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Effect of Aqueous Extract of *Cassia alata* Linn. on Oral Glucose Tolerance Test in Normal and STZ Induced Diabetic Mice

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Authors' contributions

This work was carried out in collaboration between all authors. All authors have equal contribution in preparing the article. All authors have read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To check the effect of the aqueous extract of an antidiabetic plant *Cassia alata* Linn. on Oral Glucose Tolerance Test (OGTT) in glucose induced normal, and diabetic mice and to check if there is any toxic effect of the extract.
Study Design: OGTT was carried out in two main groups (normal and diabetic) of mice, with 3 sub-groups. The effect of CAAE (*Cassia alata* Aqueous Extract) on OGTT in normal mice group was assessed at different time intervals (0 min, 30 min, 60 min, 90 min and 120 min). Oral acute toxicity test of the extract was performed in three groups of mice.
Place and Duration of the Study: The study was conducted at Assam University, Department of Biotechnology, Assam, India, between February and August, 2014.
Methods: OGTT was performed following the method of Badole 2006, in STZ induced diabetic mice group and normal mice group. Acute oral toxicity test was performed based on OECD guidelines, with reference to behavioural aspects, in Swiss Albino mice.

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Results: CAAE did not produce any mortality and sign of lethality throughout the study period of 14 days. In normal mice the percentage change in BGL ranges from 91.42% - 40.85% in NC (Normal Control) mice; 84.85% - 21.90% in GTNM Glibenclamide treated Normal Mice); and 88.66% - 43.28% in AqETNM (Aqueous Extract treated Normal Mice). In Diabetic group the percentage change in BGL ranges from 66.51% - 46.38% in DC (Diabetic Control) mice; 53.51% - 12.50% in GTDM (glibenclamide treated Diabetic Mice); and 55.21% - 0.33 in AqETDM (Aqueous Extract treated diabetic Mice).
Conclusion: The toxicity test result indicates that the extract is nontoxic and can be used in further bioactivity test. The study reveals the positive effect of extracts in maintaining glucose homeostasis in mice.

Keywords: Oral glucose tolerance test; Cassia alata; antidiabetic plant; toxicity test; STZ.

1. INTRODUCTION

Diabetes mellitus (DM) or simply diabetes is a metabolic disorder clinically characterized by hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1].

The purposes of acute toxicity testing are to obtain information on the biologic activity of a chemical. The information on acute systemic toxicity generated by the test is used in hazard identification and risk management in the context of production, handling, and use of chemicals [2]. The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose- specific toxic effects of an investigational product [3].

A test widely used for glucose tolerance classification is the Oral Glucose Tolerance Test (OGTT). The OGTT, which for its simplicity would be a method suitable for large studies, provides information on insulin secretion and action but does not directly yield a measure of insulin sensitivity [4]. The main reason for performing OGTT is to diagnose Impaired Glucose Tolerance (IGT) or diabetes by virtue of the 2-h value. Both of these are risk factors for Cardiovascular Disease (CVD) and IGT predicts the development of diabetes [5]. Researchers performed OGTT to check the effect of the antidiabetic plant extracts on glucose tolerance in glucose induced normal or diabetic mice or both [6-8].

The alarming increase in prevalence of diabetes and rate of mortality due to diabetes was reported by many studies [9-13]. The prevalence of diabetes is predicted to double globally in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India [12]. The high cost and poor availability of current therapies in rural parts of India necessitates the need of indigenous, inexpensive botanical sources of antidiabetic crude or purified drugs [9]. *Cassia alata* Linn. is considered as a herbal source for the treatment of diabetes in North East India [14-16]. The significance of the present research is to validate the antihyperglycemic property of this plant as a part of management of diabetes in developing countries like India. Consequently, scientific investigation of traditionally used antihyperglycemic plant is needed for the better antidiabetic drugs and to ensure safety use of the plants.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant specimen was authenticated by the Botanical Survey of India (BSI), Shillong, India. The voucher number of identified plant sample is BSI/ERC/2014/Plant identification/618. The fresh leaves of *Cassia alata* Linn. (CA) were collected from Sangaiprou village, district of Imphal, Manipur, India. The collected leaves were washed, air dried under shade, powdered and CA Aqueous Extract (CAAE) was prepared using Soxhlet. Extracts were filtered, evaporated and further dried using desiccators.

2.2 Experimental Mice

Swiss albino mice (8 week of age) weighing 20-30 g were obtained from Pasteur Institute, Shillong, Meghalaya, India and were allowed to acclimatize to their surroundings for 2 weeks. Mice were housed in standard laboratory condition (temperature 20℃ to 24℃, 45 to 65% humidity and 12 hour light/dark cycle).

