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EFFECTS OF *JUSTICIA GENDARUSSA* LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Keywords:

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ABSTRACT: *Justicia gendarussa* belongs to the Acanthaceae family. The leaves and young shoots are anti-periodic, antispasmodic, cardiotonic, carminative, diaphoretic, emetic and febrifuge. The presence of phytochemicals such as tannins, alkaloids, proteins, flavonoids, triterpenes and saponins in the ethanolic extract was revealed by the preliminary phytochemical screening, keeping in view of medicinal importance the present study is an effort to investigate the biochemical parameters of *Justicia gendarussa* leaves in streptozotocin-induced diabetic rats for 28 days. The acute toxicity studies of oral doses of ethanolic leaves extract in rats revealed that it has a high safety profile, as the extract/fractions were well tolerated by the animals. The test drug was administered for 28 days at a different dose level 50 mg/kg, and 100 mg/kg for ethanolic extract was given orally. Bodyweight urine sugar was analyzed before and after the treatment of extract, while serum glucose was analyzed every week. The lipid and lipoprotein profile from serum was analyzed after 28 days. The ethanolic extract of *Justicia gendarussa* leaves significantly prevented the loss of body weight and reduce urine sugar. The results indicated that the ethanolic extract produced significant change ($p < 0.001$) in biochemical parameters.

INTRODUCTION: Diabetes Mellitus is the commonest form of diabetes constituting 90% of the diabetic population. The countries with the largest number of diabetic patients in the year 2025 will be India, China and the United States. Therefore, it has become necessary to look for novel oral therapeutically effective treatment especially for usage in the developing as well as under-developed countries. India is a country with a vast reserve of natural resources and a rich history of traditional medicine.

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Ethno pharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity [1, 3]. *Justicia gendarussa* belongs to Family: Acanthaceae, traditionally used in the treatment of chronic rheumatism.

An infusion of the leaves is taken internally in the treatment of a wide range of conditions including pains in the head, paralysis of one side of the body and facial paralysis; lumbago, amenorrhoea, swellings, fevers, coughs, asthma, colics, eczema, cephalalgia, hemiplegia, facial paralysis, earache, and hemicranias. However, to the best of our knowledge and based on the citations of the use, the purpose of the present study was to evaluate the biochemical parameters of the ethanolic leaves extract of in streptozotocin-induced diabetic rats [2].

MATERIALS AND METHODS:

Plant Material: The fresh leaves of *Justicia gendarussa* were collected during January 2019, from the village of Nilgiris, Ooty, Tamil Nadu. The plant species were identified and authenticated by taxonomist Dr. S. Rajan, Field Botanist, Medicinal Plant Collection and Survey. The Unit, Department of Ayush, Emerald, Ooty.

Laboratory Animals: Healthy, adult Wistar rats of both sexes (180-220 g) were selected for the present study. Animals were maintained under standard laboratory condition and the experimental study was approved by Institute Animal Ethics Committee, and all the animal experiments were carried out as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India. Approval No: XII/ GSMCOP/ PCOL/ 03/ CPCSEA/ IAEC/ 18.8.2019)

Preparation of Extract: The collected fresh plant materials were dried in shade for two days and then dried in a hot air oven at 25 ºC for three days, and they were made into coarse powder with the use of mixer grinder. The powder of leaves of *Justicia gendarussa* obtained was weighed separately and transferred to a round-bottomed flask and then went with Soxhlet extraction using 95% ethanol for 24 h. Then the extract of ethanol was concentrated, and then the marc was stored for fractionation [4].

Preliminary Phytochemical Studies: The preliminary phytochemical screening of extract was performed to identify the presence of alkaloids, proteins, triterpenoids, saponins, flavonoids, and tannins.

Acute Toxicity Study: The study was carried out according to the OECD guidelines 425. Female Wistar rats of weight (180-220 g) were taken for the study and kept for overnight fasting. The next day, bodyweight was taken, and standardized *Justicia gendarussa* leaves extract and fractions were administered orally at a dose of 2000 mg/kg in distilled water.

Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12, and 24 h. Feed was given to the animals after 4 h of the dosing, and the body weight was checked at 6 h after dosing. Morbidity like convulsions, tremors, grip strength, and pupil dilatation were observed. The animals were observed twice daily for 28 days and body weight was taken [5, 6].

Preparation and Induction of Type 2 Diabetes in Rats by Streptozotocin: The Streptozotocin was made at a final concentration of 50mg/kg body weight by dissolving in citrate buffer (pH 4.5) the solution was then kept refrigerated overnight to facilitate its dissolution. Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50 mg/kg streptozotocin. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h.

The rats with permanent non-insulin-dependent diabetes mellitus (NIDDM) (250-350 mg/dL) were used for the study [6].

Experimental Design: The Wistar rats weighing 180-220 g of either sex were used for the experimental study. The animals were divided into seven groups of six animals each.

Grouping of the Animals:
Group I: Normal

Group II: Control

Group III: Positive Control (Diabetic + Glibenclamide 10 mg/kg I.P).

Group IV: EtOH extract of *Justicia gendarussa* (50 mg/kg, orally).

Group V: EtOH extract of *Justicia gendarussa* (100 mg/kg, orally).

The extract was administered for 28 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100 mg/kg each of two successive fractions (chloroform and n-butanol) made in aqueous and given by orally. The blood was collected by sinuous orbital under light diethyl ether anesthesia.

The blood was centrifuged at 3000 rpm for 10 min. Body weight, urine sugar was analyzed before and after treatment of extract/fractions, while serum glucose was analyzed every week, and lipid and lipoprotein profile from serum was analyzed after 28 days [7].

Analytical Methods:

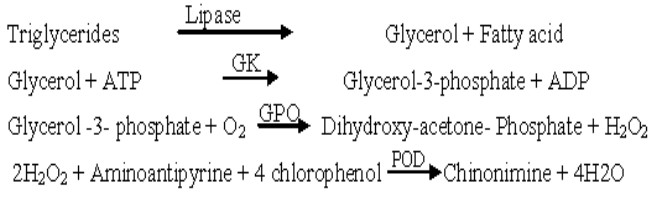
Determination of Body Weight and Urine Analysis: Bodyweight, urine sugar were analyzed before and after the treatment of extract/fractions. Urine sugar was estimated by using Reagent strips from Diastix (2802B).

Estimation of Serum Glucose: Glucose estimation in serum was assayed by using the Ecoline diagnostic kit. Glucose content is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinone-imine dye as an indicator. Measured the absorbance of the sample and standard against the reagent blank value at 500 nm. The glucose level in serum was expressed as mg/dL [8].

Estimation of Lipids and Lipoprotein Profile:

Total Cholesterol (TC): Cholesterol in serum was estimated by using an Ecoline Diagnostic Kit. Cholesterol and its esters were released from lipoproteins by detergents. Cholesterol esterase hydrolysis the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 was formed. This was converted into a colored quinoneimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase. The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. Cholesterol level in serum was expressed as mg/dL [8, 9].

Triglycerides (TG): Triglyceride level in serum was estimated using an Ecoline Diagnostic Kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. The triglyceride level in serum was expressed as mg/dL.



HDL (High-Density Lipoprotein) Cholesterol: The HDL cholesterol was separated from serum after precipitation of LDL and VLDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using the Ecoline diagnostic kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. HDL Cholesterol level in serum was expressed as mg/dL [10].

LDL (Low-Density Lipoprotein) Cholesterol: LDL Cholesterol was calculated by using the formula

LDL Cholesterol = Total Cholesterol – [HDL Cholesterol – Triglucerides / 5]

LDL Cholesterol level in serum was expressed as mg/dL.

VLDL (Very Low-Density Lipoprotein) Cholesterol: VLDL Cholesterol was calculated by the formula

VLDL Cholesterol = (Triglycerides) / 5

VLDL Cholesterol level in serum was expressed as mg/dL.

Statistical Analysis: Evaluation of statistical significance results by computer-aided program and systemic documentation. The value was presented as mean ± SEM. Data were analyzed using analysis of variance (ANOVA) and group means were compared with bonferroni multiple comparisons test using instant graph pad and prism software.

