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HYPOGLYCEMIC ACTIVITY OF LEAVES AND CALLUS OF *MEMECYLON UMBELLATUM* IN ALLOXAN INDUCED DIABETIC RAT MODEL

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ABSTRACT

Diabetes mellitus is a predominant risk factor for the development of cardiovascular, nephropathy, opthalamic, obesity, hepatic and many more associated diseases. Many chemical drugs in market has proven effective in reducing blood glucose levels, but also lead to many damages due to years for continuous medication. To reduce these side effects, the current research concentrates on the ethnobotanical prototype of using leaves and callus to reduce diabetic levels in the alloxan induced rodent model. The leaves of callus proved very effective in reducing blood glucose levels of treatment groups than in comparison to induced diabetic control and vehicle groups. Thus, ethnobotanical approach can pave a new approach in the field of drug discovery.

KEYWORDS: Callus, Diabetes mellitus, alloxan induced rodent model. Blood glucose levels.

INTRODUCTION

Diabetes mellitus is a very common and prevalent disease affecting the citizens of both developed and developing countries. Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both (Kumar *et al.*, 2002). Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Kumar *et al.*, 2002) As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy (Bearse *et al.*, 2004)

neuropathy (Seki *et al*., 2004) nephropathy (Huang *et al*., 2002) cardiovascular complications (Saely *et al.*, 2004) and ulceration (Wallace *et al*., 2003) Thus, diabetes covers a wide range of heterogeneous diseases.

Diabetes is the most common endocrine disorder and by the year 2010, its estimated that more than 200 million people worldwide will have diabetes mellitus and 300 million will subsequently have the disease by 2025 (Amos *et al.*, 1997) It is estimated that 75% of the world population is affected by this disease. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin.

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "alloxan diabetes") in these animals, with characteristics similar to type 1 diabetes in humans. Thus, testing of herbal drugs is essential for the use of complementary medicine among patients with diabetes (Szkudelski et al., 2001). Particularly herbal therapies, is widespread in the Western world as well as in many Asian countries including China and India. It seems that the use is continuously increasing despite the fact that only a small number of controlled trials dealing with either efficacy or safety of these natural products exist. With this view, an attempt is made to test for the hypoglycemic activity of leaves and callus plant in alloxan induced rodent model (Mrozikiewicz et al., 2004).

MATERIALS AND METHODS

Collection of plants

The healthy plants of M*emecylon umbellatum* (leaves and callus) were collected from Cuddalore. The collected leaves were brought to the laboratory and maintained at Department of Botany, Quaid-E-Millath Government College for Women, Tamil Nadu, India.

Authentication of plants

The plant was authenticated by Dr. P.T. Devarajan, Associate professor comparing with the herbarium voucher specimen deposited at Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous) Chennai-600 005, Tamil Nadu, India.

Materials required

The chemical and drugs used in the study were Test drug, Alloxan, Glucose, carboxymethyl cellulose (CMC), metformin, isoflurane (anaesthetic agent) formalin eosin, hematoxylin was purchased from Sigma–Aldrich, St. Louis, MO, USA. Animal restrainer (e.g., Broom restraint, Plas Labs), Micrometer, glucometer (Accu-Check, Roche, Germany) and Advia (Hematology analyzer).

Animals

All the studies were conducted as per the norms of the committee for the purpose of supervision of experiments on animals. Rats weighing 100-140g were selected and housed 6/cage with six animals per group and kept in the animal house for one week for proper acclimatization before starting the experiment under controlled conditions of illumination (12 h light/12 h darkness) and temperature ranging 20-25°C. They were housed under the above laboratory conditions, maintained on standard pellet diet and water.

Oral glucose tolerance test

a. The animals were fasted for 16-18 hours (overnight).
b. Blood basal samples (T0) were taken.
c. Animals were randomized according to their baseline blood glucose level and grouped into nine groups. The animals were also dosed according to the groups divided.

Group 1 Normal control - distilled water
Group 2 Vehicle control - 0.25% w/v (10 ml/kg) carboxymethyl cellulose (CMC)
Group 3 Metformin (500 mg/kg, p.o.),
Group 4, 5 and 6 leaves of Memecylon umbellatum (200, 400 and 600 mg/kg, p.o.)
Group 7, 8 and 9 callus of Memecylon umbellatum (200, 400 and 600 mg/kg, p.o) respectively.

