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EVALUATION OF ANTI-DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *TINOSPORA CORDIFOLIA* LEAVES AND *TRIGONELLA FOENUM-GRAECUM* SEEDS IN ALLOXAN INDUCED DIABETES IN ALBINO RATS

P. Vijaya Kumari and V. Thirumurugan*

PG and Research Department of Chemistry, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur (Dt.), Tamil Nadu, India.

***Corresponding Author Email: drv.thirumurugan@gmail.com**

ABSTRACT

The present study aims to find qualitative screening of phytoconstituents and to clarify the effect of ethanol extracts from Tinospora cordifolia leaves (TC) and Trigonella foenum-graecum (TFG) seeds on the the alloxan induced diabetic rats. The experiment included 48 albino rats. They were classified into eight groups each of 6 rats. Two groups served as control (one is normal and other one is diabetic rats) and the diabetic rats of other groups (three-seven) were orally given the doses of ethanolic extract of two study plants for four weeks. Finally, the last group of diabetic rats consumed standard drug Glibenclamide. The chemical analysis included level of blood glucose in addition to the influence of these extracts on various enzymes of glucose metabolism (ALP and chloestrol levels) showed significant decrease in the diabetic group treated with extracts of tested plants when compared with the diabetic group. Both study plants showed significant increase in AST, ALT and Insulin levels of diabeic rats. It is clear from the current data in this study that mixture of TC and TFG extract were the most efficient compared to TC or TFG alone.

KEY WORDS

Albino rats, Antidiabetic activity, Tinospora cordifolia leaves, Trigonella foenum-graecum seeds.

INTRODUCTION

Type-2 diabetes mellitus (T2D) is no doubt a major dilemma in the world today, especially in the way it deteriorates the quality of human life [1]. Moreover, the morbidity and mortality rate is increased continuously in worldwide, it has estimated 135 million people in the world with diabetes and it would rise to 380 million by the year 2025 [2]. In particular hyperglycaemia is the main characteristic feature of diabetes, due to decreased secretion or inefficient action of insulin secreted from β-cells of pancreas. It is thought to contribute various biochemical changes in cellular metabolisms, vascular complications, oxidative stress and alterations in circulating lipoproteins [3]. Commercially available oral hypoglycaemic drugs and insulin are recommended for treatment of diabetes mellitus. But there is a necessity to develop herbal medicine due to undesirable side effects, high cost and safety on long term use [4]. There are several kinds of medicinal plants that exhibit antidiabetic and antioxidant activities, but there is lack of information regarding the biochemical, haematological and safety assessment of many plants in their applications and medicinal uses. This study therefore seeks to investigate the anti-diabetic potential of *Tinospora cordifolia* leaves (TC) and *Trigonella foenum-graecum* seeds (TFG). So, the aim of the present study was to

evaluate preliminary phytochemical screening and to investigate antidiabetic potential in alloxan-induced albino rats. The various biochemical parameters like blood glucose, and serum levels of enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol and insulin level were investigated.

MATERIALS AND METHODS

Preparation of plant extract

The dried plant materials were, pulverized by a mechanical grinder, sieved through 40 meshes. The powdered materials were extracted with ethanol using soxhlet extraction apparatus. This ethanol extract was then concentrated and dried under reduced pressure. The ethanol free semisolid mass thus obtained and used for the experiment.

Phytochemical Evaluation

Phytochemical qualitative screening tests were carried out on the ethanol extract using standard procedures to identify the phytoconstituents [5, 6].

Acute oral toxicity study

The acute oral toxicity study was conducted using test guidelines on acute oral toxicity test 423 according to Organization for Economic Cooperation and Development [7]. A limit dose of 2000 mg/kg body weight was used. The signs of toxic effects and mortality were observed 3 hr. after administration then for the next 48 hr. The body weight was recorded for consecutive 30 days. Since the extracts were found safe up to the dose level of 2000 mg/kg body weight, a dose of 100 & 200 mg/kg body weight of the TC and TFG extracts were selected for screening of the antidiabetic activity.

Antidiabetic activity

Animals used: Wistar Albino rats (150-180 g) were selected for these studies. Six rats were taken for each group. The rats were used after an acclimatization period of seven days to the laboratory environment. They were provided with food and water ad libitum.

