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Research Article

INVIVO ANIMAL MODEL FOR SCREENING OF ANTI DIABETIC ACTIVITY

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ABSTRACT

The Antidiabetic activity of *Kigelia africana(Lam.)* (Family:Bignoniaceae) and *Tabebuia rosea(Bertol) DC* (Family:Bignoniaceae) were investigated in Alloxan induced diabetic albino rats. A comparison was made between both the plant extracts and a known antidiabetic drug Glibenclamide (5 mg/kg body weight). The dried leaves of *Kigelia africana* and *Tabebuia rosea* were subjected to extraction by continuous hot percolation using methanol as solvent and were subjected to standardization using pharmacognostical and phytochemical screening. Dose selection was made on the basis of acute oral toxicity study (200 mg/kg body weight) as per OECD and CPCSEA guidelines. Oral administration of extracts of *Kigelia africana* (200mg/kg) and *Tabebuia rosea*(200mg/kg) for 7 days resulted in a significant reduction in blood glucose levels. Alloxan induced diabetic rat model was used for the evaluation of antidiabetic activity. Activity is more for *Tabebuia rosea* in comparision with *Kigelia africana. Tabebuia rosea* methanolic extract (TRME) and *Kigelia africana* methanolic extract (KAME) showed significant ($p<0.001$) antidiabetic activity. The blood glucose levels of these plant extracts on seventh day of the study were TRME (108.2±4.11) KAME (127.5±2.56) in comparison of diabetic control (414.2 ± 5.03). In glucose loaded rats, TRME exhibited glucose level of (129± 3.55) after 30 min and (91± 1.366) after 90 min, whereas the levels in KAME treated animals were (126.5 ±4.12) after 30 min and (88.33±1.647) after 90 min. These extracts also prevented body weight loss in diabetic rats. The drug has the potential to act as an antidiabetic drug.

Keywords: Kigelia africana, Tabebuia rosea, antidiabetic activity , Alloxan, Acute oral toxicity.

INTRODUCTION

MATERIALS AND METHODS

Animals

Healthy adult albino rats of Wister strain of either sex between the age of 2-3 months and weighing 150-200 grams were used for the present study. The animals were housed individually in polypropylene cages, maintained under standard conditions (12 hours light and12 hours dark cycle, 25 ±5°C and 40-60% humidity). They were fed with standard rat pellet diet (National Institute for Nutrition, Hyderabad) and provided water ad libitum. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg. no. (1447/PO/a/11/CPCSEA).

Chemicals

Alloxan monohydrate, Glibenclamide, Dextrose, Tween-80, Auto analyzer (Analytical technological limited) and One-touch (Horizon). All the other chemicals and reagents used were of analytical grade.

Plant Material

Fresh leaves were collected from Chittoor district, Andhra Pradesh, India and authentified by Dr. K. MadhavaChetty, Professor, Department of Botany S.V. University, Tirupathi, Andhra Pradesh, India.

Preparation of Plant Extraction

The collected leaves were shade dried and powdered in a grinder mixture to get coarse powder. The powdered leaves were defatted with petroleum ether and later extracted with methanol. The extract was evaporated to dryness, gave a residue of 40 % w/w.

Phytochemical Screening:

A preliminary phytochemical screening of methanolic extracts of *Kigelia africana* and *Tabebuia rosea* was carried by using standard procedures[1-3].

Acute Oral Toxicity Studies

Acute oral toxicity studies [4] of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India. Administration of the stepwise doses of extracts of *Kigelia africana* from 40 mg/kg body weight up to the dose 2000 mg/kg body weight caused no considerable signs of toxicity in the tested animals. One tenth of upper limit dose were selected as the level for examination of anti-diabetic activity.

Experimental model

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 120 mg/kg body weight intraperitonially[5]. After 1 hour of Alloxan administration, the animals were given feed ad libitum, and 5% dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 72 hours blood glucose was measured by One-touch glucometer. The diabetic rats (glucose level 200-300 mg/dl) were separated and divided into six different groups for experimental study, with each group containing six animals.