2.3 Toxicity Test

The aqueous extract of *Cassia alata* Linn. was analyzed for the acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423 [17]. The test was performed using healthy young adult female Swiss albino mice, nulliparous and non-pregnant. Mice were divided into 4 groups containing 3 mice each as follows –

Group 1: received 1000 mg/kg b.w. CAAE
Group 2: received 2000 mg/kg b.w. CAAE
Group 3: received 3000 mg/kg b.w. CAAE
Group 4: Control mice

The test substance was administered by gavage using specially designed mice oral needle following a period of 5 hours fasting, animals were weighed and the test substance was administered orally at a dose of 1000, 2000, 3000 mg/kg body weight. The volume of the test substance administered was 1ml/kg body weight. The animals were observed individually after dosing, with special attention given during the first 4 hours and daily thereafter, for a period of 14 days.

2.4 Oral Glucose Tolerance Test (OGTT)

Oral glucose tolerance test was carried out following the method described by Badole et al. 2006 [18]. All the mice were divided into two main groups, with 3 sub-groups, each sub-group containing 3 mice. The divided 2 main groups are –

Group 1: Normal mice group

Sub-group 1: normal control mice
Sub-group 2: received standard drug glibenclamide
Sub-group 3: received CAAE

Group 2: Diabetic mice group

Sub-group 1: normal control mice
Sub-group 2: received standard drug glibenclamide
Sub-group 3: received CAAE

Diabetes was induced to Group 2 by intra-peritoneal injection (IP) of Streptozotocin (STZ) (40 mg STZ/kg body weight for 3 consecutive days). The blood glucose levels were measured 2 days after STZ administration, from tail-vein by glucometer. Mice with blood glucose level above 200 mg/dL were considered as diabetic [19]. Blood glucose level (BGL) before glucose load was recorded as BGL at 0 min. Without delay, glucose solution (2 gm/kg body weight) was administered to all groups orally. After 30 min time glibenclamide (10 mg/kg body weight) and extracts (200 mg/kg body weight) were administered orally to respective groups. The blood glucose level was measured at 30 mins, 60 min, 90 mins and 120 mins after glucose administration.

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity Test of the Extracts

CAAE did not produce any mortality and sign of lethality throughout the study period of 14 days even when the limit dose was maintained at 3000 mg/kg body weight. There was no sign of tremors, convulsions, salivation, diarrhoea, lethargy, sudden or drastic decrease of body weight and coma. And also there were no changes in eyes, respiratory circulation, sleep, etc. Hence, testing the extracts at a higher dose may not be necessary and the extracts were non-toxic. There was no drastic decrease of body weight of the mice. The percentage change in body weight of the mice is presented in Fig. 1.

Acute oral toxicity of the extracts was performed base on the Organization for Economic Co-operation and Development (OECD) guideline [17]. Acute toxicity (LD50) test gives a clue on the range of doses that could be used in subsequent toxicity/bioactivity testing and estimating the therapeutic index of drugs and xenobiotics [20]. In the toxicity test performed, there was no sign of any lethality over the period of 14 days, even at the highest concentration (3000 mg/kg body weight of mice). It is an indication that the extracts have no adverse effects. The median lethal dose (LD50) was indeterminable since there was no mortality. In an acute toxicity test, 3000 mg/kg body weight is the limit dose and any sample nontoxic at this level is considered as safe [17]. Decrease in body weight at high dose extract indicates its toxic potential [21].

3.2 Oral Glucose Tolerance Test (OGTT)

Oral Glucose tolerance test of the extracts was performed in two groups, in normal group and STZ induced diabetic group.

3.2.1 Effect of CAAE on OGTT in normal mice

The effect of CAAE on OGTT in normal mice group was assessed at different time intervals and is depicted in Table 1. The Blood Glucose Level (BGL) at 0 min, 30 min, 60 min, 90 min and 120 min were compared with the initial baseline blood glucose level of their respective groups. The BGL after the glucose load reached a peak at 30 min and decreased subsequently over time, in all the groups of mice. The percentage change in BGL at 30 min, 60 min, 90 min and 120 min, from initial/baseline BGL (0 min) are – 91.42%, 60.87%, 46.72% and 40.85% respectively in NC mice; 84.85%, 53.94%, 42.11% and 21.90% respectively in GTNM; and 88.66%, 71.89%, 50.34% and 43.28% respectively in AqETNM (Fig. 2).

3.2.2 Effect of CAAE on OGTT in STZ induced diabetic mice

The effect CAAE on OGTT in STZ induced diabetic mice was measured at different time intervals and is depicted in Table 2.