After dosing the animals as specified, a glucose load of 2g/kg and either vehicle (CMC) or were simultaneously administered orally.

d. Blood glucose concentrations were measured at 15,30, 60, 90 and 120 min (T15–T120) post treatment by tail cut method using glucometer (Accu-Check, Roche, Germany)

e. The reduction in blood glucose produced by the compounds metformin and testing drugs were calculated using the area under the curve method with basal value as the zero. (AUC 0–120 min).

Alloxan induced diabetic model (Vivek kumar et al., 2010).

a. After one week of the acclimatization, rats were injected once with low-dose of Alloxan (80mg/kg,i.p.) to induce partial insulin deficiency. The glucose value was noted using glucometer before Alloxan injection. This is considered as Basal value.
b. After 48-96 hrs of Alloxan injection, the rat's fasting blood glucose value (glucometer) were noted using tail flick method. The animals would display hyperglycemia and glucose intolerance.
c. Animals with similar degrees of hyperglycemia (mostly above 95mg/dl) were considered and according to their glucose value, animals were randomized and divided to groups as follows

- Group 1 – Normal Group
- Group 2 - Vehicle control (Untreated)
- Group 3 - Diabetic control (Untreated)
- Group 4 – Diabetic group + Metformin (350 mg/kg, p.o),
- Group 5 –Diabetic group + leaves of Memecylon umbellatum (300mg/kg) and
- Group 5 – Diabetic group + callus of Memecylon umbellatum (300 mg/kg)

Animal blood collection

Animals were kept fasting for 12 hours on the day before glucose estimation. Then the animals tail were flicked for fasting blood glucose levels along with body weight and feed intake were noted on the Day 0 and every week (week 1, 2, 3 and 4) of the entire observation period of 28 days. Blood plasma were collected for analysis of biochemical parameters and whole blood were collected for analysis of haematological parameter.

Estimation of insulin and calculation of HOMO-IR index

The major function of insulin is to counter the concerted action of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. Because there are numerous hyperglycemic hormones, untreated disorders associated with insulin generally lead to severe hyperglycemia and shortened life span. The estimation of insulin is done using

ELISA method Insulin resistance was determined using the homeostasis model assessment index for insulin resistance (HOMA-IR) (Mathews et al., 1985) using the following formula:

HOMA-IR index = [fasting glucose (mmol/L)×fasting insulin (µU/ml)]/22.5)

RESULTS AND DISCUSSION

OGTT

The present study was undertaken to assess the antidiabetic effect of leaves and callus of *Memecylon umbellatum.* In the present study, the oral treatment of leaves of *Memecylon umbellatum* decreased the blood glucose levels in diabetic rats. The leaves (200, 400 and 600 mg/kg) showed significant ($P < 0.001$) decrease in blood glucose level at 15,30, 60, 90 and 120 min. Continuous treatment with the leaves and callus (200, 400 and 600 mg/kg) one hour prior to glucose dose oral dose showed a significant ($P < 0.001$) decrease in the blood glucose level in diabetic rats (Figure no. 1). Maximum reduction of blood glucose level occurred at the dose of 600 mg/kg. p.o. Even though, the drugs showed short onset and short duration of antihyperglycemic action at 600mg/kg p.o the dose concentration of 300mg/kg p.o was selected based on the guidelines and as well as to match the standard metformin concentration of 350mg/kg.

Alloxan induced diabetic model

The initial body weight ranging 125 - 130 were noted on the day 0 for all groups of the study. It was found that the drug has the ability to maintain body weight of 131.78±1.77 (leaves) and 131.73±1.75 (callus) which is equal to that of normal mean body weight of 132.17±2.85 (Table no. 1), this may be due to the ability to recover the appetite and stabilise the feed intake (Table no. 1). This reduces the chances of obesity induced hyperglycemic effects.

The possible mechanism by which herbal drugs brings about its antihyperglycemic action may be through potentiation of the pancreatic secretion of insulin from islet β-cell or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly demonstrated by the increased levels of insulin in tested drug groups 22.09±0.73 (leaves) and 23.50±0.76 (callus) in comparison to the decrease levels in diabetic control 14.16 ± 0.88 (Figure no. 2A). The calculated HOMO IR index (Figure no. 2B) showed increase in tested groups leaves (8.02±0.41) and callus (8.40±0.56) in comparison to Metformin (7.73±0.45) and C-peptide in diabetic rats treated with drugs. In this context, a number of other products have also been reported to have an antihyperglycemic and insulin-release stimulatory effect.

