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## Hypoglycaemic Activity of *Tragia tennifolia* (Euphorbiaceae) Extract in Rats

G.A. Koffuor, E. Woode and P. Amoateng

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences,  
College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

**Abstract:** The hypoglycaemic effect of the ethanolic *Tragia tennifolia* Extract (TTE) was studied in Sprague-Dawley rats subjected to Oral Glucose Tolerance Test (OGTT), Fasting Plasma Glucose Test (FPGT) and alloxan-induced diabetes mellitus. Acute toxicity studies were also conducted on Sprague-Dawley rats. TTE (53 mg kg<sup>-1</sup>, p.o.) significantly ( $p \leq 0.01$ ) reduced the peak plasma glucose concentration and the AUC of the OGTT curve, by 47.0 and 44.9%, respectively 1 h after its administration and this was comparable to that produced by glibenclamide (0.14 mg kg<sup>-1</sup>) and metformin (12.14 mg kg<sup>-1</sup>). TTE like glibenclamide, significantly ( $p \leq 0.01$ ) reduced the fasting plasma glucose by 29.1% after 5 h compared to control groups. The AUC for TTE-treated rats was reduced by 14.8%. In alloxan-induced DM rats, insulin (8.5 unit/kg/day) significantly ( $p \leq 0.01$ ) reduced hyperglycaemia by 68.6% whereas TTE, glibenclamide and metformin had no significant effects. Acute toxicity studies showed no evidence of toxicity after an oral administration of TTE (0.1-0.5 g kg<sup>-1</sup>). These results indicate that TTE may be potentially useful in Non-insulin Dependent Diabetes Mellitus (NIDDM) but not for Insulin Dependent Diabetes Mellitus (IDDM).

**Key words:** *Tragia tennifolia*, hyperglycaemia, oral glucose tolerance test, noninsulin-dependent diabetes mellitus, alloxan-induced diabetes mellitus

### INTRODUCTION

*Tragia tennifolia* Benth. (Euphorbiaceae) is an herbaceous, mostly twining or trailing plant found in Ghana and other West African countries. In Ghanaian traditional medicine, the leaf-mash is used to treat sores, as an abortifacient or promote child delivery (Burkill, 2004; Irvine, 1967) other parts have also been used as remedy for impotence (Dokosi, 1998), rheumatism and arthritis (Irvine, 1967); an infusion of the warmed leaves are drunk as remedy for diarrhea, colitis or dysentery. The plant has been reported to decrease the anti-diabetic drug dosage when used together as infusions or fresh juice (Stuart, 1979).

The prevalence of diabetes for all age-groups is projected to rise to 366 million in 2030 in developed countries (Rathmann and Giani, 2004; Shaw *et al.*, 2010; Wild *et al.*, 2004). This estimate is expected to double in urban populations in developing countries (Wild *et al.*, 2004). In developing countries, high cost of effective medicines and lack of appropriate health facilities have led to the poor management of life threatening and chronic diseases like diabetes. Thus a greater percentage of the population relies on traditional medicines (Calixto, 2000; Okwu and Uchegbu, 2009; Sofowora, 1993). More than 400 plant species and 100 polysaccharides isolated from plants have been reported to have hypoglycaemic effects (Ernst, 1997; Wang and Ng, 1999; Yuan *et al.*, 1998).

Despite the global and local need of cost effective drugs for the management of diabetes, only a few of these plant sources have been explored.

The present study reports of the hypoglycaemic effects of *T. tennifolia* on models of hyperglycaemia in Sprague-Dawley rats, thus supporting its local use in the management of diabetes mellitus.

### MATERIALS AND METHODS

**Plant sample collection:** Quantities of the whole plant of *Tragia tennifolia* were collected from the botanical garden at the Faculty of Pharmacy, KNUST in September 2000 and identified by the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

**Preparation of extract:** Whole plants of *Tragia tennifolia* were collected; air-dried and powdered. The powdered material (300 g) was Soxhlet extracted with 70% v/v ethanol. The extract was then evaporated to a brown semi-solid mass under reduced pressure and kept in a desiccator until required. This is subsequently referred to as the extract or TTE. Suitable quantities of the dried product were dissolved in normal saline for use.