Experimental Design

Different groups of rats were used to study the effects of KAME and TRME. The rats were divided into six groups each consisting of six rats.
Group-I: Normal/control animals received 1% tween80, 1ml per orally.
Group-II: Alloxan (120mg/kg body weight) induced diabetic animals received in 1% tween80, 3ml/kg body weight per orally.
Group-III: Alloxan (120g/kg body weight) induced diabetic animals received Glibenclamide 5mg/kg body weight perorally.
Group-IV: Alloxan (120mg/kg body weight) induced diabetic animals received TRME 200mg/kg, body weight per orally.
Group-V: Alloxan (120mg/kg body weight) induced diabetic animals received KAME 200mg/kg, body weight per orally.
Significant hyperglycemia was achieved within 48 hrs after Alloxan (120mg/kg b.w. i.p.) injection induced diabetic rats with more than 200mg/dl of blood glucose were identified as to be diabetic and used for the study.

In acute study all the surviving diabetic animals and normal animals were fasted overnight Blood samples were collected from the fasted animals prior to the treatment with above scheduled and after administration, at each day up to 7 days.

Body Weight Measurement

Body weight was measured totally four times during the course of study period[6] [i.e., before Alloxan induction (initial values), and on the first, fourth, and seventh days of the treatment period], using a weighing scale.

Statistical Analysis

The results of the study were subjected to one way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. Values with *P*<0.05 were considered significant.

RESULTS

Phytochemical Screening

Phytochemical screening of the extracts of *Kigelia africana*and *Tabebuia rosea* showed the presence of various chemical constituents, mainlyAlkaloids, Tannins, Flavonoids, Carbohydrates, Lignins, Proteins in *Kigelia africana*and Flavonoids in *Solanum pubescence* may be responsible for its Anti-diabetic properties.The results obtained were comparable and satisfied the standard literature.

Acute Oral Toxicity Studies

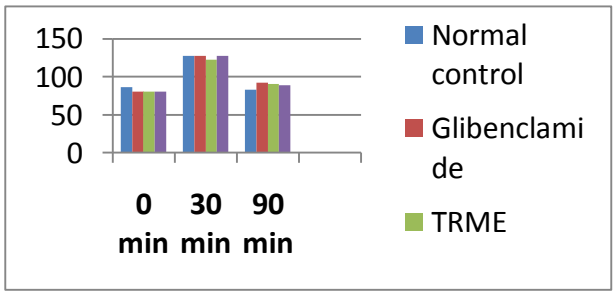
In acute toxicity study, none of the studied methanolic extracts of leaves showed any significant toxicity sign when observed for the parameters during the first 4 hours and followed by daily observations for 14 days and mortality was also not observed. The drug was found to be safe at the tested dose level of 2000 mg/kg b. w. One-tenth of this dose level was taken as effective dose. All the extracts were experimented at the same dose of 200 mg/kg b. w. In order to ascertain a scientific base for the usefulness of this plant in the treatment of diabetes. It was decided to evaluate experimental design of antidiabetic activity by Alloxan-induced model.

Oral glucose tolerance test

The effect of different extracts on glucose tolerance test in normal rats is shown in Table-1. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from fasting value and then subsequently decreased. The methanolic extracts of *Kigelia africana* and *Tabebuia rosea* exhibited remarkable blood glucose lowering effect at 90 min. *africana* and *Tabebuia rosea* exhibited remarkable blood glucose lowering effect at 90 min.

Table 1: Effect of Methanolic extract of *Kigelia africana*and *Tabebuia rosea* on blood glucose level in Oral glucose tolerance test in normal rats.

	0 min	30 min	90 min
Sample	**Blood Glucose Levels (mg/dl)**		
Normal control	86 ± 1.065	127.2 ± 4.23	83.17 ± 1.24
Glibenclamide	80.50 ± 1.335***	127 ± 4.203***	92.17 ± 0.94***
TRME	80.33 ± 0.667***	122.5± 0.284***	90.33± 2.12***
KAME	80.83± 0.4774***	127.2 ±4.23***	88.83±0.6007***