The biochemical parameter analysis showed increase in glucose value of 168.17±5.38 in diabetic control and after treatment with leaves and callus showed a decrease in glucose level 147.33±7.55 and 144.83±6.15 respectively. Similarly significant decrease is observed in Triglyceride level from 74.0±28.0 (in diabetic control) to 51.0 ±13 (leaves) and 52.0±8.0 (callus) and whereas the callus showed significant decrease in Total cholesterol value of 109.00±11.83 than leaves 117.50±7.20 in comparison to diabetic control 112.83±7.65 (Table 2). No significant changes occur in albumin, GGT, calcium, creatinine, globulin, BUN, protein and potassium levels. The remaining biochemical parameter of AST, ALT, ALP showed less significant variation in levels in comparison to normal and diabetic control.

No haematological parameter showed significant variation, which indicates that leaves and callus given orally for a period of 28 days did not alter hematological parameters (RBC, WBC, PLT, hemoglobin, percentage hematocrit, MCV, MCH and MCHC) and were within the normal physiological ranges. There was no detectable hematological toxicity at therapeutic dose.

The literature review indicated hepatoprotective property of herbal extracts and the improvement of liver function and subsequent increase in uptake of blood glucose and its utilization may be another mechanism of action of the extract. Other possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors (Vivek kumar et al., 2010). Estimation of insulin level and insulin receptor may give more insight into the mechanism of the anti-diabetic activity exhibited by the extract.

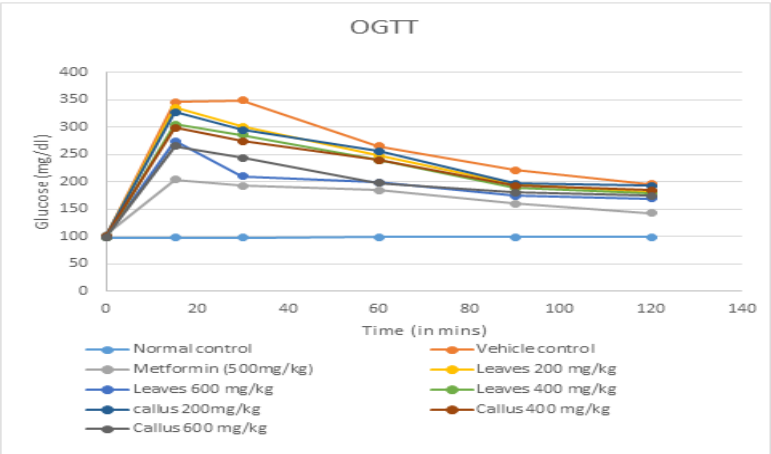

Figure no. 1 OGTT results

Results are expressed as Mean ± S.D; Drug A and Drug B indicates the leaves and callus of *Memecylon umbellatum.*

Table no. 1 Effect of Leaves and callus of *Memecylon umbellatum* (300 mg/kg, p.o.), Metformin (350 mg/kg, p.o.) on body weight and feed intake in the experimental groups.

Groups	Initial body weight (g)	Final body weight (g)	Cumulative feed intake (g)
Normal control	125.63 ± 0.95	132.17±2.85	62.96±0.79
Vehicle control	125.17 ± 1.09	132.4±1.15$$	64.07±1.84
Diabetic control	129.88 ± 1.02	134.25±0.99	63.07±1.19
Metformin 350mg/kg	129.43 ± 2.63	134.43±2.44	61.29±0.85
Leaves (Drug A) (300mg/kg)	128.7±1.54	131.78±1.77	61.50±1.43
callus (Drug B) (300mg/kg)	128.82±1.79	131.73±1.75	61.14±0.90

Results are expressed as Mean±S.D. and analyzed using one-way ANOVA followed by Dunnett test for multiple comparisons. *P≤0.05 versus normal group, #P≤0.001 versus Diabetic group, n=6.