**Animals:** Sprague-Dawley rats of either sex (200-300 g) were purchased from Noguchi Memorial Institute for

Medical Research, University of Ghana, Legon and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of six in stainless steel cages (34×47×18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 22-24°C, relative humidity 60-70% and 12 h light-dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The Departmental Ethics Committee approved the protocols for the study.

**Drugs:** The drugs and chemicals used in this study include Glibenclamide and metformin (Hovid Pharmaceuticals, Belgium) suspended in 1% w/v tragacanth; insulin (Novo-Nordisk A/S, Denmark).

**Oral Glucose Tolerance Test (OGTT):** Twenty Sprague-Dawley rats were fasted overnight and randomly put into six groups (n = 5) as follows; TTE (26.5, 53, 106 mg kg<sup>-1</sup>), Metformin-treated (12.14 mg kg<sup>-1</sup>), Glibenclamide-treated (0.14 mg kg<sup>-1</sup>, p.o.) and vehicle-treated (0.2 mL normal saline, p.o.). Thirty minutes after extract/drug/vehicle treatment, the rats received a loading dose of glucose (1.75 g kg<sup>-1</sup>, p.o.). The plasma glucose concentration of each rat was then measured after 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 h using a Glucostix® reagent strip (Bayer Diagnostics, Ames, USA) according to the manufacturer's instructions. Briefly, a drop of blood expressed from the tail vein was placed onto the Glucostix® test pads. The test strip was then inserted into the Glucometer® II after drying in air for 90 sec and the plasma glucose read. Urine glucose and ketone were semi-quantitatively determined using Uriquick™ CLINI-9SG (Stanbio lab. Inc., San Antonio Texas, USA) in accordance with the manufacturer's instructions.

**Fasting Plasma Glucose Test (FPGT):** Twenty rats (200 -250 g) were fasted overnight but allowed free access to water and then their fasting plasma glucose concentrations were determined. The animals were randomized into four groups (n = 5) and treated with vehicle, glibenclamide (0.14 mg kg<sup>-1</sup>), metformin (12.14 mg kg<sup>-1</sup>) or TTE (53 mg kg<sup>-1</sup>), respectively. Plasma glucose concentrations were measured at hourly intervals for up to 5 hours as described in OGTT.

**Alloxan-induced DM:** Rats were put in individual metabolic cages and allowed five days to acclimatize.

Food and water intake, urine and faeces output, bodyweight and plasma glucose concentration were then measured daily over a period of 7 days to establish the normal (control) conditions. DM was induced with alloxan monohydrate (140 mg kg<sup>-1</sup>, *intraperitoneally*.) as previously described (Panneerselvam and Govindaswamy, 2002). After a week, rats with plasma glucose concentration of at least 19.4 mmol L<sup>-1</sup> (350 mg dL<sup>-1</sup>) and having glycosuria (indicated by Uriquick™ CLINI-9SG) were considered diabetic. Changes in food and water intake, urine and faeces output, bodyweight and plasma glucose concentration were recorded daily for 10 days (the diabetic stage). The effects of insulin (8.5 units/kg/day), glibenclamide (0.14 mg kg<sup>-1</sup>), metformin (12.14 mg kg<sup>-1</sup>) and TTE (53 mg kg<sup>-1</sup>) on the parameters above in the diabetic rats were recorded for 7 days (the treatment stage). Treatment was then withdrawn and the animals observed for a further 4 days.

**Acute toxicity studies:** TTE was administered orally to 5 groups of rats (n = 6) at dose levels of 0.1, 0.2, 0.3, 0.5 and 1.0 g kg<sup>-1</sup>. Control group received vehicle (1 ml kg<sup>-1</sup>, p.o.). The animals were observed for up to 24 h (acute) and subsequently for 7 days to observe possible delayed toxicity.

