



Effect of Root Extract Fractions of *Kyllinga triceps* Rottb on Streptozotocin Induced Diabetic Rats

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ABSTRACT

The present study was aimed to evaluate the root extract fractions of *Kyllinga triceps* (KT) for their antidiabetic potential on streptozotocin induced diabetes in neonatal rats. Diabetes was induced by a single intraperitoneal injection of Streptozotocin (90mg/kg) to 48±2h old neonatal rats. Effect of root extract fractions (toluene, ethyl acetate, 1- butanol at 50 & 100 mg/kg.) were tested for their antihyperglycemic activity by measuring their fasting blood glucose level in diabetic rats at 0,2,4,6,8,12 & 24 h after the treatment. In sub acute study ethyl acetate fraction of KT (EAKT) was administered daily to diabetic rats orally at a dose of 100mg/kg for 28 days. Body weight of the animals and blood glucose level were observed at weekly interval during the study. Cholesterol, triglycerides, insulin, SGPT, ALP, creatinine and total proteins level in serum were also estimated at the initial and after 28 days of the treatment. As the preliminary investigation conducted in our lab on methanolic extract of the roots of KT had showed significant oral glucose tolerance with 200 mg/kg in normal rats. Oral administration of fractions of the plant significantly reduced the fasting blood glucose level in diabetic rats. Among the fractions, EAKT was found to be more effective. Further, in sub-acute study, EAKT, showed a significant anti diabetic activity by reversal of the altered afore said serum biochemical parameters. The results of the study are substantiating the traditional claim of the roots of *Kyllinga triceps* in the treatment of diabetes with a scope for development of antidiabetic herbal drug from EAKT.

Key words: Antidiabetic activity, *Kyllinga triceps*, Ethyl acetate fraction, Streptozotocin.

INTRODUCTION

Diabetes mellitus is a metabolic disorder due to disturbances in carbohydrate, protein and lipid metabolism causing hyperglycemia with complications such as retinopathy, neuropathy, nephropathy, atherosclerotic vascular disease (Dewanjee *et al.*, 2008). The treatment of diabetes mellitus is based on oral antihyperglycemic agents and insulin. The oral antihyperglycemic agents currently used in clinical practice have

characteristic profiles of serious side effects (Pickup and Williams, 1991). This leads to increasing demand for herbal products because of their effectiveness, minimal side effects in clinical experience and low cost (Valiathan, 1998). Therefore search for safe and more effective agents has continued to be an important area of active research.

Kyllinga triceps is an herb belonging to the family Cyperaceae. It grows in moist places. Fresh juice of the plant is used externally to wash the wounds. It is used in the treatment of, indigestion (Tirkey *et al.*, 2004). Decoction of roots is used in diabetes and to relieve thirst in fevers (Jitendra, 2006). The roots yield oil which is used to promote the action of the liver and relieve pruritus (Kirtikar and Basu, 1987). In

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view of the traditional claim for the roots of *Kyllinga triceps* (KT) in the treatment of diabetes, as the preliminary investigation with methanolic extract of roots of KT (MERK) conducted in our labs showed a significant glucose tolerance in normal rats. Hence an attempt was made to screen the fractions of MERK for antihyperglycemic activity in STZ induced diabetic rats and to identify a safe and efficient bioactive fraction.

MATERIALS AND METHODS

Plant material

The roots of *Kyllinga triceps* were collected in the month of August 2008 from Kakatiya University campus, Warangal, Andhra Pradesh, India, after the authentication of the plant by taxonomist Prof. V .S. Raju of the University. A voucher specimen (KU/UCPSC/No.42) has been deposited in the herbarium of the college, for future reference.

Preparation of the extract

Fresh roots (2 Kg) of the plant were shade dried and powdered. The powdered material was macerated with methanol for a week and filtered. Then the filtrate was concentrated under reduced pressure to yield a semisolid mass, i.e. methanolic extract (MERK). The so obtained MERK was suspended in water and fractionated with toluene, ethyl acetate, 1-butanol in succession. The yield of fractions was found to be 30 g for toluene fraction, 2.5 g for ethyl acetate fraction, and 10 g for 1-butanol fraction.

Animals

Wistar albino rats of either sex weighing 160-180 g were purchased from Mahaveer enterprises, Hyderabad. The animals were housed in a polypropylene cages maintained at $22 \pm 2^\circ\text{C}$, 50-70% humidity with an alternating 12 h light/dark cycle with a provision to have free access to standard food and water provided *ad libitum*. All the experiments on animals were conducted after obtaining permission from Animal Ethical Committee of the Institute.