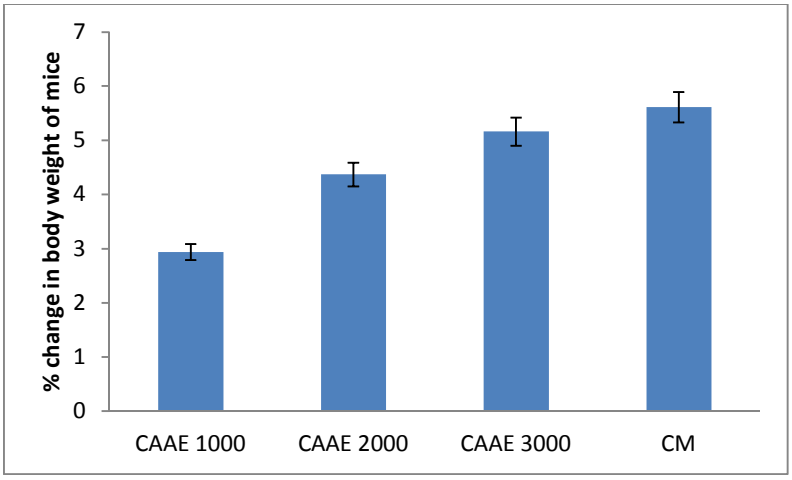

Fig. 1. Effect of acute oral toxicity test of extracts on body weight of mice
CM: Control Mice

Table 1. Effect of extracts on oral glucose tolerance test in normal group mice

Groups	BGL mg/dL at various time intervals in OGTT (normal group)				
	0 min	30 min	60 min	90 min	120 min
NC	80.56±1.62	154.21±1.23	129.6±2	118.2±1.7	113.47±2.3
GTNM	86.2±1.9	159.34±2.01	132.7±1.8	122.5±2.1	105.08±2.4
AqETNM	82.9±1.53	156.4±2.53	142.5±2.46	124.63±2.74	117.95±3.2

NC, normal control; GTNM, Glibenclamide Treated Normal Mice; AqETNM, Aqueous extract treated normal mice.

Table 2. Effect of extracts on oral glucose tolerance test in STZ induced diabetic mice group

Groups	BGL mg/dL at various time intervals in OGTT (Diabetic group)				
	0 min	30 min	60 min	90 min	120 min
DC	204.81±2.5	341.02±2.7	322.44±2.87	311.46±3.66	299.79±3.25
GTDM	225.06±1.81	346.27±1.99	253.75±2.6	218.43±3.1	186.93±3.7
AqETDM	221.46±1.3	341.56±2.43	289.35±3.87	245.32±4.1	220.73±4.05

DC, Diabetic Control; GTDM, Glibenclamide Treated Diabetic Mice; AqETDM, Aqueous extract Treated Diabetic Mice

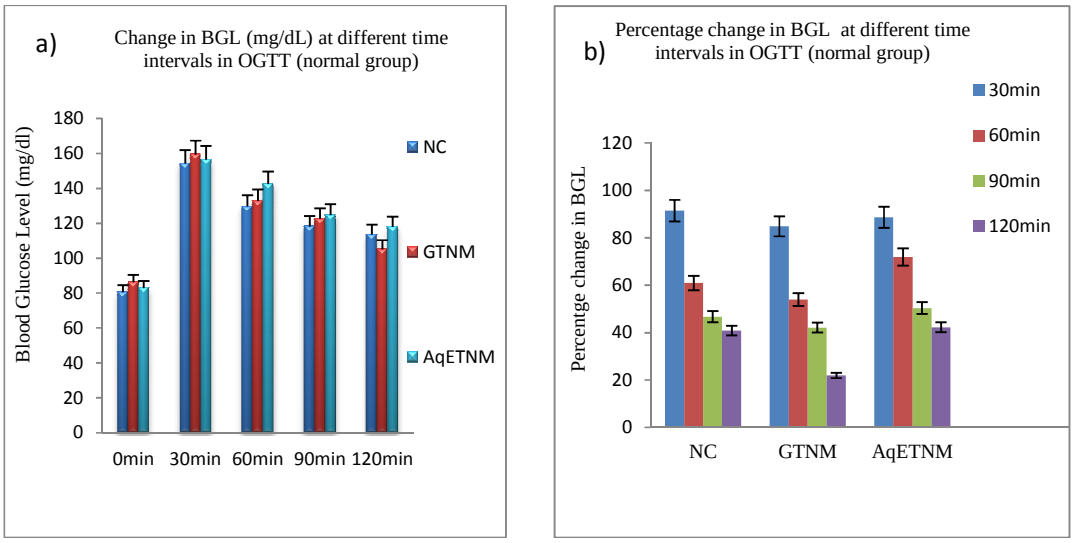

Fig. 2. Effect of extracts on OGTT in normal group mice. a) Change in BGL (mg/dL) at different time intervals, b) Percentage change in BGL at different time intervals

NC, normal control; GTNM, glibenclamide treated normal mice; AqETNM, Aqueous extract treated normal mice.

