EFFECT OF LEAVES OF *MUNTINGIA CALABURA* L., IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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Summary

Hypoglycemic and antihyperglycemic effect of methanolic extract of *Muntingia calabura* L. leaves was evaluated in normoglycaemic, glucose loaded and alloxan-induced (135 mg/Kg body weight, i.p) diabetic rats. The extract (500 mg/kg body weight) significantly lowered the blood glucose levels to an extent comparable to that produced by standard antidiabetic drug (Glipizide 5 mg/Kg body weight) in both normal and diabetic rats. The extract (500 mg/kg body weight) increased the glucose tolerance in glucose loaded rats. The results suggest that methanolic extract of *Muntingia calabura* L. leaves possess significant antidiabetic activity.

Keywords: Hypoglycemic, antihyperglycemic effect, *Muntingia calabura* L., glucose tolerance, glucose loaded, antidiabetic activity.

Introduction

Diabetes mellitus is a chronic metabolic disorder, caused by insulin deficiency, often combined with insulin resistance. According to World Health Organization (WHO) estimate, there are 177 million people worldwide suffering from diabetes and this figure is likely to be more than double by 2030[1]. Presently, synthetic oral hypoglycemic agents, insulin products and some herbal medicines are in practice to control the disorder and there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents[2,3].

Muntingia calabura L., (Family: Elaeocarpaceae) is a fast-growing tree of slender proportions, native to the American continent and is widely cultivated in warm areas of Asian region, including Malaysia. This plant has several vernacular names like straw berry tree, Jamaican cherry (English), Chinese cherry (or) Japanese cherry (India) and cherry chettu (Telugu)[4].

An ethyl acetate soluble extract of dried leaves of *Muntingia calabura L.*, reported to exhibit quinone reductase induced activity in assay with cultured cells[5]. The leaves of *Muntingia calabura L* plant were reported to be rich in flavanoidal compounds, which may possess anti-diabetic and other activities[6]. So far there is no scientific evidence available of its traditional use in diabetes. Hence, the present study was aimed to determine the same using alloxan-induced diabetes rats.

Material and Methods

Chemicals

Alloxan monohydrate of Sigma Aldrich Company, USA was procured from S.D. Fine Chem. Ltd., Mumbai. Glipizide was obtained as gift sample from Dr.Reddy's Laboratories Pvt Ltd., Hyderabad and GOD-POD (Glucose Oxidase–Peroxidase) kit was purchased from local supplier. All other reagents and chemicals used were of analytical grade.

Plant Material

Leaves of *Muntingia calabura* were collected in the month of September from the trees located in the Roman Catholic Church, Station Ghanpur, Warangal, Andhra Pradesh, India and authenticated by Prof. V. S. Raju, Department of Botany, Kakatiya University, Warangal. A voucher specimen was deposited in the herbarium of the University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India.

Preparation of Extract

The leaf (2 kg) part of *M. calabura* were air dried and crushed in a mixer. The material so obtained was macerated with cold methanol (5 L) at room temperature in round bottom flask for 3 days with intermittent stirring. After 3 days, the contents of the flasks were filtered and the filtrate was concentrated under reduced pressure. The yield of *M. calabura* leaf extract was 6.55% w/w.

Animals

Male Wistar rats weighing between 180 – 220g procured from National Institute of Nutrition (NIN), Hyderabad, were used in the study. They were maintained under standard laboratory conditions with ambient temperature of 22±3^{o}C, relative humidity 50±5% and 12-h light/12-h dark cycle. Rats were fed with a commercial pellet diet (Hindustan Lever, India) and water *ad libitum*. The animals were acclimatized to these conditions by maintaining them at the experimental conditions for about seven days prior to dosing. They were fasted for 18 h prior to the experiment (allowing access to water) and, during the experiment, food and water were withdrawn. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of University College of Pharmaceutical Sciences, Kakatiya University, Warangal, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preliminary Phytochemical Screening
The methanolic extract of *M. calabura* L. leaves was tested for the presence of flavanoids, steroids, glycosides, carbohydrates, phenolic compounds and alkaloids using the general chemical tests[7].