Preparation of diabetic rats: Hyperglycaemia was induced by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate (SD fine Chemicals Pvt. Ltd., Biosar) 150 mg /kg body weight (b/w), to overnight fasted rats. Control rats receive similar volume of vehicle, normal saline (2 ml/kg b/w) alone. Animals that did not developed Hyperglycaemia after 48 hr. of alloxan injection were rejected and new animals were used. All the protocols and experiments were conducted in compliance with the ethical principles and guidelines proved by the ethical committee. Ethical approval no is 63/CS/Pharm/ CPCSEA/10/16.

Experimental Design: Eight groups of rats, six in each received the following treatment schedule.

Group I: Normal control (saline).
Group II: Alloxan treated control (150 mg /kg, b/w).
Group III: Alloxan + TC extract (100 mg /kg, b/w)
Group IV: Alloxan + TC extract (200 mg /kg, b/w)
Group V: Alloxan + TFG extract (100 mg /kg, b/w)
Group VI: Alloxan + TFG extract (200 mg /kg, b/w)
Group VII: Alloxan + TC + TFG extract (100 + 100 mg /kg, b/w)
Group VIII: Alloxan + Gliblenclamide (5mg / kg, b/w)

Plant extracts and standard drug glibenclamide (5 mg / kg b/w) and saline were administered with the help of feeding cannula.

Biochemical assay of Blood glucose, Serum cholesterol, AST, ALT and ALP:

Blood glucose levels were estimated by the methods of Sasaki et al [8]. Serum cholesterol was estimated by the methods of Zlatkis et al [9]. Estimation of AST, ALT by using Reitman and Frankel method [10] and ALP through Kind and Armstrong method [11].

Insulin estimation:

At the end of the 30th day, 3 hr. after the last dose of the solvent, glibenclamide or the extract, blood samples were withdrawn from the orbital sinus of rats, under light ether anaesthesia, into heparinized tubes. Samples were centrifuged at 3500 rpm for 15 min to separate the plasma. The plasma samples were separated and kept at 20°C for analysis when required. The plasma insulin was analysed by Enzyme Linked (ELISA) method using Boehringer Mannheim kit [12]. 0.1 ml of plasma protein was injected into the plastic tubes coated with anti-insulin antibodies. Phosphate buffer and anti-insulin Peroxidase (POD) conjugate was added to form anti-insulin antibody-POD conjugate. Substrate- chromogenic solution was then added to form indicator reaction. A set of standards were also treated in the similar manner. After the

development of colour, the absorbance was read at 420 nm. The values were expressed as IU/ml of plasma.

Histological assay

On the 30th day, pancreatic tissues were taken from animals, which were fasted overnight, under ether anaesthesia. The whole pancreas from each animal was removed after killing the animals, was placed in 10 % formalin solution, and immediately processed by the paraffin technique. Sections of 5 μm thickness were cut and stained by haematoxylin and Eosin (H and E) for histological examination. The photomicrographs of histological studies are taken.

Statistical analysis

The values were expressed as mean ± S.E. Data were tabulated and discussed using Students't' test. P values <0.01, 0.001 were considered as significant.

RESULTS AND DISCUSSION

The phytochemicals screening results shows both plant extracts having phytosterol and flavonoids (Table 1). Alkaloids present in TC leaves and TFG seeds extract and phenolic compounds present in TC leaves extract only.

The LD_{50} values for both extract which was found to be greater than 2000 mg/kg b/w for TC and TFG (Table 2). One tenth of this value was chosen as initial dose in this work. The increasing prevalence of T2D threatening the quality of human life demands extensive and qualitative research into development of efficient anti-diabetic agents free of adverse effects. Hence, medicinal plants are constantly being investigated using animal model of the disease with the anticipation of developing a comparatively safe anti-diabetic plant-based product [13, 14]. In this study, reported the anti-diabetic potential of the TC and TFG in an animal model of T2D.