Drugs and Chemicals

Glibenclamide is a generous gift from Orchid laboratories, Chennai, India. Streptozotocin was purchased from Sigma-Aldrich Company, Germany. Glucose, Serum glutamate pyruvic transaminase (SGPT), Alkaline phosphatase (ALP), total protein, creatinine, triglycerides, total cholesterol levels were studied by 'Merck Micro lab 300' analyzer by using Merck analytical kits. All other chemicals used were of analytical grade.

Acute toxicity study

Acute toxicity study for the extracts was carried out according to the method described in the literature (Litchfield J. T and Wilcoxon F. A., 1949). Toluene fraction (TLKT), ethyl acetate fraction (EAKT), butanol fraction (BLKT) of methanolic extract of roots of KT suspended in 5% gum acacia solution in doses of 100-2000 mg/kg were administered orally to albino rats of either sex. The animals were observed continuously for any change in autonomic 'or' behavioral responses for first few hours and later at 24 h interval for a period of 72 h. At the end of this period, the mortality if any in each group was noted.

Effect of root extract fractions of KT on fasting blood glucose level

Streptozotocin was dissolved in citrate buffer (pH 4.5) and injected to 48 ± 2 h neonatal rats intraperitoneally at a dose of 90 mg/kg. After 8 weeks of streptozotocin administration, the diabetic rats (glucose level > 180 mg/dl) were separated and divided into 8 groups of six animals in each and treated orally in the following manner (Angel et al., 1996). Group I served as diabetic control, received 5% gum acacia, group II served as standard received glibenclamide 10mg/kg, group III & IV, V & VI and VII & VIII received toluene, ethyl acetate, butanol fractions respectively at doses of 50 and 100 mg/kg. Blood samples were collected just before and 2, 4, 6, 8, 12 and 24 h after administration of the test samples and were analysed for blood glucose content by using glucose oxidase method (Trinder, 1969).

Table 1. Effect of root extract fractions of *Kyllinga triceps* on blood glucose level in streptozotocin induced diabetic rats.

Group	Dose	Blood glucose level (mg/dl) at different hours after the treatment						
		0 hr	2 hr	4hr	6hr	8 hr	12 hr	24 hr
Diabetic control	-	212.6±7.2	215.6±6.2	219.5±5.8	220.3±6.4	222.8±7.4	222.1±9.3	225.5±7.1
Glibenclamide	10mg	235.8±8.7	186.0±6.6*	151.8±9.4**	123.8±3.3**	148.5±7.9**	179.5±6.2**	201.8± 8.4
TLKT	50mg	201.1±1.1	164±4.4**	143.8±4.0**	156.8±3.7**	165.8±2.3**	181.5±4.6**	188.6±2.5
TLKT	100mg	204.8±9.0	181.6±5.1*	135.1±2.6**	153.5±7.0**	157.0±5.8**	167.3±8.4**	193.1±6.6
EAKT	50mg	238.3±8.1	192±9.8**	151.8±8.7**	141.6±8.2**	160.8±8.6**	191.6±9.9**	220±5.4
EAKT	100mg	242.3±9.3	194±8.0**	146.1±11.2**	120.5±7.5**	146.3±9.0**	184.8±6.6**	199±4.1
BLKT	50mg	240.0±8.3	233.6±8.2*	200±7.0**	158±10.7**	174±9.1**	193.3±6.4**	226±8.3
BLKT	100mg	245±8.3	225.8±4.3	194±5.2**	145.6±7.9**	170±3.1**	195.1±8.8**	232.5±9.3

Statistically significant * p<0.05, ** p<0.01, ***p<0.001 compared to diabetic control at the respective time point; Data represented as mean±SD. TLKT: Toluene fraction of *Kyllinga triceps*, EAKT: Ethyl acetate fraction of *Kyllinga triceps*, BLKT: 1- Butanol fraction of *Kyllinga triceps*.

Sub acute treatment

The diabetic rats (glucose level > 180 mg/dl) were divided into 3 groups of six animals in each. Group I served as diabetic control received 5% gum acacia, group II served as standard received glibenclamide at a dose of 10mg/kg; group III received EAKT at a dose of 100 mg/kg. The test extract fraction (EAKT) was administered to the animals once in a day for 28 days. During the study period, the body weight of the animals and blood glucose level were recorded after 7, 14, 21 and 28 days of treatment. Cholesterol, triglycerides, insulin, SGPT, ALP, creatinine and total proteins level in serum were estimated at the initial and after the 28 days of the treatment.

Statistical analysis

All the values were expressed as Mean ± SD. The data was statistically evaluated using one way analysis of variance (ANOVA) followed by Dunnetts t-multiple comparison test using Graph pad Prism 3 computer software. P value of 0.05 or less was considered to be significant.