**Statistical analysis:** Data are presented as Mean±SEM. (n = 5-10) and analysed by One-way ANOVA followed by Newman-Keuls test for column graphs. GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. The p≤0.05 was considered statistically significant in all analysis. The graphs were plotted using Sigma Plot for Windows Version 11.0 (Systat Software Inc., Germany).

## RESULTS

**Oral Glucose Tolerance Test (OGTT):** After the glucose load (1.75 g kg<sup>-1</sup>), plasma glucose level peaked 4.3 fold (2.37-12.54 mmol L<sup>-1</sup>) within 1 h (Fig. 1a). Glibenclamide (0.14 mg kg<sup>-1</sup>) and metformin (12.14 mg kg<sup>-1</sup>) decreased this peak to 6.39 mmol L<sup>-1</sup> (51% reduction) and 5.89 mmol L<sup>-1</sup> (47% reduction), respectively (p≤0.01). TTE (26.5, 53 and 106 mg kg<sup>-1</sup>, p.o.) also reduced the peak plasma glucose value by 2.63 mmol L<sup>-1</sup> (21% reduction), 5.89 mmol L<sup>-1</sup> (47% reduction) and 6.45 mmol L<sup>-1</sup> (51.5% reduction), respectively (Fig. 1). The AUC values for drug-treated rats compared with vehicle-treated rats are as shown in Table 1. TTE (53 mg kg<sup>-1</sup>) reduced the AUC under the OGTT curve by 45%.

**Fasting Plasma Glucose Test (FPGT):** In the vehicle-treated control group, there was no significant

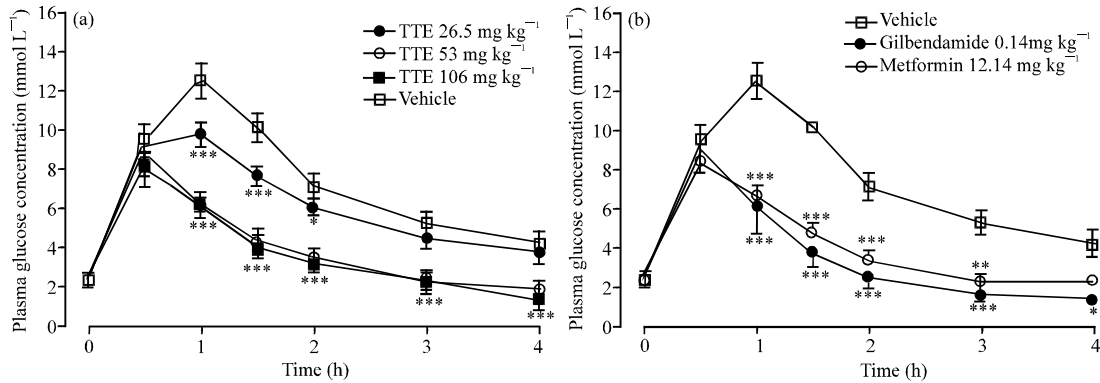


Fig. 1: Effect of (a) TTE (26.5-106 mg kg<sup>-1</sup>) and (b) Gilbenclamide (0.14 mg kg<sup>-1</sup>) and Metformin (12.14 mg kg<sup>-1</sup>) on plasma glucose concentration of normal rats in an oral glucose tolerance test. Values are Means±SEM (n = 5). \*p<0.05, \*\* p<0.01, \*\*\*p<0.001 compared to vehicle-treated group (Two-way ANOVA followed by Bonferromi's post hoc test)

Table 1: The area under the curve (AUC) values for curves in the OGTT and FPGT

Drugs	OGTT		FPGT	
	AUC	% reduction in AUC	AUC	% reduction in AUC
Normal saline	29.42		14.62	
Glibenclamide (0.142 mg kg <sup>-1</sup> )	14.15	51.9	11.97	18.1
Metformin (12.14 mg kg <sup>-1</sup> )	16.36	44.4	14.42	1.4
TTE (53 mg kg <sup>-1</sup> )	16.19	45.0	12.40	13.8