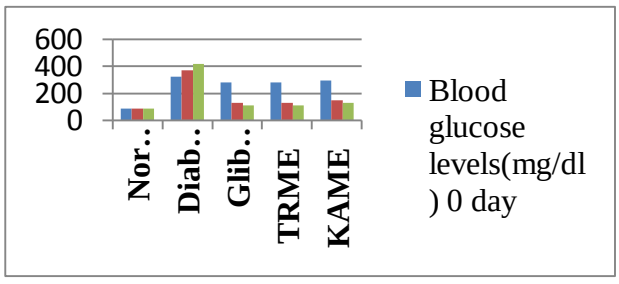
The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's *t*-test. The blood glucose values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Alloxan induced diabetic model

As expected in the diabetic control, there was severe hyperglycemia as compared to the normal animals. Compared to the diabetic control, the TRME and KAME lowered the elevated blood glucose levels only in subacute treatment Table-2. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly, bringing it nearly back to normal, whereas TRME and KAME significantly (p<0.01) decreased fasting blood serum glucose in diabetic rats on 3rd and 7th days as compared to initial (0 hr) blood serum glucose levels. When TRME and KAME were compared for their antidiabetic activity in comparison to active control, particularly Glibenclamide, the results showed that their potential was lesser but significant (**p<0.01) than the standard drug at subacute level.

Table 2: Effect of Methanolic extract of *Kigelia africana*and *Tabebuia rosea* on blood glucose level of alloxan induced diabetic albino rats.

Group	Treatment	Blood Glucose Levels (mg/dl) 0 day	3rd day	7th day
I	Normal Control	83.67 ± 2.48	83.5 ± 1.83	84.17 ± 2.15
II	Diabetic Control	323.3 ± 12.92	369.7 ± 7.06	414.2 ± 5.03
III	Glibenclamide	277.3 ± 5.22*	127 ± 4.2***	107.2 ± 4.11***
IV	TRME	277.3± 5.22*	129.8 ± 5.16**	108.2±4.11***
V	KAME	295.3±3.95*	146.8 ±5.57**	127.5±2.56***

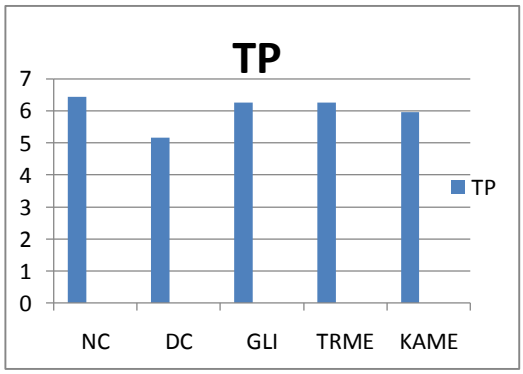


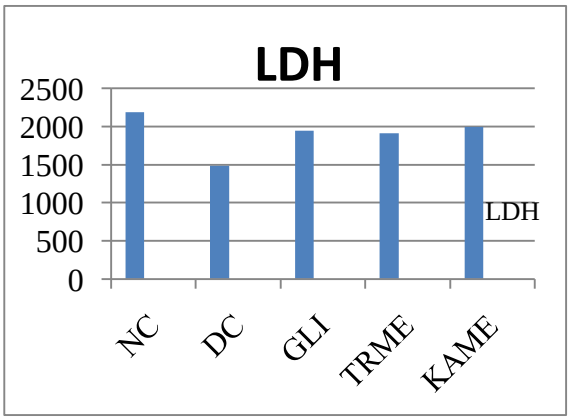
The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's *t*-test. The blood glucose values of groups III, IV, V and VI are compared with control animals, values ***p<0.001, **p<0.01, *p<0.05

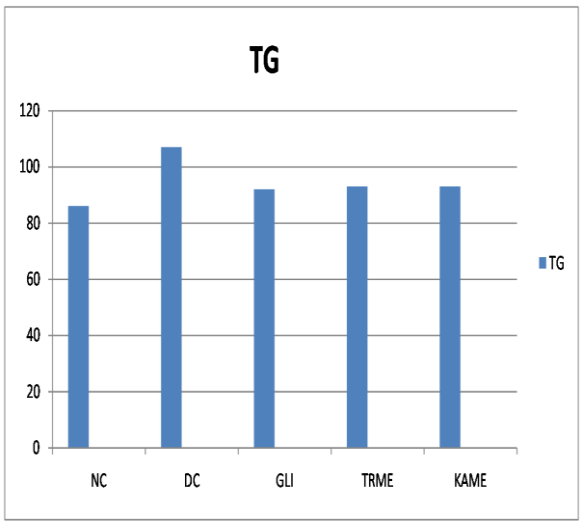
Table 3: Effect of Oral administration of the methanolic extracts of *Kigelia african a*and *Tabebuia rosea* on serum profile in experiment rats after 7 days.

