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BIOCHEMICAL STUDIES ON THE EFFECT OF ETHANOLIC EXTRACTS OF *TRICHOSANTHES DIOICA* AND *CLITORIA TERNATEA* IN STREPTOZOTOCIN INDUCED DIABETIC MALE WISTAR RATS

R. Kavitha

Department of Biotechnology, Periyar University PG Extension Centre, Dharmapuri - 636701, Tamil Nadu, India.

Keywords:

Trichosanthe dioica, *Clitoria ternatea*, Antidiabetic, Streptozotocin, Blood glucose, Insulin

Correspondence to Author:
R. Kavitha

Department of Biotechnology, Periyar University PG Extension Centre, Dharmapuri - 636701, Tamil Nadu, India.

E-mail: erokavi_vasu@yahoo.com

ABSTRACT: To investigate the effect of ethanolic extracts of leaf and fruit of *Trichosanthe dioica* (*T. dioica*) and leaf of *Clitoria ternatea* (*C. ternatea*) on the biochemical parameters in streptozotocin (STZ) induced diabetic Wistar albino rats. Healthy male adult Albino rats of Wistar strain were randomly divided into eleven groups of six rats each were assigned into normal and diabetic groups (Group I to XI). Diabetes was induced in albino rats by administration of STZ (60 mg/kg i.p). Group I and II were kept as normal control and diabetic control. The other diabetic groups from group III to group X were treated with either individual and combined ethanolic extracts of *T. dioica* and *C. ternatea* at a dose of 200 and 400 mg/kg of body weight (bw) were administrated at a single dose per day and group XI was treated with glibenclamide at a dose of 600 µg/kg body weight for 28 consecutive days. The effect of ethanolic extracts of leaf and fruit of *T. dioica* and leaf of *C. ternatea* on blood glucose, serum insulin, glycosylated hemoglobin (HbA1c), urea, creatinine, SGOT, SGPT, LDH, ALP, total protein, albumin and globulin were evaluated in the diabetic rats. In the acute toxicity study, ethanolic extracts of leaf and fruit of *T. dioica* and leaf of *C. ternatea* were non-toxic and safe up to 2000 mg/kg body weight in rats. A significant decrease in blood glucose, HbA1c and other biochemical parameters levels and increase in serum insulin level were observed in diabetic rats treated with ethanolic extracts of *T. dioica* (leaf and fruit) and *C. ternatea* (leaf) compared to diabetic control rats. These findings suggest that the combined extract of *T. dioica* fruit and *C. ternatea* leaf have potent antidiabetic activity in STZ induced diabetic rats.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder characterized by greater or lesser impairment in the metabolism of carbohydrates, proteins and lipids. It is a multi factorial, non-communicable disease where there is too much glucose circulating in the blood stream. This occurs because either the pancreas cannot produce enough insulin or the cells in the body have become resistant to insulin. It causes some serious health issues including kidney failure, nerve damage, heart diseases, stroke, non-traumatic lower limb amputations and blindness [1].

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The World Health Organization (WHO) reported that the estimated incidence of diabetes mellitus for the year 2016 is 422 million. The greatest increase in prevalence is occurring in low- and middle-income countries including in Asia and Africa. The global increase in the prevalence of diabetes in developing countries is due to population growth,

ageing, urbanization, less physically demanding work, food habits like consumption of food with high energy dense but nutrient poor often high in carbohydrates, saturated fats and an increase of obesity [2].

Currently available therapies for the management of diabetes include insulin therapy and various oral hypoglycemic agents. But they are unable to lower glucose concentration to within normal range and people with diabetes may need to take these medicines for the rest of their lives [3]. Hence there is a need to search for newer antidiabetic agents having high therapeutic efficacy with minimum side effects. This may be fulfilled by treating diabetes with plant derived antidiabetic agents.

Herbal medicine is the use of plants (herbs) to treat a range of disorders and enhance well-being. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites such as alkaloids, glycosides, terpenoids, flavonoids, phenolics *etc*., which are sought after because they are known to exhibit numerous biological activities that promote health effects [4]. However, the study of plants for antidiabetic activity may give new pharmacological approaches in the treatment of diabetes mellitus.

Trichosanthes dioica Roxb. and *Clitoria ternatea* L. are being used as popular remedy for the treatment of diabetes mellitus in Ayurveda and Siddha medicine. In the present investigation, the first plant *Trichosanthes dioica* (*T. dioica*) Roxb. belongs to the Cucurbitaceae family. It is often called green potato, young and unripe fruit is valued by Europeans next to potatoes and brinjals. The fruits are green with white or no stripes whereas leaves are 7.5 cm long, ovate-oblong, cordate, acute, sinuate denate, rigid and rough on both surfaces [5]. It has been used for constipation, bronchitis, diuretics, fever, skin infection, dysentery, diarrhoea, convalescents and cancer like conditions.