RESULT AND DISCUSSION

Acute toxicity studies

No adverse effects and no mortality of the animals were observed during the period of study, 72 h up to the dose 2000 mg/kg, of all the three fractions [TLKT, EAKT, and BLKT] of methanolic extract of roots of *Kyllinga triceps*. Hence, the two doses i.e. 50 and 100 mg/kg, of the fractions, which are less than that of the effective methanolic root extract dose in OGTT i.e. 200 mg/kg were selected for the study.

Effect of root extract fractions of KT on fasting blood glucose

The results of the study are shown in Table 1. All the three fractions of MERK (TLKT, EAKT, and BLKT) exhibited significant (P<0.05) reduction in fasting blood glucose level at the two test doses i.e. 50 and 100 mg/kg b.w. after 2 h of administration and continued the effect up to 12 h. TLKT at both the test doses exhibited maximum reduction in blood glucose level after 4 h (30.3%, 34.5%) whereas EAKT and BLKT showed the same effect after 6h of administration 41.6.0%, 50.0% and 34.1%, 40.8%,

Table 2. Effect of EAKT on body weight and blood glucose level in streptozotocin induced type 2 diabetic rats (sub acute study).

Group	Body weight (g)					Blood glucose (mg/dl)				
	Days of treatment					Days of treatment				
	1	7	14	21	28	1	7	14	21	28
Diabetic control	150.3 ±4.0	146.8 ±2.9	142.2 ±2.5	142.2 ±1.8	140 ±1.2	202.1 ±8.6	209.17 ±12.4	217.8 ±11.2	241.1 ±9.6	258.0 ±13.4
Glibenclamide (10mg/kg)	155.4 ±5.5	160.5 ±5.2	161.0 ±5.6	161.6 ±5.0	164.0 ±4.6*	212.6 ±7.2	168.4 ±1.7*	148.8 ±6.9**	131.0 ±14.0**	116.5 ±7.6**
EAKT (100mg/kg)	155.5 ±6.6	157.1 ±6.2	161.1 ±4.6	166 ±4.6	169.6 ±1.7*	225.8 ±4.3	185.5 ±34.9*	168.3 ±2.9**	154.6 ±3.9**	126.0 ±6.3**

Statistically significant * $p < 0.05$, ** $p < 0.01$ compared to diabetic control at the respective time point; Data represented as mean \pm SD. EAKT: Ethyl acetate fraction of *Kyllinga triceps*

respectively. However, among the three fractions EAKT at 100 mg/kg was found to be more effective ($P < 0.01$) in reducing the fasting blood glucose level in STZ induced diabetic rats, which is well comparable to that of the effect of reference drug, glibenclamide (10 mg/kg, $p < 0.01$).

Effect of EAKT on different parameters in sub-acute study (28 days)

Body weight

There was a gradual diminution in body weight of animals in diabetic control group. The animals of extract (EAKT) treated and reference drug treated groups showed a gradual increase in the body weight after 7 days of treatment. The increase in the body weight was observed till the end of the study (28 days). The significant ($P < 0.01$) effect of EAKT (100mg/kg) on body weight of the animals was comparable to that of the reference drug, glibenclamide (10 mg/kg). The results are shown in Table 2.

Bio chemical Changes

EAKT and reference drug, lowered the blood glucose level ($p < 0.01$) gradually after 7 days of the treatment and continued the effect up to the end of the study ($p < 0.01$). The maximum effect was observed after 28 days showing 44% reduction in blood glucose level. The significant antihyperglycemic effect of the fraction was well comparable to that of the reference drug, Glibenclamide (45.2% reduction) 10mg/kg at

each time interval of the study. The results are shown in Table 2.

EAKT at 100 mg/kg showed a significant ($p < 0.01$) effect on cholesterol, triglycerides, SGPT, ALP and creatinine level in serum by reducing their elevated level while increasing the diminished serum insulin and total protein levels in STZ diabetic rats, and it was comparable to that of the reference drug. The results are shown in Table 3.

The results of the study indicate that all the three root extract fractions (TLKT, EAKT, BLKT) of KT are non toxic up to a dose of 2000 mg/kg and have significant ($p < 0.01$) anti hyperglycemic activity in STZ induced diabetic rats. Among the three fractions, EAKT at 100 mg/kg b.w. was found to have significant ($p < 0.01$) effect on fasting blood glucose level after 6h of administration. This effect was well comparable with that of glibenclamide (10 mg/kg), the reference hypoglycemic drug of sulphonylurea type (Rang *et al.*, 1995). As the treatment of 48 h old rats with STZ produces a relatively moderate increase and decrease in fasting blood glucose level and insulin level respectively i.e. a rat model of type2 diabetes (Abdel-Zaher *et al.*, 2005), the antihyperglycemic effect of all extracts in the study could be explained in terms of potentiating glucose induced insulin secretion.