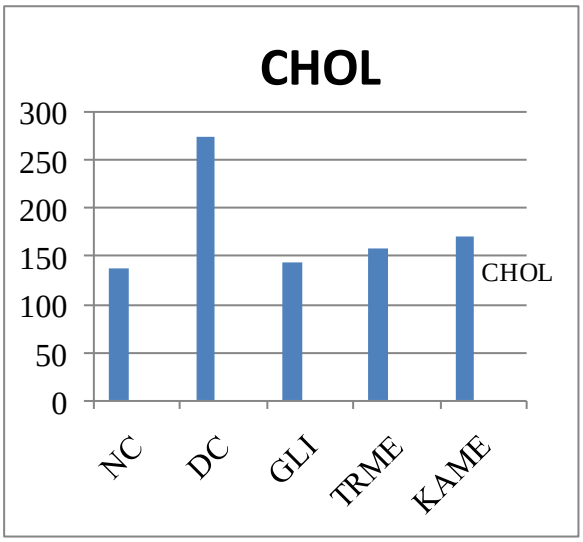
G	TG	TP	LDH	Chol	Creat	ALP	AST	ALT	Alb	BUN	HDL	LDL
NC	86.17 ± 1.88	6.45 ± 0.16	2192 ± 80	139.3 ± 2.98	0.53 ± 0.03	114.7 ± 1.61	50.67 ± 2.04	90.5 ± 0.99	4.145 ± 0.11	24 ± 1.5	43.5 ± 0.763	70.83 ± 0.98
DC	107.0 ± 2.68	5.16 ± 0.14	1485 ± 15.0	274.5± 1.94	1.30 ± 0.11	315.3 ± 8.83	91.33 ± 1.70	165.2 ± 4.67	2.75 ± 0.29	60.80 ± 0.89	32.17 ± 0.6	109.5 ± 1.765
Gli	92.17 ± 1.70***	6.27 ± 0.08***	1944 ± 26.15***	144.7 ± 3.21***	0.55 ± 0.03***	130.3 ± 3.99***	61.17 ± 1.53***	110.0 ± 4.96***	3.66 ± 0.15***	30.46 ± 1.33***	45.5 ± 1.118***	72.83 ± 1.376***
TRME	93± 2.12***	6.27±0.13***	1911± 22.85***	159.5±4.24	0.52± 0.06***	133.5± 2.64***	62.17 ± 2.272***	107.0 ± 2.04***	3.662 ± 0.13***	29.25±1.26***	46.67± 0.666***	75.± 1.673***
KAME	93.17 ± 2.613***	5.96± 0.12***	1995±13.29***	171.2±2.044	0.79± 0.048***	135±3.16***	71± 2.596***	141.7± 1.83***	3.623± 0.16***	39.74± 0.30***	41± 0.730***	84.17± 1.515***

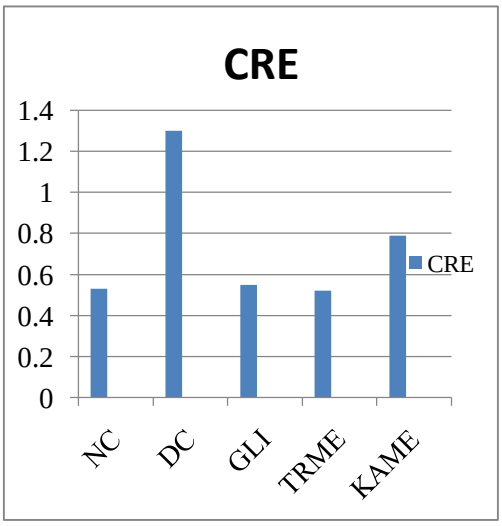
Values are expressed as mean ± SEM, n=6. Statistical significance test for comparison was done by ANOVA, followed by Dunnett's *t*-test.***p<0.001, **p<0.01, *p<0.05.