Table 1: Phytochemical qualitative screening of ethanolic extract of TC and TFG

Phytoconstituents	TC leaves extract	TFG seeds extract
Alkaloids	+	+
Reducing sugar	-	+
Phytosterol	+	+
Fixed oil & Fats	-	-
Phenolic compounds & Tannins	+	-
Proteins & Amino acids	+	-
Gums & Mucilage	+	+
Flavonoids	+	+
Lignins	-	-
Saponins	-	+

(+) indicates present, (-) indicates absent

Table 2: The LD_{50} value of the ethanolic extracts of TC and TFG

Test sample	LD_{50} (mg/kg, b/w)
TC	>2000
TFG	>2000

Table 3: Effect of TC and TFG Extracts on Blood glucose levels in different animal groups

Groups	Blood Sugar (mg/100ml)			
	Initial	Day 7	Day 14	Day 28
I	85.1±1.8	86.0±0.2	86.4±1.9	87.9±5.7
II	88.0±2.0	370±20.5	421±5.9	403±9.2
III	87.4±2.9	239±16.7*	177±4.5*	119±3.9*
IV	85.0±3.4	190±16.3*	154±1.5**	97.9±6.7**
V	88.5±6.1	245±14.6*	197±12.9*	139±4.9**
VI	90.4±5.9	205±16.3*	169±11.2*	109±7.4**
VII	87.4±4.3	185±9.7*	139±9.5*	99±5.5**
VIII	83.0±4.9	172.0±0.8*	128.0±9.5**	91.7±7.3**

Values are expressed as Mean ± S.E, n = 6 by students't' test; * P < 0.01 Vs Control; ** P < 0.001 Vs Control

Administration of the TC leaves extract and TFG seeds for 28 days reduced the blood glucose levels in alloxan diabetic rats (Table 3). After 28 days the blood glucose level in diabetic rats were 403 ± 9.2 m g/100 ml and the blood glucose level in TC + TFG extract (100 + 100mg/kg, b/w) treated diabetic rats were very much decreased as 99 ± 5.5 m g/100 ml.

The results of AST, ALT and ALP enzyme activities were summarized in (Table 4). After 30 days the AST and ALT levels in normal rats are 82.5± 2.3 IU/l and 85.1±0.4 IU/l, whereas the diabetic control group decreased the AST and ALT levels 60.1±1.1 IU/l and 65.8± 1.2 respectively. The Plant TC + TFG extract (100 + 100 mg/kg, b/w) treated diabetic rats were increased the AST and ALT (80.4 ± 4.7 and 85.0 ± 4.5 IU/l) enzyme concentration as less than or equal to normal rats (82.5 ± 2.3 and 85.1 ± 0.4 IU/l)). Glibenclamide treated diabetic rats also increased the AST and ALT levels as 78.2 ± 4.1 IU/l and 84.8 ± 1.4 IU/l respectively. ALP concentration were increased in diabetic rats (14.0 ± 0.2 IU/l) then normal level (6.00 ± 2.1); it was almost decreased in mixture extract treated animals (6.3 ± 2.1 IU/l).

Cholesterol levels were increased in diabetic rats (225.0 ± 2.7 µg/ml) then normal level (145.0 ± 2.9 µg/ml); it almost decreased in mixture extract level 149.0 ± 4.5µg/ml nearer to standard drug glibenclamide range is 139.0 ± 5.9 µg/ml (Table 5).

Sulphonylureas cause hypoglycemia by stimulating insulin secretion from the pancreas and these compounds are potent in mild alloxan induced diabetes [15]. Insulin normal level 137.2 ± 6.3 significantly increased in plant TC + TFG extract treated diabetic rats (139.0±1.6 µg/ml), compared to alloxan treated rats (54.3 ± 2.5 µg/ml). The administration of standard anti-diabetic drug Glibenclamide significantly increased (140.0 ± 2.1) in diabetic control (Table 5).

Table 4: Effect of TC and TFG extracts on AST, ALT and ALP levels in Alloxan induced diabetic rats