OGTT: Oral glucose tolerance test, FPGT: Fasting plasma glucose test and AUC: area under curve

change in plasma glucose throughout the experimental period. Metformin (12.14 mg kg<sup>-1</sup>) had a similar effect to the vehicle-treated control. Fasting plasma glucose in glibenclamide (0.14 mg kg<sup>-1</sup>) and TTE-treated (53 mg kg<sup>-1</sup>) rats however decreased significant (p< 0.01) after 2-3 h of drug administration (Fig. 2). The AUC values for TTE-treated rats compared to vehicle-treated reduced by 14.8% (Table 1).

**Alloxan-Induced DM:** Plasma glucose concentration increased significantly (p<0.001) from 4.7±0.32 to 21.35±0.20 mmol L<sup>-1</sup> after induction of DM (urine glucose also increased from trace quantities to 1500 mg dL<sup>-1</sup>). Treatment of the diabetic rats with insulin (8.5 units kg<sup>-1</sup>) caused a 3 fold reduction (p<0.001) in glycemic levels from 21.35±0.20 to 6.55±1.24 mmol L<sup>-1</sup> (Fig. 3a). However, plasma glucose concentration increased significantly again to 19.81±0.96 after insulin withdrawal. Glibenclamide (0.142 mg kg<sup>-1</sup>), metformin (12.14 mg kg<sup>-1</sup>) and TTE (53 mg kg<sup>-1</sup>) did not reduce significantly (p<0.05) plasma glucose concentration during treatment (Fig. 3a). Urine glucose levels were between 1500-2000 mg dL<sup>-1</sup>.

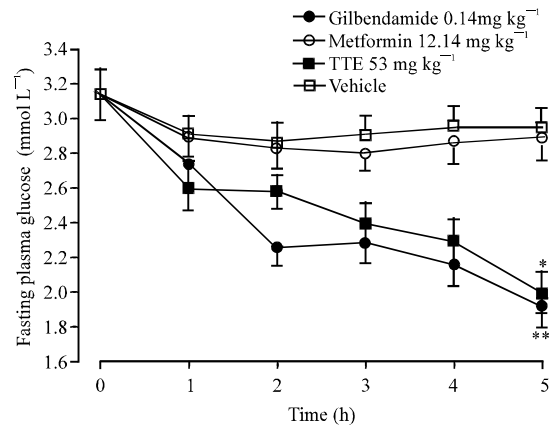


Fig. 2: Effect of TTE (53 mg kg<sup>-1</sup>), Gilbenclamide (0.14 mg kg<sup>-1</sup>) and Metformin (12.14 mg kg<sup>-1</sup>) on plasma glucose concentration of normal rats in an oral glucose tolerance test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 compared to vehicle-treated group (Two-way ANOVA followed by Bonferromi's post hoc test)

Insulin reduced food intake on treating diabetic rats but glibenclamide, metformin and TTE did not reduce significantly food intake (Fig. 3b). Daily water intake increased (p<0.001) 3 fold (32.3 to 86.8 mL) and urine output increased (p<0.001) 6 fold (11.1 to 63.6 mL) compared to vehicle-treated control. Aside insulin which significantly decreased (p<0.001) water intake and urine output, there were no significant changes (p<0.05) on treating with glibenclamide, metformin and TTE (Fig. 4a, b). There was however, no significant changes in body weight before and after induction of diabetes and treatment with all the drugs (Fig. 5).