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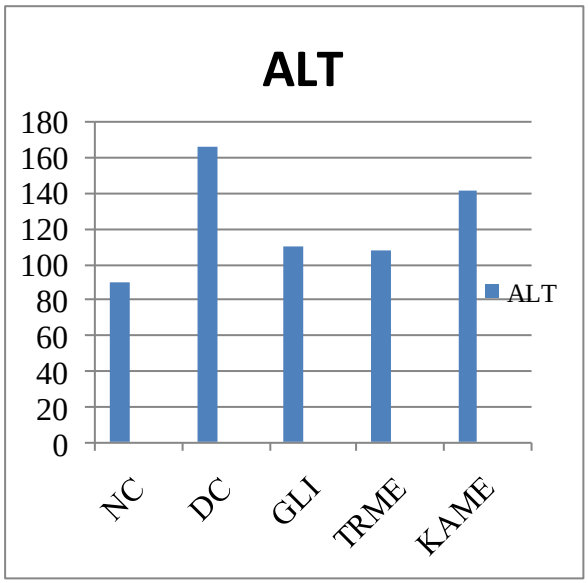
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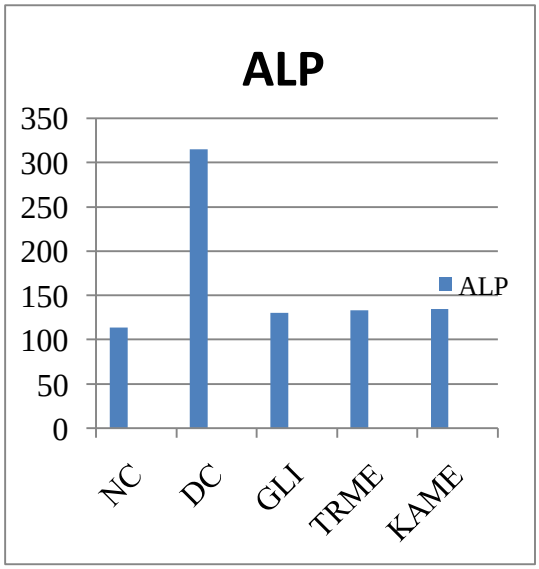
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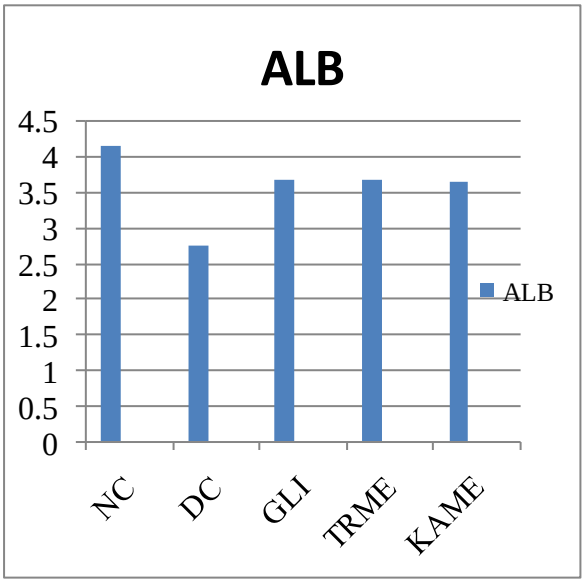
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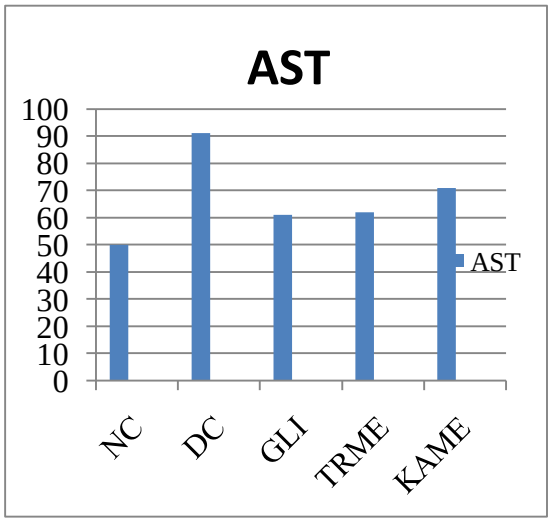
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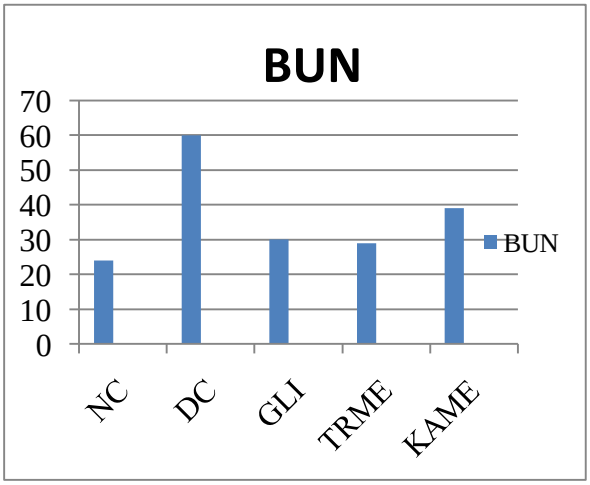
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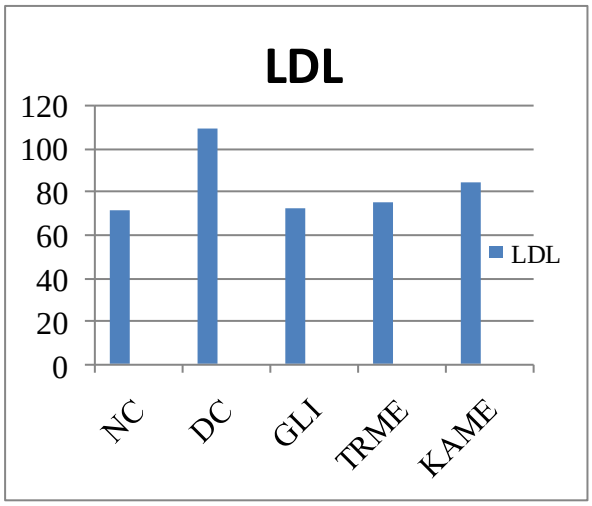
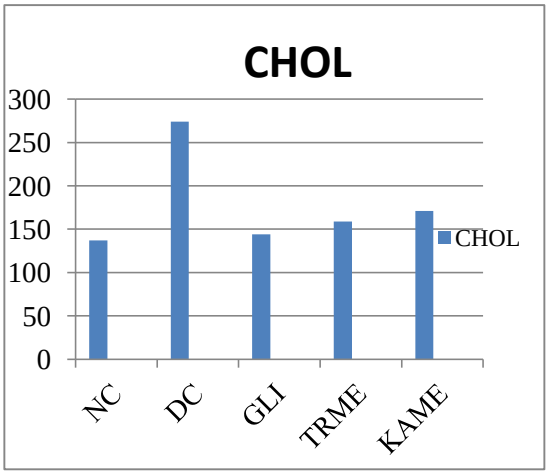