In the sub acute study, administration of EAKT at 100 mg/kg brought about beneficial changes

Table 3. Effect of EAKT on serum biochemical parameters in streptozotocin induced type 2 diabetic rats (Sub acute study).

Group	Serum Cholesterol (mg/dl)		Triglycerides (mg/dl)		SGPT(IU/L)		ALP(IU/L)		Creatinine (mg/dl)		Insulin (μ IU/ML)		Total protein (mg/dl)	
	1 st day	28 th day	1 st day	28 th day	1 st day	28 th day	1 st day	28 th day	1 st day	28 th day	1 st day	28 th day	1 st day	28 th day
Diabetic control	131.73 \pm 4.5	122.4 \pm 6.7	168.5 \pm 12.6	170.5 \pm 10.6	77.36 \pm 2.0	79.4 \pm 4.5	301.7 \pm 15.1	350.9 \pm 11.2	1.9 \pm 0.15	2.26 \pm 0.3	8.98 \pm 0.4	8.6 \pm 0.5	4.3 \pm 0.4	4.2 \pm 0.4
Glibenclamide	110.83 \pm 10.4	83.0 \pm 5.2**	168.6 \pm 9.5	131.0 \pm 8.4**	72.6 \pm 0.3	26.13 \pm 3.9**	294.5 \pm 13.0	162.5 \pm 13.8**	2.2 \pm 0.42	1.3 \pm 0.2**	8.8 \pm 0.9	16.9 \pm 0.9**	5.6 \pm 0.54	7.5 \pm 0.5**
EAKT	157.0 \pm 7.6	113.3 \pm 3.2**	170.0 \pm 6.1	120.8 \pm 5.0**	73.0 \pm 3.5	48.1 \pm 5.7**	305.6 \pm 7.8	204.0 \pm 7.8**	2.08 \pm 0.5	1.1 \pm 0.4**	8.7 \pm 0.6	15.3 \pm 1.1**	4.1 \pm 0.7	7.0 \pm 0.6**

Statistically significant * $p < 0.05$, ** $p < 0.01$ compared to diabetic control at the respective time point; Data represented as mean \pm SD. EAKT: Ethyl acetate fraction of *Kyllinga triceps*.

on body weight as well as on different serum biochemical parameters. A significant improvement in body weight indicates the ability of EAKT to prevent loss of body weight in diabetic rats. (Xie *et al.*, 2003) As the effect was quite similar with that of reference drug, glibenclamide, it can be said that EAKT do not have any effect on degradation of depot fat and it can maintain the bodyweight. This effect of EAKT could be due to its ability to reduce hyperglycemia. The significant ($p < 0.01$) antihyperglycemic effect of EAKT observed in this study was supported by significant changes in other serum parameters such as decrease in cholesterol, triglycerides, SGPT, ALP, creatinine and increase in insulin and total protein.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in streptozotocin induced diabetic rats. In insulin deficient subjects, it fails to activate the enzyme lipoprotein lipase and causes hypertriglyceridemia (Abdel-Zaher *et al.*, 2005). Hence, it is possible that the mechanism of reduction of serum lipid levels with EAKT may be through insulin release or by enhancing insulin sensitivity in the tissues, which was also evident from the increase in serum insulin level. The effect of EAKT was similar as that of the reference drug, glibenclamide a insulin secretagogue (Sharma *et al.*, 1997) indicating that the antihyperglycemic effect of EAKT may

be due to its stimulatory effect on insulin secretion. The diminution of serum GPT, ALP and creatinine level with EAKT further strengthens the antidiabetogenic effect of EAKT as the hepatoprotective enzymes (SGPT, ALP) and creatinine levels are elevated in STZ induced diabetic rats (Ghosh and Suryawanshi, 2001) due to damage to the structural integrity of the liver and degenerative condition in the kidney (Nair *et al.*, 2007).

CONCLUSION

The results of the study revealed that the roots of *Kyllinga triceps* are endowed with antidiabetic properties, which substantiates the traditional claim of the roots for treatment of diabetes. The ethyl acetate fraction of methanolic extract of roots of *Kyllinga triceps* (EAKT) has antihyperglycemic potential without any toxic effects and thus it could be beneficial in the management of type 2 diabetes. However detailed chemical and biological studies on EAKT are needed to isolate and characterize the biologically active principles with their mechanism of action for developing *Kyllinga triceps* to be an effective and safe antidiabetic drug.

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