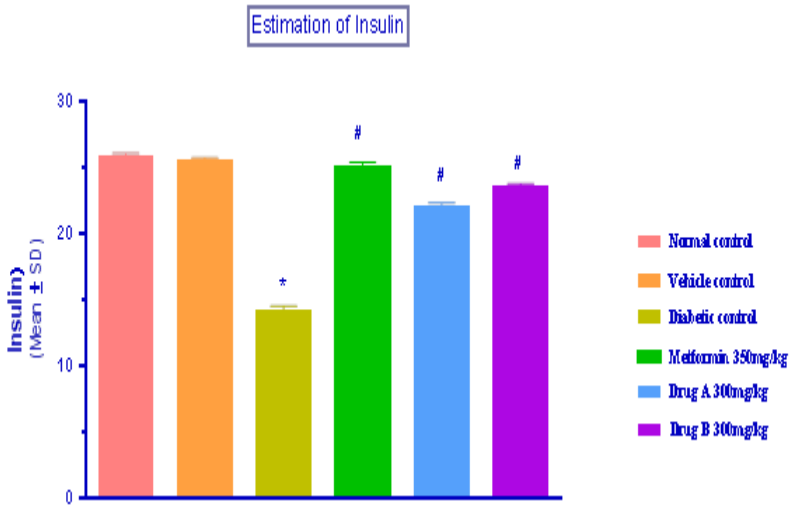

Figure no. 2 A Estimation of insulin levels

Results are expressed as Mean±S.D. and analyzed using one-way ANOVA followed by Dunnett test for multiple comparisons. *P≤0.05 versus normal group, #P≤0.001 versus Diabetic group, n=6. Drug A and Drug B indicates the leaves and callus of *Memecylon umbellatum.*

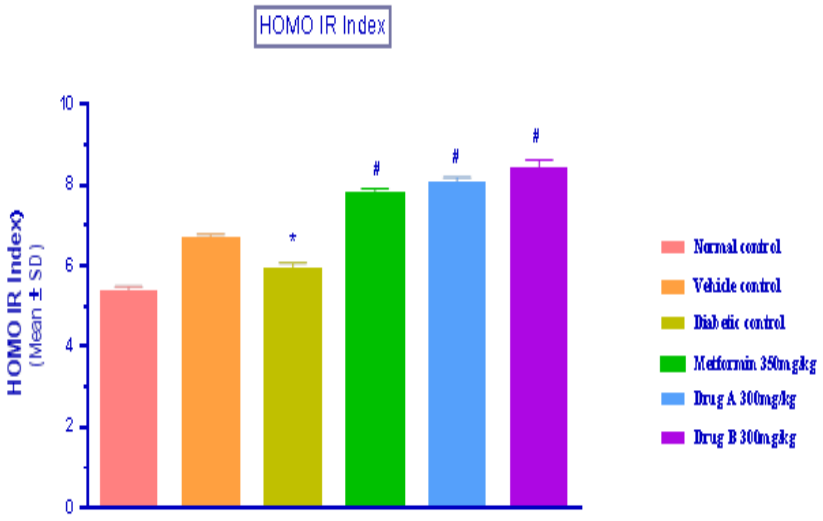

Figure no. 2 B HOMO-IR index

Results are expressed as mean±S.D and analyzed using one-way ANOVA followed by Dunnett test for multiple comparisons. *P≤0.05 versus normal group, #P≤0.001 versus Diabetic group Drug A and Drug B indicates the leaves and callus of *Memecylon umbellatum*.

Table no. 2 Effect of leaves and callus of *Memecylon umbellatum* (300 mg/kg, p.o.), Metformin (350 mg/kg, p.o.) on Biochemical parameters in the experimental groups

Group Details	Trig mg/dl	T.Chol mg/dl	ALP (IU/l)	ALT (IU/l)	AST (IU/l)	Glu mg/dl
Normal control	88.33±37.58	86.67±10.69	147.83±28.24	43.5±1.97	45.5±4.32	83.67±4.72
Vehicle control	95.67±39.16	101.17±12.73	142±39.83	49.67±3.5	87.17±7.41	105.5±3.45
Diabetic control	74±28.0	112.83±7.65	129±18.5	64.17±48.59	105.17±4.17	168.17±5.38
Metformin	93±37.0	114±7.85	126±29.4	57.5±2.35	103.83±3.06	125.83±3.87
Leaves (300 mg/kg)	51±13	117.5±7.2	114±8.7	52.17±6.24	94.5±20.78	147.33±7.55
Callus (300 mg/kg)	52±8.0	109±11.83	108±20.0	58.5±6.41	77.67±19.75	144.83±6.15

CONCLUSION

Using medicinal drugs to treat alloxan-induced diabetic rats results in activation of ß-cells and insulinogenic effects. Alloxan is well known for its selective pancreatic islet β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms. Intraperitoneal administration of Alloxan (80 mg/kg) effectively induced diabetes in normal rats, as reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss when compared with normal rats. It was also observed and demonstrated that oral administration of leaves of *Memecylon umbellatum* could reverse the above mentioned diabetic effects, thus proving leaves of callus can effectively reduce diabetes effectively in comparison to the chemical drug metformin.

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