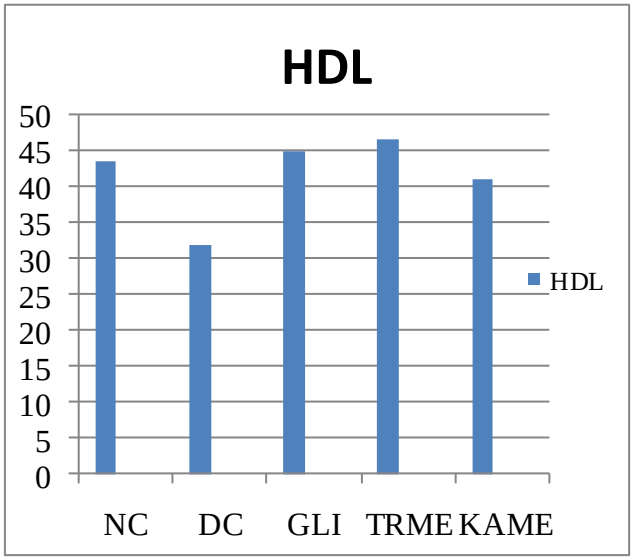
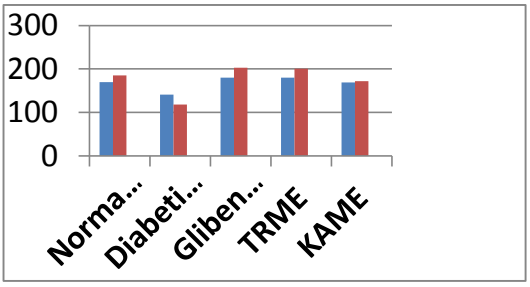


