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PHARMACOLOGICAL SCREENING OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTS OF JUSTICIA TRANQUEBARENSIS ON STREPTOZOCIN INDUCED RATS.

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Abstract

Non-communicable diseases like diabetes mellitus are becoming major health problem these days. There is an increasing demand by patients to use the natural products (plants) with anti-diabetic activity due to the severe side effects associated with the use of insulin and oral anti-diabetic drugs. One such plant is *Justicia tranquebareinsis* which has been traditionally used for the treatment of diabetes mellitus. The aim of this study was, therefore, to evaluate hypoglycemic activity of ethanol extract of *Justicia tranquebareinsis* in streptozotocin-induced diabetic rats, respectively and to carry out acute toxicity test. Two doses (200 mg/kg and 400 mg/kg) of the ethanol extract of *Justicia tranquebareinsis* were administered to normal glucose loaded and diabetic rat to study blood glucose lowering effect. Ethanol extract of *Justicia tranquebareinsis* (200mg/kg and 400mg/kg, p.o.) showed significant tolerance ($P < 0.05$) to oral glucose load at 1 and 2 hrs after glucose load. The extract also produced significant ($P < 0.05$) blood glucose reduction at 4 hours after its administration in normoglycemic mice. The extract at 400mg/kg dose level produced significant ($P < 0.05$) reduction in blood glucose level at 2, 3 and 4 hours of treatment in streptozotocin (45 mg/kg) induced diabetic mice. Acute oral toxicity studies of ethanol extract of *Justicia tranquebareinsis* in rats showed no death or signs of toxicity at the dose of 2000 mg/kg indicating the safety nature of the extract. The results of the experiments showed that ethanol extract of *Justicia tranquebareinsis* has significant antihyperglycemic activity in streptozotocin induced diabetic rat and improvement in glucose tolerance and slight hypoglycemic activity in normal rat justifying the traditional claim for its use in diabetes.

Keywords: *Justicia tranquebareinsis*, Streptozotocin, hypoglycemic and antihyperglycemic activities.

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Introduction

Diabetes mellitus is a major global health problem. World health organization (WHO) estimated the number of cases of diabetes worldwide to be about 347 million [1]. Most common cause for this abnormality is obesity and the associated metabolic syndrome [2, 3]. WHO projects that diabetes will be the 7th leading cause of death in 2030.

Diabetes exerts a significant burden in developing countries especially in Sub-Saharan region, and this is expected to increase due to economic growth, adoption of 'western' lifestyles and their accompanying risk factors – smoking, high-fat diets, lack of exercise [4]. Many diabetic patients face significant challenges accessing diagnosis and treatment, which contributes to the high mortality and prevalence of complications observed [5]. In Ethiopia, prevalence of diabetes is increasing and estimated to be 2% nationally related to lifestyle changes and the resulting surge in obesity [6]. Estimated number of cases of diabetes in Ethiopia to be 1,386.64 and more than one-third, but only less than half, of diabetic patients receive standard diabetes care [6,7]. A large number of plants have proved their efficacy in the management of diabetes especially hyperglycemia [8,9]. For example, the popular hypoglycemic drug glucophage (metformin) is derived from *Galega officinalis* [10]. Keeping this in view, the present study aimed to investigate the phytochemical analysis and anti-diabetic activity of *Justicia tranquebariensis* extract.

Materials and Methods

Collection of plant material

The plant powder of *Justicia tranquebariensis* was collected from Chittoor District, Andhra Pradesh, India.

Preparation of plant extract

The dried leaves were ground into powder and sieved. The obtained powder was stored in a tightly closed amber-colored bottle. The powdered plant material was divided into six equal parts with solvents Petroleum Ether, Ethanol and Distilled water. The mixtures were filtered and the filtrates were subjected to evaporation using water bath to remove the solvents. The dried extracts were used for phytochemical screening. Based on *Justicia tranquebareinsis* ethanol solvent was selected for further tests for anti-diabetic activity study.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Trease and Evans (1989) and Harborne (1973) [11, 12].