Acute Oral toxicity test
After an overnight fast of 18 h, *Muntingia calabura* leaf extract (MC) was administered orally in doses of 300, 500 and maximum dose of 2000 mg/kg to groups of rats (n = 6) and closely observed for the first 2-3 hr for signs of toxicity and percentage mortality was noted beginning with 24 h up to a period of 14 days[8].

Effect on Euglycemic wistar rats[9]
Rats were divided in four groups of six rats each. The blood glucose concentration of fasted rats of all groups was determined at zero time. All groups were treated orally in the following manner. Group I (control rats, 5% gum acacia); group II (MC 300mg/kg); group III (MC 500mg/kg), group IV Glipizide (5 mg/kg). Serum glucose concentration of rats of all groups was determined at 2, 4, 6, and 8 hours later.

Effect on Oral Glucose Tolerance Test (OGTT)[10]
Fasted rats were divided into three groups with six rats each and were treated orally in the following manner. Group I (control rats 5% gum acacia); group II (MC 500mg/kg) and group III (Glipizide 5 mg/kg). Half-an-hour later all the rats were orally loaded with 2 g/kg of 25% (w/v) glucose solution. Blood samples were collected through retro-orbital plexus immediately prior to commencement of treatment and at 30 minutes intervals up to a period of 120 minutes after glucose challenge.

Effect on Alloxan Induced Diabetic Rats[11]
Diabetes was induced by single intraperitoneal injection of 135 mg/kg of freshly prepared alloxan monohydrate in sterile saline in overnight fasted rats. After 72h of injection, the blood samples from all surviving rats were estimated for glucose levels. Rats with blood glucose levels of 250 mg/dL and above were considered as diabetic and selected for further experiments. Care is exercised throughout the induction period to prevent occurrence of sudden hypoglycemic states by intermittent feeding with 10% dextrose solution and standard pellet diet. Diabetic rats were divided into three groups of six rats each. The blood glucose concentration was determined at zero time. Group I (diabetic control rats); group II (MC 500mg/kg), group III (Glipizide - 5mg/kg). All groups were treated orally. Serum glucose concentration of rats of all groups was determined at 2, 4, 6 and 8 hours later.

Collection of blood and determination of blood glucose
In all these experiments, approximately 0.5 ml blood was drawn each time from the retro-orbital plexus using aseptic technique. The collected blood was allowed to clot for 5min at room temperature and serum was separated after centrifuging at 3000 rpm for 10 min. The glucose concentration in the serum samples was analyzed by the Glucose oxidase/Peroxidase (GOD/POD) method[12] using Lyphozyme assay Kit (Beacon Diagnostics Ltd., Navasari, India) and optical density read at 510 nm using Elico UV-Visible spectrophotometer SL 164 (Elico Pvt. Ltd., Hyderabad, India).

Statistical evaluation

Data were expressed as mean ± standard error of mean. Statistical comparisons were made by using one-way ANOVA followed by Newman-Keuls multiple comparison test. The blood sugar levels following administration of each extract at each dose level and standard drug were compared with those of control at each time point. The results were considered statistically significant if

$P < 0.05$.

Results

In preliminary phytochemical analysis, *Muntingia calabura* leaf extract showed positive result for flavanoids, steroids/triterpenoids and their glycosides. There were neither signs of toxicity nor mortality recorded up to a dose of 2000mg/kg body weight (b.wt) of the extract.

Hypoglycemic activity in euglycemic rats.

Results of serum glucose levels measured at 0, 2, 4, 6, and 8 hr in control group, standard group (Glipizide 5 mg/Kg) and extract treated groups (300 and 500 mg/kg b.wt) were presented in Table 1. Percentage reduction in serum glucose levels with respect to 0 hr and significance is expressed with respect to control group. In normal fasted rats, both the doses produced significant hypoglycaemic effects ($P<0.05$) after 6 hr and 4-8 hr respectively. *Muntingia calabura* leaf extract produced significant effect at 6 hr ($p<0.01$) at a dose of 500 mg/kg b.wt. At this dose it reduced the blood glucose level of the fasted normal rats from an initial mean value of 83.19 mg/dL at 0 hr to a mean value of 62.62 mg/dL (24.81 %) at the end of the 6 h. It is noteworthy to mention that animals treated with glipizide (5 mg/kg) showed a significant reduction in blood glucose level after 2 hr ($p<0.05$) and up to 8 hr ($p<0.01$) with more pronounced effect observed at 6hr ($p<0.001$).