Groups	AST(IU/l)			ALT(IU/l)			ALP(IU/l)		
	Initial	15 Days	30 Days	Initial	15 Days	30 Days	Initial	15 Days	30 Days
I	85.5±1.5	86.46±0.53	82.5±2.3	84.12±1.2	84.2±0.37	85.1±0.4	5.58±1.1	5.56±1.12	6.00±2.1
II	81.08±1.07	65.5±1.0	60.1±1.1	85.1±1.2	70.9±1.75	65.8±1.2	6.12±0.4	18.8±0.58	14.0±0.2
III	85.2±0.54	83.4±1.8	84.3±2.1*	83.2±0.5	81.88±1.9	83.0±2.2*	7.21±0.4	8.1±0.2	9.0±0.7*
IV	85.95±5.7	88.0±1.4*	90.0±1.5*	85.7±1.2	85.4±1.6	84.6±2.5*	6.8±0.4	7.9±0.3	7.8±0.6*
V	84.7±2.3	85.4±1.8*	85.9±2.1*	83.7±3.6	81.78±4.9	81.3±2.4*	7.01±0.6	8.03±0.4	9.0±1.3*
VI	85.42±4.3	86.0±5,2*	87.0±2.4*	84.6±5.2	86.4±2.9	85.9±5.2*	6.1±0.5	7.2±0.39	6.8±0.6*
VII	84.51±5.7	81.7±3.4*	80.4±4.7*	84.9±3.4	87.4±1.6	85.0±4.5*	5.9±2.9	6.6±3.1	6.3±2.1*
VIII	82.8±3.2	80.4±2.9*	78.2±4.1*	85.1±1.1	88.0±2.6	84.8±1.4*	6.7±1.0	6.4±1.1	6.8±1.0*

Values are expressed as Mean ± S.E, n = 6 by students't' test; * P < 0.01 Vs Control

Table 5: Effect of TC and TFG extracts on Cholestrol and Insulin in Alloxan induced diabetic rats

Groups	Cholesterol (µg/ml)			Insulin (µg/ml)		
	Initial	15 Days	30 Days	Initial	15 Days	30 Days
I	141.0±1.9	141.6±6.7	145.0±2.9	136.9±5.6	136.5±4.7	137.2±6.3
II	141.4±2.9	216.5±6.9	225.0±2.7	136.1±7.9	57.6±3.7	54.3±2.5
III	147.0±1.9	165.0±3.9*	175.0±4.7*	137.4±2.7	79.17±2.4*	102.0±1.8**
IV	143.0±1.4	146.2±3.1**	151.0±3.9**	136.8±1.9	119.6±4.7**	121.0±1.8**
V	142.0±1.9	161.0±5.9*	171.0±4.5*	134.3±2.1	97.7±2.4**	115.0±1.8**
VI	140.0±1.4	147.5±3.4**	151.0±6.9**	136.9±3.9	129.7±2.7**	136.0±1.8**
VII	142.0±2.5	142.5±6.7**	149.0±4.5**	136.5±4.9	131.3±5.7**	139.0±1.6**
VIII	143.4±1.5	140.0±3.7**	139.0±5.9**	135.9±2.1	130.0±2.8**	140.0±2.1**

Values are expressed as Mean ± S.E, n = 6 by students't' test; * P < 0.01 Vs Control; ** P < 0.001 Vs Control

In this study, continuous treatment with the plant extracts caused significant decrease in blood glucose levels of treated rats compared to untreated diabetic rats. Diabetes were characterised by a severe loss in body weight due to loss or degradation of structural proteins [16]. This condition was alleviated by the treatment of the diabetic rats with the plant extracts as the treated rats were healthy and agile at the end of the study. Some plants are reported to exert hypoglycaemic action by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin [17]. While others act through extra pancreatic mechanisms by inhibition of hepatic glucose production or corrections of insulin resistance [18, 19].

Measurements of enzymatic activities of alkaline phosphates are of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in diseased conditions. The increment in the activities of plasma AST, ALT and ALP indicated that diabetes may induce hepatic parenchymal injury and hepatic dysfunction ad leakage of these enzymes from the liver cytosol into the blood steam [20-22]. On the other hand, treatment of the diabetic rats with their TC or TFG extracts brought back the activity of AST, ALT and ALP enzymes to normal level. The TC and TFG extracts have clearly demonstrated an increase in insulin levels as evidenced in the treatment groups and have utilized one of the above mechanisms in exerting its antidiabetic effect.

Histopathology results

The Histopathalogical studies on the pancreatic tissue before and after treatments have been done (Fig.1 (a-h)). Normal animals showed normal acini and normal cellular population in the islets of Langerhans in pancreas. Extensive damage to the islets of Langerhans and reduced dimensions of islets were found in diabetic control animals. Restoration of normal cellular population and size of islets with hyperplasia were seen in extract treated groups. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia were observed in extract TC and TFG treated animals proving antidiabetic potential of the plant extracts.