Acute oral toxicity study

Acute oral toxicity test was performed as per OECD (Organization for Economic Cooperation and Development) guideline-425 (limit test) [13, 14]. Five female albino rats (one animal in each step) were randomly selected. The animals were kept fasting for 4 hours providing only water. Aqueous extract of *Justicia tranquebariensis* were administered orally at a dose of 2000 mg/kg. The rats were observed continuously for the first 4 hrs and then periodically up to 24 hrs for toxic manifestations like: drowsiness, restlessness, writhing, convulsion, piloerection and mortality if any. The rats were observed for two weeks.

Anti-diabetic activity of Ethanolic extract of *Justicia tranquebariensis* on streptozotocin-induced diabetic rats:

Male Albino rats (n=6) of 5 groups of control, positive control, negative control, test 1&2. Group 1(control) treated with Normal saline; Group 2 (Positive control) treated with Streptozotocin induced diabetes; Group3 (Negative control) treated with Metformin. Group 4 and 5 identified as Test 1 & 2 treated with 200 and 400mg/kg of ethanolic extracts of *Justicia tranquebareinsis* along with the inducing agent as STZ.

Blood glucose concentration in diabetic rats

The blood glucose concentration (mg/dl) was measured at 0 to 6 days after the treatment with ethanolic leaves and roots extracts of *Justicia tranquebareinsis*. At the end of 6 days, animals were deprived of food for overnight and blood samples were collected via tail vein for measurement of blood glucose levels [15, 16].

Results and Discussion

Qualitative Phytochemical screening

The qualitative phytochemical screening of various extracts of Justicia tranquebareinsisis shown in Table 1. In qualitative screening, the phytochemical analysis was performed, which confirmed the presence of alkaloids, glycosides, flavonoids, phenols, and tannins.

Table 1. Phytochemical analysis of various extracts of *Justicia tranquebareinsis*

S.No.	Phytoconstituents	PEJT	EEJT	AEJT
1	Alkaloids	-	+	+
2	Glycosides	-	+	+
3	Carbohydrates	-	+	+
4	Steroids	+	-	-
5	Proteins	-	+	+
6	Flavonoids	+	+	+
7	Saponins	-	+	+
8	Tannins	-	+	+
9	Triterpenoids	-	-	-
10	Fixed oilsandfats	-	-	-
11	Phenols	-	+	+

'-' Absent,'+' Present

PEJT – Petroleum ether extract of Justicia tranquebareinsis; EEJT – Ethanolic extract of Justicia tranquebareinsis; AEJT – Aqueous extract of Justicia tranquebareinsis

Acute Toxicity Studies

This study helps us to determine the therapeutic index and the extract was confirmed to be safe. Acute toxicity study was performed as per the OECD guidelines. The extracts were administered orally at a dose of 50, 300, 500, 1000 and 2000mg/kg b.w. in 0.5% carboxy methyl cellulose (CMC). No gross observational changes were recorded during the period of 14 days observation. The results are tabulated in Table 2.

Table 2. The effects of ethanolic extract of Justicia tranquebareinsis (EEJT) on general behavioral observation in acute toxicity studies

S.No.	General Behavior	Observation after drug administration
1	Sedation	-
2	Hypnosis	-
3	Convulsion	-
4	Stupar reaction	-
5	Change in Motor activity	-
6	Muscle relaxant	-
7	CNS stimulant	-
8	CNS depressant	-
9	Change in Skin color	-
10	Lacrimation	-