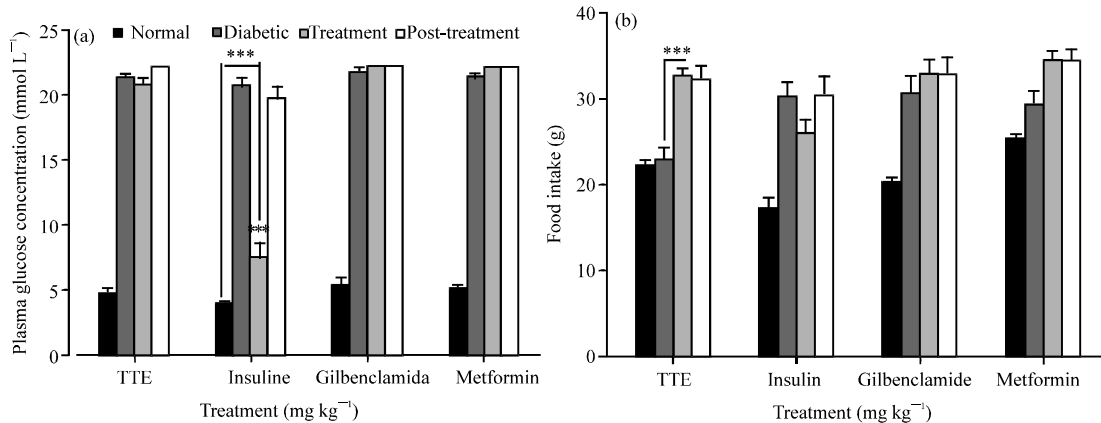


Fig. 3: Changes in (a) plasma glucose concentration (mmol L<sup>-1</sup>) and (b) food intake (g) with respect to normal rats, alloxan-induced diabetic rats and the results of treatment of the diabetic rats with TTE (53 mg kg<sup>-1</sup>), Gilbenclamide (0.14 mg kg<sup>-1</sup>) and Metformin (12.14 mg kg<sup>-1</sup>) and post-treatment effects. Each bar represents the mean±SEM. (n = 5). \*\*\*p<0.001 (One-way ANOVA followed by Newman-Keuls post hoc test)