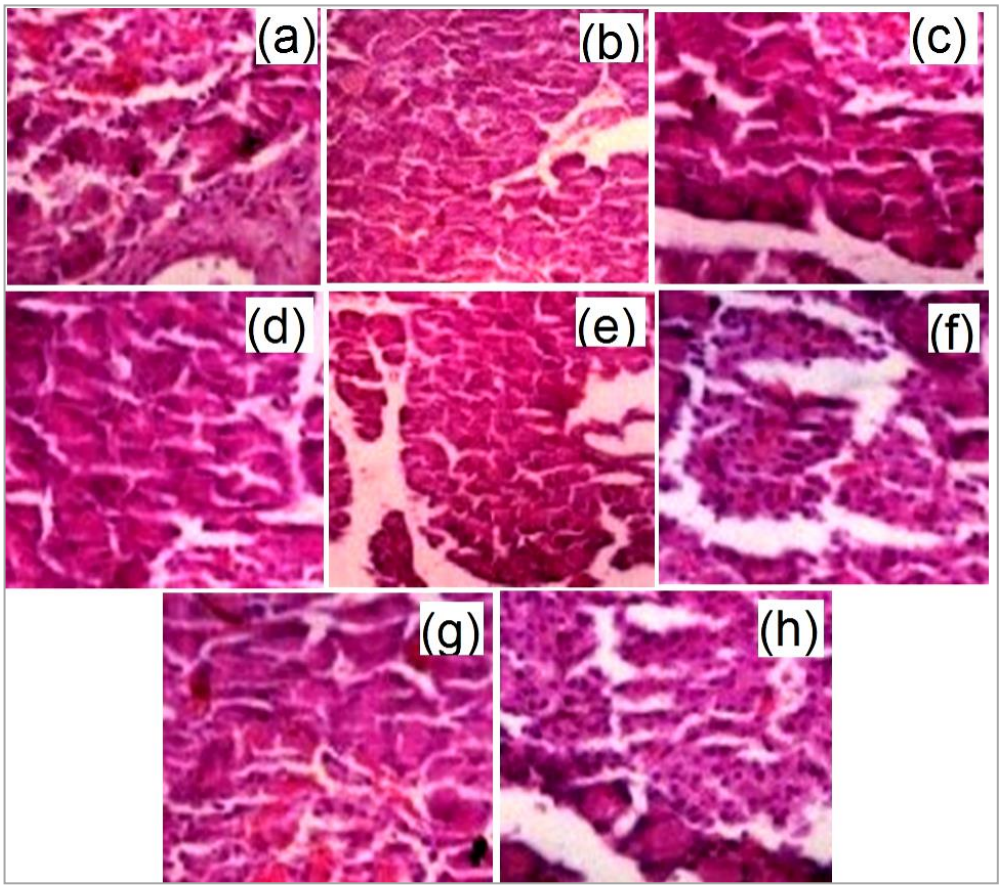

Fig.1.(a) Normal – Control: Showed normal acini, and normal cellular population in the islets of langerhans in pancreas; **(b) Diabetic – Control:** Extensive damage to the islets of langerhans and reduced dimensions of islets; **(c) TC Extract – 100 mg/kg:** Partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia **(d) TC Extract – 200 mg/kg:** Restoration of normal cellular population, size of islets with hyperplasia;(e) **TFG Extract 100 mg/kg**: Partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia; **(f) TFG Extract 200 mg/kg:** Partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia; **(g) TC + TFG Extract 200 mg/kg:** Restoration of normal cellular population, size of islets with hyperplasia; **(h) Gliblenclamide 5 mg/kg:** Restoration of normal cellular population, size of islets with hyperplasia

CONCLUSION

Plants are natural antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as flavonoids, tannins, phenolic, and alkaloids that improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose. This study showed that the daily administration plant extracts resulted in reduction in blood glucose levels in the animal model of T2D. The beneficial effects of the leaves fractions could be attributed to improved insulin sensitivity and beta-cell function as a result of alkaloids, flavonoids, saponins and/or tannins present in the fractions. These phytochemicals are known to possess anti-diabetic activities and at least one or more of them were present in the fraction. The data from this study gives credibility to existing reports that both plants were valuable in the ethno-therapeutic management of diabetes mellitus.

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***Corresponding Author:**
V. Thirumurugan*
Email: drv.thirumurugan@gmail.com