Table 4: Effect of the methanolic extracts of *Kigelia africana* and *Tabebuia rosea* on body weight after treatment in diabetic rats.

Group	Treatment	Average body weight (g) ±SEM	
		Initial value	Day 7
I	Normal control	170 ± 1.36	185.8 ± 2.15
II	Diabetic control	141.3 ± 1.99	118.3 ± 2.09
III	Glibenclamide	180 ± 4.5***	203.8 ± 3.18***
IV	TRME	180± 4.5***	201.3 ± 2.30***
V	KAME	169.8±2.4***	172±4.08***

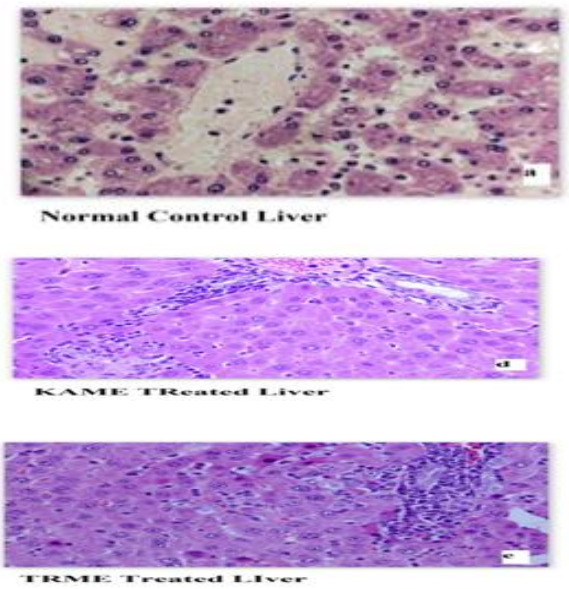


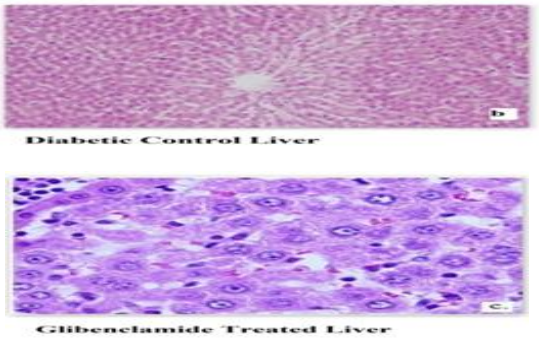
The values are expressed as mean ± SEM. n=6 animals in each group Statistical significant test for comparison was done by ANOVA, followed by Dunnett's *t*-test. The Average body weight values of groups are compared with normal control animals, values ***p<0.001, **p<0.01, *p<0.05.