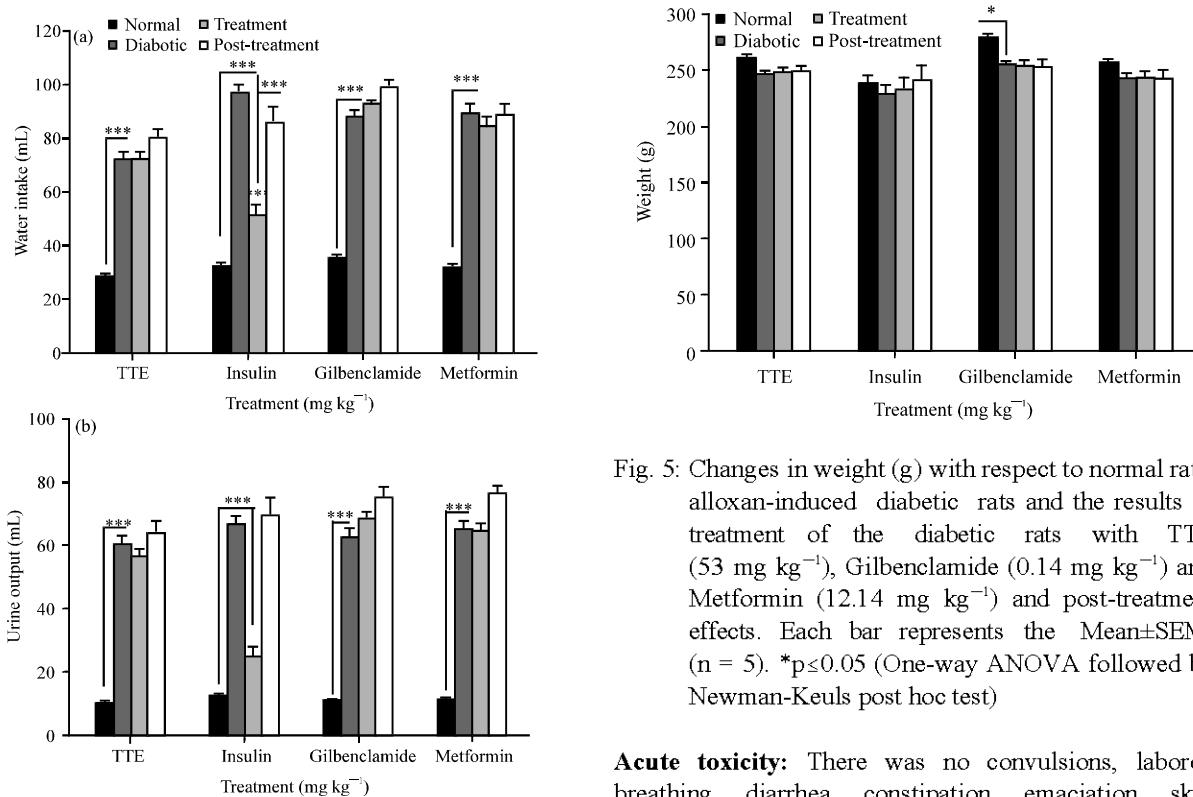


Fig. 4: Changes in (a) water intake (mL) and (b) urine output with respect to normal rats, alloxan-induced diabetic rats and the results of treatment of the diabetic rats with TTE (53 mg kg<sup>-1</sup>), Gilbenclamide (0.14 mg kg<sup>-1</sup>) and Metformin (12.14 mg kg<sup>-1</sup>) and post-treatment effects. Each bar represents the mean±S.E.M. (n = 5). \*\*\*p<0.001 (One-way ANOVA followed by Newman-Keuls post hoc test)

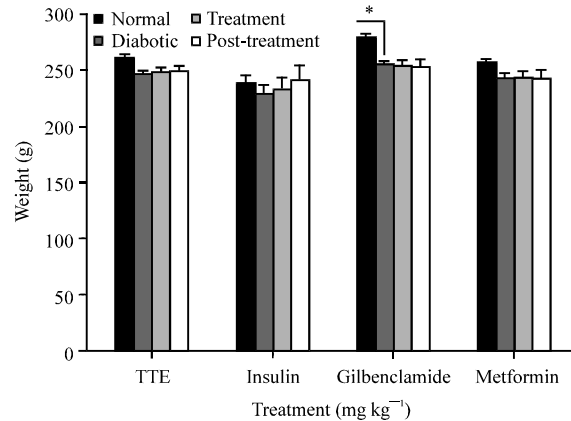


Fig. 5: Changes in weight (g) with respect to normal rats, alloxan-induced diabetic rats and the results of treatment of the diabetic rats with TTE (53 mg kg<sup>-1</sup>), Gilbenclamide (0.14 mg kg<sup>-1</sup>) and Metformin (12.14 mg kg<sup>-1</sup>) and post-treatment effects. Each bar represents the Mean±SEM. (n = 5). \*p<0.05 (One-way ANOVA followed by Newman-Keuls post hoc test)

**Acute toxicity:** There was no convulsions, labored breathing, diarrhea, constipation, emaciation, skin eruptions, abnormal posture, bleeding (from the eyes, nose, or mouth), sedation, ataxia, flaccid paralysis polyuria, polydipsia, polyphagia and anorexia rhinorrhoea/nasal congestion, loss of autonomic reflexes, decreased locomotory activity, neuromuscular incoordination and collapse hyperesthesia, hypothermia, twitching, spasticity, writhing, respiratory depression, ocular discharge, prostrate posture and mortality.

## DISCUSSION

The significant reduction in AUC values caused by glibenclamide, metformin and TTE in OGTT indicates hypoglycaemic effect. Glibenclamide inhibits ATP-dependent potassium channels on pancreatic beta cells which augment insulin secretion and increases sensitivity to insulin of the insulin-dependent tissues i.e., the liver, muscles and adipose tissues resulting in glucose utilization while metformin enhances glucose utilization by peripheral tissues and also a delay in the absorption of glucose (Sethu *et al.*, 2002). Metformin stimulates the hepatic enzyme AMP-activated protein kinase (Zhou *et al.*, 2001). If insulin secretion is induced before glucose loading, plasma glucose concentration will not rise significantly above normal ranges 2 h postprandial. Plasma glucose concentration falls rapidly as the extent of utilization would be high and could even reduce to within fasting plasma glucose ranges in relatively short hours postprandial. TTE could possibly be exerting its hypoglycaemic effect by mechanisms similar to that of glibenclamide and metformin.