Table 1: Effect of *Muntingia calabura* leaf extract on blood sugar levels in euglycemic rats

S.No	Group	Serum glucose levels (in mg/dl)				
		0 hr	2 hr	4 hr	6 hr	8 hr
1	Control	81.07±8.86	80.02±8.52 (1.25%)	77.83±8.9 (4.02%)	77.06±7.72 (4.84%)	78.38±7.91 (3.21%)
2	Standard	79.56±11.23	62.15±11.7* (21.4%)	53.43±16.63* (34.16%)	51.33±11.0*** (35.87%)	58.02±15.22* (26.6%)
3	MC-300	84.43±5.99	76.03±3.88 (9.76%)	73.57±3.43 (12.71%)	69.07±3.81* (18.05%)	74.83±5.73 (11.3%)
4	MC-500	83.19±5.22	71.55±8.0 (14.2%)	65.53±7.4* (21.4%)	62.62±6.04** (24.81%)	66.93±7.58* (19.7%)

MC-300 is a *Muntingia calabura* leaf extract at dose of 300mg/kg b.wt
MC-500 is a *Muntingia calabura* leaf extract at dose of 500mg/kg b.wt

Values are expressed as Mean ± S.D (% reduction); (n=6)
*indicates $P<0.05$, ** indicates $P<0.01$, *** indicates $P<0.001$ with control

Selection of dose of *Muntingia calabura* leaf extract for oral glucose tolerance test (OGTT) and Alloxan induced diabetes

Muntingia calabura L. leaf extract at 500 mg/Kg b.wt. produced more pronounced hypoglycemic effect in normal rats. Hence the extract at that dose is subjected for OGTT in non-diabetic normal rats and alloxan induced diabetic rats.

Hypoglycaemic effect on oral glucose tolerance test (OGTT)

Effect of *Muntingia calabura* leaf extract on blood glucose levels of normal fasted rats after a glucose load (1.5 g/kg) are outlined in Table 2. In control group, the blood glucose increased rapidly 1 hr after administration of glucose, and thereafter decreased gradually. When 500 mg/kg b.wt of extract is administered orally before glucose loading, a significant ($P < 0.01$) reduction in the rise of blood glucose at 60 min to 120 min when compared to the control group is observed. In the case of standard (glipizide 5 mg/kg) the glucose levels reached the fasting values at the end of 60 minutes.

Table 2. Effect of *Muntingia calabura* leaf extract on oral glucose tolerance test in normoglycemic rats

S.No	Group	Serum glucose levels (in mg/dl)				
		Fasting	30min	60min	90min	120min
1	Control	85.20±2.4	159.95±5.27	144.73±7.86	125.47±9.61	79.94±2.36
2	Standard	72.24±6.54	93.23±4.57*	72.09±2.98*	62.63±5.02*	55.03±4.43**
3	MC-500	84.98±4.37	128.54±7.78	116.46±6.94*	94.76±10.11**	79.82±5.37*

Values are expressed as Mean ± S.D; (n=6)
*indicates P<0.01, ** indicates P<0.001 with control

Hypoglycaemic effect on alloxan induced diabetic rats

Effect of *Muntingia calabura* leaf extract on blood glucose levels in alloxan induced diabetic rats are outlined in Table 3. The results indicate that 500 mg/kg dose of methanolic extract of leaves significantly (p<0.001) reduced the hyperglycemia induced by alloxan. The percent of reduction in blood glucose levels was maximum at the end of 6 hr for all groups. Further, the effect produced by *Muntingia calabura* L. leaf extract is comparable to that of the standard.