'-' Absent,'+' Present

Antidiabetic Activity of ethanolic extract of *Justicia tranquebareinsis*

The blood glucose level was increased significantly in Streptozotocin (STZ) treated group when compared to control group. The STZ induced rats were treated with the

ethanolic extract of *Justicia tranquebareinsis* (EEJT) 200mg/kg/p.o and 400mg/kg/p.o for the duration of 21 days. Treatment with ethanolic extract of *Justicia tranquebareinsis* at the dose of 200 mg/kg/p. o showed marginal reduction in the blood glucose level in the second week of treatment. Treatment with ethanolic extract of *Justicia tranquebareinsis* at the dose of 400mg/kg/p.o. showed significant decrease in the blood glucose level from first week ($p<0.01$), which further reduced in the second and third weeks ($p<0.001$), respectively. Treatment with Glibenclamide (Standard drug) (5mg/kg b.w/ p.o) produced a significant ($p<0.001$) decrease in blood glucose level from first week to third week. The results are tabulated in Table 3.

Table 3. Effect of ethanolic extract of *Justicia tranquebareinsis* on blood glucose levels in STZ induced diabetic rats

Groups Treatment/Dose	0 day (mg/dL)	After 3 days (mg/dL)	After 7 days (mg/dL)	After 14 days (mg/dL)	After 21 days (mg/dL)
Normal control	97.32± 1.32	95.61± 0.96***	96.18± 1.45***	96.64± 1.34***	98.82± 0.98***
Diabetic Control (STZ)	265.18± 2.74	281.16± 1.48	271.22± 2.64	219.47± 2.46	209.24± 1.98
EEJT (200mg/kg b.w.)	264.16± 2.81	247.94± 2.13**	208.27± 3.16**	176.61± 3.37**	130.46± 2.96**
EEJT (400mg/kg b.w.)	260.49± 3.72	221.62± 3.64**	160.65± 2.69***	130.60± 2.99***	110.79± 3.16***
Standard Glibenclamide (5mg/kg b.w.)	253.13± 3.19	200.22± 3.17***	152.53± 2.89***	118.84± 2.76***	98.26± 3.14***

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01, ***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnett's'test.

Estimation of liver enzymes

The effect of ethanolic extract of *Justicia tranquebareinsis* at doses of 200 & 400 mg/kg b.w. on liver enzymes. It was found that the ethanolic extract of *Justicia tranquebareinsis* showed significant ($p<0.001$) protection on liver parameters. The ethanolic extract of *Justicia tranquebareinsis* has not elevated the normal liver profiles (AST, ALP and ALT) on dose dependent manner. The standard drug Glibenclamide (5mg/kg/p. o) showed significant ($p<0.001$) protection in AST, ALP, ALT when compared STZ induced diabetic animals. The results are tabulated in Table 4.

Table 4. Effect of Ethanolic extract of *Justicia tranquebareinsis* on liver profile on STZ induced diabetic rats.

S.No.	Groups Treatment/Dose	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
1	Normal control	89.25± 7.32***	83.65± 7.26***	25.42± 1.45***
2	Diabetic control	168.86± 9.12	161.82± 9.17	128.57± 7.82
3	EEJT (200mg/kg b.w.)	121.56± 8.32*	87.41± 7.20**	44.81± 2.46*
4	EEJT (400mg/kg b.w.)	100.89± 7.86**	89.28± 6.77***	34.58± 2.92**
5	Standard Glibenclamide (5mg/kg b.w.)	85.84± 6.16***	89.75± 6.74***	28.86± 1.72***

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01, ***p<0.001Vs control. Statistical significance test for comparison was done by one way ANOVA followed by Dunnett's'test.

Estimation of renal profile

The serum urea level was significantly ($p<0.001$) increased in STZ induced diabetic rats when compared to control rats. Serum urea level of diabetic rat treated with ethanolic extract of *Justicia tranquebareinsis* [EEJT] 200 mg/kg/p.o and 400 mg/kg/p.o showed significant decrease ($p<0.001$) and ($p<0.001$) in serumurea level when compared to STZ induced diabetic rat. Glibenclamide (5 mg/kgb.w/p.o) treatment showed significant ($p<0.001$) decrease in serum urea when compared to STZ induced diabetic rats. The results are tabulated in Table 5.