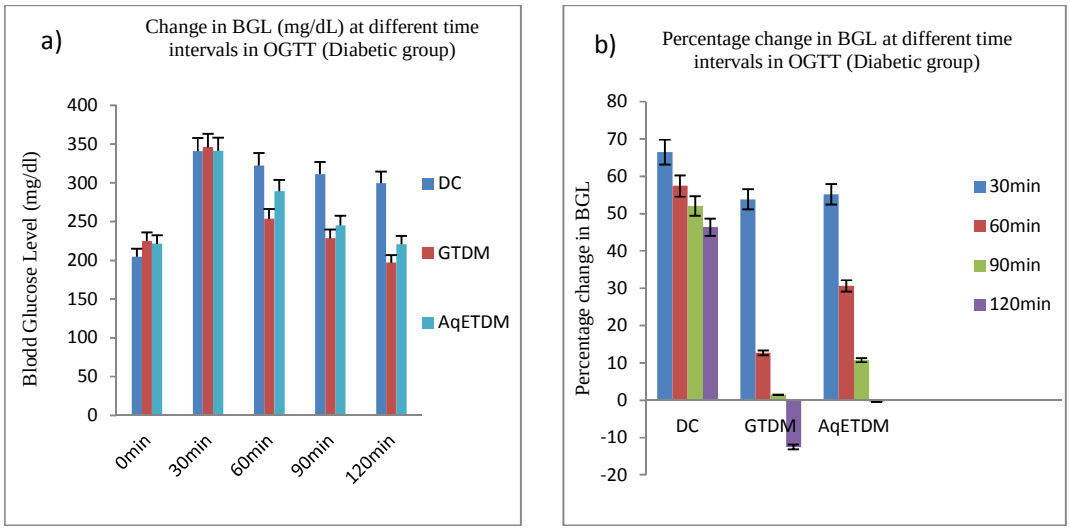

Fig. 3. Effect of extracts on OGTT in diabetic group mice. a) Change in BGL (mg/dL) at different time intervals, b) Percentage change in BGL at different time intervals

DC, Diabetic Control; GTDM, Glibenclamide Treated Diabetic Mice; AqETNM, Aqueous Extract Treated Diabetic Mice

The blood glucose level at 30 min, 60 min, 90 min and 120 min were compared with the initial baseline (0 min) blood glucose level of their respective groups. The BGL after the glucose load reached a peak at 30 min and decreased subsequently over time, in all the groups of mice. The percentage change in BGL at 30 min, 30 min, 90 min and 120 min, from initial/baseline BGL (0 min) are – 66.51%, 57.43%, 52.07% and 46.38% respectively in DC mice; 53.51%, 12.75%, 1.50% and -12.50% respectively in GTDM; and 55.21%, 30.65%, 10.77% and -0.33 respectively in AqETDM (Fig. 3 above).

The oral glucose tolerance test (OGTT) measures the body's ability to metabolize glucose [22]. In OGTT, both normal group and diabetic group, by comparing the bar groups of 0 min with the bar groups of 30 min (Figs. 2a and 3a), it indicates that oral induction

of glucose in mice resulted in 1 to 1.5 fold increase in blood glucose level. In the OGTT (normal group), after glucose load, the BGL reaches a peak and was eventually decreased to near normal indicating a normal glucose metabolism and further indicates that the extracts do not exhibit hypoglycaemic activity in normal mice based on Kar [23]. In diabetic group a significant reduction was observed in extract treated group. However, it was not as effective as the standard drug glibenclamide which have 26.71%, 10% and 13.79% reduction potential at 60min, 90min and 129 min respectively. The extract showed a better inhibitory activity, compared with the diabetic control suggesting that the extracts could decrease the postprandial glucose level probably by inhibiting the activity of α-amylase and α-glucosidase which is in agreement with the work of Wan [24], or might be by enhancing the secretion of insulin in response to glucose load and increased peripheral utilization of glucose [23,25].

4. CONCLUSION

In acute oral toxicity test there was no sign of toxicity and lack of drastic change in body weight of mice at the end of the test. It indicates that the extract is nontoxic and can be used in further bioactivity test. It is an indication that the extracts have no adverse effects. In our OGTT, it was observed that aqueous extract of *Cassia alata* Linn. enhanced glucose utilization. The aqueous extract was able to reduce blood glucose level on STZ induced diabetic mice. The present study showed preliminary idea on hypoglycaemic activity of the plant. The study reveals the positive effect of extracts in maintaining glucose homeostasis in mice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

In-vivo antidiabetic activity assessment was performed after the approval from Institutional Animal Ethical Committee (approval number: IEC/AUS/2013-031 dt-20/3/13). The principles of animal care of the committee were followed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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