Table 3: Effect of *Muntingia calabura* leaf extract in alloxan induced diabetic rats

S.No	Group	Serum glucose levels (in mg/dl)				
		0 hr	2 hr	4 hr	6 hr	8 hr
1	Control	381.05 ±12.41	370.9±7.70 (2.63%)	361.3±9.76 (5.15%)	350.03±11.05 (8.12%)	357.13±10.6 (6.25%)
2	Standard	340.34 ±8.77	242.5±6.95* (28.7%)	230.5±11.96* (32.2%)	215.13±8.62* (36.75%)	246.63±6.14* (27.5%)
4	MC-500	385.78 ±8.73	358±8.15 (7.16%)	331.4±9.92* (14.09%)	283.74±11.8* (26.47%)	314.24±12.9* (18.53%)

MC- 500 is *Muntingia calabura* leaf extract at a dose of 500mg/kg body weight
Values are expressed as Mean ± S.D (% reduction); N=6
* indicates P<0.001, with control group.

Discussion

In light of results, the study indicates that *Muntingia calabura* leaf extract have hypoglycemic/antihyperglycemic activity in normal and alloxan induced diabetic rats.

Methanolic extracts of leaves at dose of 500 mg/kg exhibited significant and consistent ($p<0.001$) reduction in blood glucose levels in normoglycemic euglycemic rats and alloxan induced diabetic rats at 4 hr and was maximum at the end of 6 hr. This effect is comparable to that produced by standard drug, Glipizide (5 mg/kg b.wt). *Muntingia calabura* L. leaf extract (500 mg/kg b.wt) is well tolerated and markedly reduced the progressively elevated blood glucose levels in glucose loaded rats. The hypoglycemic effect was significantly ($P<0.001$) pronounced from 60 to 90 min when compared to control group. This indicates that the extract may be acting by direct stimulation of existing ß-cells to release insulin or increase the glucose uptake. Our investigations revealed that the *Muntingia calabura* L. leaf extract effectively regulated the blood glucose levels in normal euglycemic rats and alloxan induced diabetic rats indicating the presence of antidiabetic activity.

Phytochemical analysis of *Muntingia calabura* leaf extract revealed the presence of flavanoids, steroidal/triterpenoidal compounds and their glycosides. The significant anti-diabetic activity of *Muntingia calabura* leaf extract may be due to the presence of flavanoids, as the literature reveals that the plant flavanoids have anti-diabetic activity[6].

The present study reveals that *Muntingia calabura* leaves extract possess significant antidiabetic activity. Further study need to be carried out to rule out its mechanism(s) of action, and the isolation of phytoconstituents responsible for this activity.

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References

1. World Health Organization, global strategy on Diet, physical activity and health. WHO; 2003.
2. Holman RR, Turner RC. Oral agents and insulin in the treatment of NIDDM. In: Pickup J, Williams G, eds. Textbook of Diabetes, Blackwell, Oxford. 1991:467-469.
3. Kameswara Rao B, Giri R, Kesavalu MM, Appa Rao CH. Herbal medicine in the management of diabetes mellitus. Manphar Vaidhya Patrika 1997;1:33-35.
4. Fosberg, FR, Sachet M-H, Oliver RL. 1979. A geographical checklist of the Micronesian dicotyledonae. Micronesica 15:1-295.
5. Talalay P. Chemoprotection against cancer by induction of phase 2 enzymes. Bio factors 2000;12:5-11.

6. Perez GRM, Zavala SMA, Perez GC. Antidiabetic effect of compounds isolated from plants. Phytomedicine 1998;5:55-75.
7. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, New Delhi, India. 1994; 107-113.
8. OECD/OCDE. 425 guideline. OECD guidelines for testing of chemicals. Acute Oral Toxicity – Up-and-Down-Procedure. 2001. 1-26.
9. Masiello P. Animal models of type 2 diabetes with reduced pancreatic β-cell mass. The International Journal of Biochemistry & Cell biology. 2006;38:873-893.
10. Cretti A, Brunato B, Zenti MG, Tosi F, Muggeo M, Bonora E, Bonadonna RC. A novel tool to assess ß-cell function during the oral glucose tolerance test (OGTT). Diabetes 2000; 49(Suppl. 1): A89.
11. Vogel HG, Vogel WH, eds. Drug Discovery and Evaluation, Pharmacological assays. Springer-Verlag, Berlin, 1977: 1-755.
12. Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol, 1969;22(2):246.