Table 5. Effect of ethanolic extract of *Justicia tranquebareinsis* on renal profile in STZ induced diabetes rat

S.No	Groups Treatment/Dose	Serum Creatinine (mg/dl)	Blood Urea (mg/dl)
1	Normal control	1.17± 0.18***	25.83± 2.36***
2	Diabetic control	2.48± 0.26	41.24± 2.71
3	EEJT (200mg/kg b.w.)	1.52± 0.13**	30.71± 2.74*
4	EEJT (400mg/kg b.w.)	1.31± 0.19***	27.76± 2.41**
5	Standard Glibenclamide (5mg/kg b.w.)	1.37± 0.20***	23.84± 2.18***

Estimation of lipid profile

The serum total cholesterol, triglyceride, LDL, VLDL level was significantly increased where as HDL was significantly decreased in STZ induced diabetic rat when compared to

control rats. Serum total cholesterol, triglyceride, LDL, VLDL level of diabetic animal treated with ethanolic extract of *Justicia tranquebareinsis* [EEJT] 200 mg/kg/p.o and 400 mg/kg/p.o showed significant decrease ($p<0.001$) and HDL level of diabetic rats treated with EEJT showed significant increase ($p<0.01$), when compared to STZ induced diabetic animals. Glibenclamide (5 mg/kg/p.o) also showed a significant decrease ($p<0.001$) in serum total cholesterol, triglyceride, LDL, VLDL level and HDL was significantly increased when compared to STZ induced diabetic rat. The results are tabulated in Table 6.

Table 6. Effect of ethanolic extract of *Justicia tranquebareinsis* on lipid profile in STZ induced diabetic rats

S.No.	Groups Treatment/Dose	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	Normal control	66.58± 1.85***	34.42± 1.82***	13.65± 0.72***	59.72± 3.26***	6.75± 0.32***
2	Diabetic control	77.51± 1.46	44.21± 2.36	12.31± 0.85	74.35± 2.84	9.59± 0.20
3	EEJT (200mg/kg b.w.)	71.84± 1.57**	36.43± 1.84**	15.26± 0.72*	64.45± 3.42**	7.98± 0.38*
4	EEJT (400mg/kg b.w.)	68.28± 1.68***	35.43± 2.31***	14.18± 0.81***	63.45± 2.78***	7.19± 0.42**
5	Standard Glibenclamide (5mg/kg b.w.)	67.51± 3.33***	35.74± 2.72***	14.40± 0.86***	63.35± 2.84***	7.40± 0.38***

Conclusion

The plant, *Justicia tranquebareinsis* was selected for the evaluation of antidiabetic potential in STZ induced diabetic rat model. The dried plant material was extracted by hot percolation using ethanol as a solvent in Soxhlet apparatus and the preliminary phytochemical screening was performed and it shows that the ethanolic extract of *Justicia tranquebareinsis* contained alkaloids, glycosides, flavonoids, phenols, and tannins.

Safety profile is essential for the drugs obtained from the plant origin [17]. The level of toxicity can be evaluated by toxicological studies. From the results it was concluded that the ethanolic extract of *Justicia tranquebareinsis* showed no changes in the general behavior of the experimental animals and showed no toxic effect up to a dose of 2000mg/kg bow

The ethanolic extract of *Justicia tranquebareinsis* exhibited significant anti diabetic effect in a dose dependent manner which was comparable to the standard drug Glibenclamide. The result also showed significant decrease in the liver Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine amino transferase (ALT), serum urea level, cholesterol, triglycerides, VLDL and LDL in STZ induced diabetic animals when compared to control group. It is concluded that the ethanolic extract of *Justicia tranquebareinsis* showed significant anti-diabetic effect in STZ induced diabetic rats. Further studies are necessary to examine the underlying mechanism of hypoglycemic effect and to isolate the active compound (s) responsible for antidiabetic activity.

Conflicts of interest

The authors declare no conflicts of interest.

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Both are contributed equally

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