The degree of hypoglycaemia produced by glibenclamide and TTE in fasted rats increases significantly with time over the dose administered. This effect was however insignificant with metformin. The maintenance of blood glucose levels in the fasting state is largely dependent on hepatic glucose production. Any agent that reduces hepatic glucose production therefore, will produce a hypoglycaemic effect (El-Shabrawy and Nada, 1996). Glibenclamide enhances insulin secretion from pancreatic  $\beta$ -cells which cause hepatic glucose utilization i.e., glycogen formation. Insulin release antagonizes the activity of glucagon with a resultant reduction in fasting plasma glucose (Sethu *et al.*, 2002).

Direct stimulation of glycogen formation, reduction in gluconeogenesis, slowing of glucose absorption and induction in glucagon secretion takes place only when plasma glucose levels are rising. Metformin which exerts its hypoglycaemic effect mainly by these mechanisms will insignificantly affect fasting plasma glucose levels. Since TTE lowers fasting plasma glucose significantly, it may have suppressive action on hepatic glycogenolysis as the maintenance of blood glucose in the fasting state depends largely on hepatic glucose production.

The effect of TTE, glibenclamide and metformin on plasma glucose concentration in the alloxan-induced diabetes suggested that TTE may not be useful in insulin-dependent DM. However, insulin produced significant reduction in blood glucose levels. Alloxan is a diabetogenic agent known to induce diabetes mellitus by

destroying pancreatic  $\beta$ -cells of the islet of Langerhans where insulin is produced and secreted (Singh and Gupta, 2007). There could therefore be a partial or complete lack of insulin resulting in the metabolic disorder characterized by hyperglycaemia. When insulin is lacking, uptake and utilization of glucose by insulin-sensitive sites are shut down. Glucose therefore persists in plasma (hyperglycaemia) and spills into the urine (glucosuria). There is energy and water loss mainly through urine as renal threshold for glucose conservation (about  $180 \text{ mg dL}^{-1}$ ) is exceeded. There is osmotic diuresis resulting in polyuria, dehydration and thirst (Ganong, 1995).

The results confirm observations made that in insulin-dependent diabetes mellitus there is hyperglycaemia, glucosuria, polyphagia, polydipsia, polyuria (English and Williams, 2004). Insulin treatment showed significant reduction and reversal in hyperglycaemia, glycosuria, polyphagia and polyuria when alloxan-induced diabetic rats were treated with exogenous insulin. Injected insulin mimics the activity of endogenous insulin and causes a reversal of the catabolic features of insulin deficiency and enhances anabolic activity in the liver. It also enhances protein and glycogen synthesis in the muscles and increases triglyceride formation from glucose, to be stored in adipose tissues: Hence, the fall in plasma glucose concentration (Herfindal and Gourley, 1996). It is clear from the results obtained that glibenclamide and metformin do not significantly reverse the condition of hyperglycaemia and hence glycosuria, polyphagia and polyuria in alloxan-induced DM. This is consistent with findings on the mechanism of action of glibenclamide and metformin in insulin-dependent DM. TTE had effects similar to glibenclamide.

## CONCLUSION

The present study demonstrates that TTE produces hypoglycaemic effects in the oral Glucose Tolerance Test (OGTT) and Fasting Plasma Glucose Test (FPGT) but had no significant effect in the alloxan-induced diabetes. Thus TTE is useful in insulin-independent diabetes rather than insulin dependent type. The results of the acute toxicity studies suggests that TTE is safe in rats up to a dose of  $0.5 \text{ g kg}^{-1}$ .

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