Body Weight Measurement

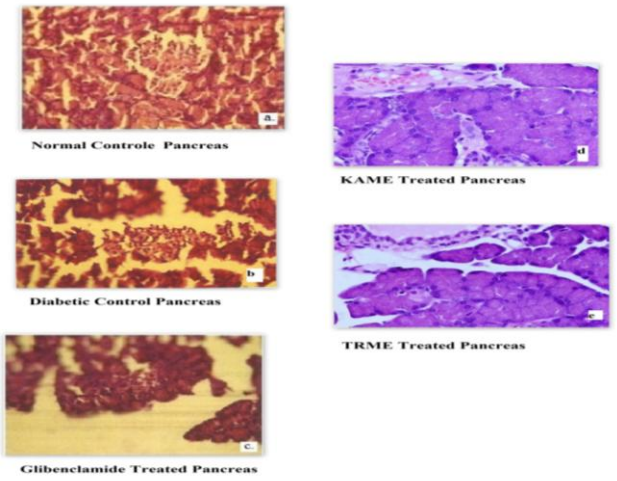
In the present study, diabetic rats had lower body weights and high blood glucose level as compared to normal rats. In spite of increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. As shown in Table-4, treatment with TRME and KAME improved the average body weights of rats, which indicates that control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

Histopathology





Photomicrograph of liver stained with haemotoxylin and Eosin (magnification x 400). a) Group-I (Normal control) Hepatocytes are normal, b) Group-II (Diabetic control) with shrunken nuclei, granular cytoplasm and dilated sinusoids, c) Group-III (Glibenclamide) Normal hepatocytes, dilated central veins are seen, d) Group-V (KAME) Focal areas of necrosis are seen and hepatocytes are normal. Perivascular round cell collection (PVRCC) and portal tract infiltration with lymphocytes are also seen, e)Group-IV (TRME) hepatocytes are normal. Occasional dilated central veins are seen along with occasional focal areas of necrosis.



Photomicrograph of Pancreas stained with haemotoxylin and Eosin (magnification x 400). a) Group-I (Normal control) Normal islets with acni, b) Group-II (Diabetic control) islet cells with fatty infiltration shows damaged and atrophic islet with acni, c) Group-III (Glibenclamide) islet cells are small, d) Group-V (KAME) islets with normal structural intactness with their nucleus, e) Group-IV (TRME) islets with normal round and elongated.

DISCUSSION

In the recent times many traditionally used medicinally important plants were tested for their antihyperglycemic potential by various investigators in experimental animals. I have undertaken a study on *Kigelia africana*and *Tabebuia rosea* for their antidiabetic activity.

The present experiment was continuous post treatment for 7 days with the TRME and KAME where TRME showed more potential hypoglycemic activity than in KAME in OGTT and normoglycemic rats and alloxan induced diabetogenic rats.

Preliminary phytochemical screening revealed that KAME showed positive response to Alkaloids, Tannins, Flavonoids, Carbohydrates, Lignins, Proteins and in TRME, the response was positive to Flavonoids. The increased level of glycosylated haemoglobin (HbA1c) is directly proportional to the decreased level of haemoglobin in diabetic control experimental rats. HbA1c is used as most reliable marker and standard diagnosis practices for estimating the degree of protein glycation during diabetes mellitus[8]. Proglycation is a non-enzymatic reaction between excess glucose present in the blood and free amino groups on the globin component of haemoglobin. Measurement of HbA1c level provides information of long term glycemic status and to correlate with various complications related to Diabetes mellitus. On oral administration of TRME and KAME, the TRME, is more significantly decreased the Hb1c level possibly due to normoglycemic control mechanisms in experimental rats which also reflect the decreased protein glycation condensation reactions and the reports obtained is concordant with the previous result[9].

A marked increase in serum concentration of TC, TG, LDL and decreased HDL was observed with diabetic rats than normal control group which is often linked with hyperlipidaemia. Hyperlipidaemia certainly contributes to major risk factor for cardio vascular diseases[10, 11]. During diabetic state, insulin deficiency contributes to derangements of various metabolic and regulatory mechanisms in body. At normal state insulin activates the lipolytic hormones action on the peripheral fat depots which hydrolyses triglycerides and prevents mobilization of free fatty acids[12, 13]. However, insulin deficiency inactivates the lipoprotein lipase which promotes liver conversion of free fatty acids into phospholipids and cholesterol and finally discharged into blood which resulted into elevated serum phospholipid level[14, 15]. My result of this study reveals that the administration of TRME and KAME not only lowered TC, TG and LDL, but also enhanced the cardioprotective lipid HDL.

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