RESULTS:

Effect on Body Weight and Fluid Intake: Gradual increase in body weight in untreated control while the diabetic control continues to lose the weight However treated diabetic group gained 16%, 11%, 16%, 15% as compared to diabetic control and body weight of diabetic treated towards normal range ($P<0.001$). On the other hand, the administration of ethanolic extract/fractions. Ethanol (EtOH) (100 mg/kg and 200 mg/kg b.w.), of *Justicia gendarussa* decrease urine sugar level respectively. In diabetic control, animal urine sugar remaining that +4 level, but there was a decrease of 2% to 3% in urine sugar in case of treated diabetic rats. The change in body weight and urine sugar in all group of animals were given in **Table 5**.

Effect of *Justicia gendarussa* on Serum Glucose: The initial blood glucose levels of the diabetic rats selected for the study wherein the range of 240-300

mg/dL. In the untreated control (diabetic) rats, the blood glucose level increase to 379 mg/dL on the 7th day the glucose levels on the 14th and 21st days of the animals, which survived were 410 mg/dL respectively. In the *Justicia gendarussa* treated rats, the blood glucose level suddenly decreased (P<0.001, P<0.01); thus, the *Justicia gendarussa* treatment restore the serum glucose levels almost nearer to a normal value and comparable to that of positive control (P<0.001). The changes in serum glucose estimation in all groups of an animal were given in **Table 6**.

Serum Lipid Profile and Lipoprotein Profile: Effect of extract of *Justicia gendarussa* on the control and experimental animals. STZ diabetic rats group were found to have significantly increased HDL, LDL, VLDL, TG, TC, levels as compared to the control group (P<0.001, P<0.01) HDL cholesterol was also reduced significantly in diabetic rats after treatment of ethanolic extract of *Justicia gendarussa*.

The positive control was significantly preventing the increasing the serum TC, TG, HDL, LDL, VLDL, as compared to the diabetic group. The diabetic treated group was significantly increased in HDL cholesterol levels as compared to diabetic group. (P<0.001, P<0.01). Thus, the *Justicia gendarussa* treatment restores all these changes near to normal value. The change in serum lipid and lipoprotein profile was tabulated in **Table 7**.

TABLE 5: EFFECT OF ADMINISTRATION OF FEEDING, THE ETHANOLIC EXTRACT OF *JUSTICIA GENDARUSSA*, LEAVES ON BODY WEIGHT AND URINE SUGAR ANALYSIS IN NORMAL AND DIABETIC RATS FOR 28 DAYS

S. no.	Group	Body Weight (g)		Urine sugar	
		Before treatment	After treatment	Before treatment	After treatment
1	Normal	195 ± 1.87	221.5 ± 1.840	Nil	Nil
2	Control	201.76 ± 2.23	168.5 ± 2.433###	+4	+4
3	Diabetic + Glibenclamide (10 mg/kg)	206.66 ± 1.745	222.43 ± 1.86***	+4	+1
4	Diabetic + EtOH (50 mg/kg)	207 ± 1.926	219.17 ± 1.077***	+4	+2
5	Diabetic + EtOH (100 mg/kg)	198 ± 2.176	214.5 ± 1.476***	+4	+1

All values are expressed as mean ± S.E.M (n = 6). ***P<0.001 as compared to diabetic control. ###P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests

TABLE 6: EFFECT OF ADMINISTRATION OF FEEDING THE ETHANOLIC EXTRACT OF *JUSTICIA GENDARUSSA* LEAVES ON SERUM GLUCOSE ESTIMATION IN NORMAL AND DIABETIC RATS

S. no.	Group	Serum glucose (mg/dL)				
		0 day	7th day	14th day	21th day	28th day
1	Untreated control	84.83 ± 5.41	85.63 ± 5.87	84.66 ± 5.77	84.83 ± 5.09	84.73 ± 5.07
2	Diabetic control	297.16 ± 17.18	357.53 ± 4.7##	413.83 ± 16.61###	410 ± 2.045###	408 ± 2.039###
3	Diabetic + Glibenclamide (10 mg/kg)	281.23 ± 2.43	205.33 ± 1.145**	165 ± 1.29***	114.83 ± 1.302***	112.83 ± 1.107***
4	Diabetic + EtOH (50 mg/kg)	279 ± 7.57	225.45 ± 1.195**	164.66 ± 1.406***	116.5 ± 1.232***	113.5 ± 1.210***
5	Diabetic + EtOH (100 mg/kg)	283.56 ± 4.49	223.73 ± 1.194**	164.16 ± 1.406***	115 ± 0.966***	111.5 ± 0.879***

All value is expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01 as compared to diabetic control. ##P<0.01, ###P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests

TABLE 7: EFFECT OF ETHANOLIC EXTRACTS ON SERUM LIPID AND LIPOPROTEIN PROFILE IN STREPTOZOTOCIN INDUCED RATS

S. no.	Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
1	Untreated control	86.5 ± 0.73	54.83 ± 1.138	54.80 ± 0.693	19.28 ± 0.46	8.678 ± 0.41
2	Diabetic control	155.58 ± 0.70###	184.5 ± 10.69###	21.33 ± 0.44###	37.73 ± 6.75###	38.40 ± 2.76##
3	Diabetic + Glibenclamide (10 mg/kg)	68.93 ± 2.71***	115.95 ± 5.21***	32.61 ± 2.32***	16.29 ± 1.29***	24.87 ± 0.17**
4	Diabetic + EtOH (50 mg/kg)	124..88 ± 0.23***	63.76 ± 1.43***	35.63 ± 3.86***	51.58 ± 1.50***	13.72 ± 0.68**
5	Diabetic + EtOH (100 mg/kg)	126.92 ± 0.21***	68.96 ± 4.53***	37.47 ± 2.675***	53.09 ± 0.77***	13.78 ± 0.70**

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01, as compared to diabetic control ###P<0.001, ##P< 0.01, as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests

DISCUSSION: A major challenge to curing liver, pancreas, and kidney injuries are to find novel chemical entities with less toxicity and greater effectiveness than those used in current chemo-therapy. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease. Insulin and sulphonylurea drugs (glibenclamide) causes hypoglycemia when taken in excessive doses and overt hypoglycemia is the most worrisome effect of this drug. However, Biguanides do not cause hypoglycemia even when taken in excessive doses. One of the Biguanide, Metformin does not stimulate insulin secretion and act by reducing hepatic glucose production through inhibition of gluconeogenesis and to a lesser extent by enhancing insulin sensitivity in the tissues. In the present study, *Justicia gendarussa* extract did not influence serum levels of insulin in diabetic as well as normoglycemic rats. Other parameters of diabetes such as body weight and urine sugar were also affected by treatment with *Justicia gendarussa*. Treatment with *Justicia gendarussa* inhibits the reduction in body weight and urine sugar by diabetes as the treatment altered the body weight and urine sugar of diabetic animals towards normal.

Hyperlipidemia is a recognized complication of Diabetes Mellitus characterized by elevated levels of cholesterol, triglycerides, and changes in lipoprotein composition. The results of our present study clearly show that *Justicia gendarussa* ethanolic extract EtOH (50, 100 mg/kg not only lowered the TC, TG, VLDL and LDL levels but also enhanced the cardioprotective lipid HDL in normal and diabetic rats after 28 days of treatment. In the present study, the extract not only decreases the TC level but also enhances the HDL cholesterol significantly.

High level of triglycerides and more importantly LDL cholesterol is a major cause of coronary risk factors. Administration of leaves of extract to diabetic rats for 28 days lowered TC and LDL cholesterol levels respectively. In the present study, we conclude that *Justicia gendarussa* leaves demonstrated that the ethanolic extract decreased blood glucose, improve lipid metabolism and maintain body weight and urine sugar in STZ induced diabetic rats.

CONCLUSION: The present study reveals that investigation of the biochemical parameters of *Justicia gendarussa* leaves in streptozotocin-induced diabetic rats is proved by a significant change in biochemical parameters.

Further, works are being carried out to isolate and identify the active principle involved in the anti-diabetic activity of plant extracts.

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CONFLICTS OF INTEREST: There is no conflicts of interest during this research.

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