The second plant *Clitoria ternatea* (*C. ternatea*) Linn. belongs to the Fabaceae family. The leaves are pinnate, with 5-7 elliptic to lanceolate leaflets, 3-5 cm long and shortly pubescent underneath. It was traditionally used to treat infertility, urinogenital disorder, bronchitis, purgative, diuretic, diabetes mellitus, gonorrhea and rheumatism [6, 7, 8]. The preliminary phytochemical analysis of ethanolic extracts of *T. dioica* and *C. ternatea* showed the presence of flavonoids, phenolic compounds, alkaloids, glycosides and tannins [9, 10]. It is well known that different phytochemical constituents from these plants were reported for many pharmacological activities including antidiabetic activity.

Hence, the present study was undertaken to investigate the effect of above mentioned two different herbal plants are used individually and in combination to evaluate blood glucose, insulin, HbA1c, urea, serum creatinine, liver marker enzymes, protein, albumin and globulin in streptozotocin induced diabetic rats.

MATERIALS AND METHODS:

Chemicals: Streptozotocin (STZ) was purchased from Siga Chemical Company (USA). Glibenclamide was obtained from Aventis Pharmaceuticals Limited (India). All the chemicals and reagents used in the experiments were of analytical grade obtained from BDH (England and India), E. Merck (Germany), Siga Chemical Company (U.S.A), LOBA - Chemie Indo Austranol Co., (India) whenever necessary the solvents were redistilled before use.

Plant Materials: Fresh unripe fruit and leaf of *T. dioica* and the leaf of *C. ternatea* were collected from SKM Herbal Research Centre, Erode, Tamil Nadu, India. With the help of local flora, a voucher specimen (no. VOCB 2307 and VOCB 2453) was retained in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu for further reference.

Preparation of Plant Extracts: Freshly collected leaf and fruit of *T. dioica* and leaf of *C. ternatea* were washed with distilled water and the fruits were cut into small pieces and shade dried for two weeks. After drying, the plant materials were coarsely powdered separately. Powdered plant samples about 500 g was extracted in Soxhlet extractor using 1250 ml of ethanol for 24 h. All the extracts were filtered through Whatmann no. 41 filter paper separately and the extracts were concentrated in vacuum at 60 ºC using a rotary

evaporator. To evaporate the remaining solvent, the extracts were kept in an oven at a temperature of 40 - 50 ºC for 8 h that were used for further study.

Animals: Normal healthy male adult albino rats of Wistar strain (160 - 180 g) were procured from Nandha College of Pharmacy. The entire process was approved by the Institutional Animal Ethics Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals, (CPCSEA), India (Proposal number: NCP/IAEC/ PHD/01/2007-2008), Nandha College of Pharmacy, Erode, Tamil Nadu, India.

Induction of Experimental Diabetes: Diabetes was induced by single dose intraperitoneal administration of streptozotocin at a dose of 60 mg/kg body weight in 0.1 M citrate buffer (pH 4.5) and then injected into the tail of the sixty rats. The injection volume was prepared to contain 1 ml/kg body weight [18]. After 72 h of STZ administration, the blood glucose content was measured. The animals with blood glucose levels ≥ 250 mg/dl were considered to be diabetic and used for the experiment [11].

Experimental Design of Animals: In the present investigation, the rats were divided into eleven groups of six rats in each group as follows: Group I: Normal control rats received normal saline (0.9% sodium chloride); Group II: STZ-induced diabetic control rats received normal saline; Group III: Diabetic rats received ethanolic leaf extract of *T. dioica* (200 mg/kg body weight); Group IV: Diabetic rats received ethanolic leaf extract of *T. dioica* (400 mg/kg body weight); Group V: Diabetic rats received ethanolic fruit extract of *T. dioica* (200 mg/kg body weight); Group VI: Diabetic rats received ethanolic fruit extract of *T. dioica* (400 mg/kg body weight); Group VII: Diabetic rats received ethanolic leaf extract of *C. ternatea* (200 mg/kg body weight); Group VIII: Diabetic rats received ethanolic leaf extract of *C. ternatea* (400 mg/kg body weight); Group IX: Diabetic rats received combined ethanolic extracts of *T. dioica* leaf (200 mg/kg body weight) and *C. ternatea* leaf (200 mg/kg body weight); Group X: Diabetic rats received combined ethanolic extracts of *T. dioica* fruit (200 mg/kg body weight) and *C. ternatea* leaf (200 mg/kg body weight); Group XI: Diabetic rats received standard drug glibenclamide (600 µg/kg body weight) for 28 d orally by using an intragastric catheter tube.

Collection of Blood: At the end of the experimental period, all rats were fasted an overnight and sacrificed by cervical decapitation. Blood was collected from the experimental animals by direct cardiac puncture for the various biochemical parameters estimation.

Biochemical Analysis: Serum glucose was measured by the method [12]. Insulin level was determined by the method [13]. HbA_1c was estimated by the method [14], urea and creatinine were determined by the method [15, 16], SGOT (Serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate pyruvate transaminase) activities were determined according to the method [17], LDH (lactate dehydrogenase) and ALP (alkaline phosphatase) activity were studied by the method [18, 19], protein, albumin and globulin were estimated according to the methods [20, 21, 22].

Statistical Analysis: All the experimental values are expressed as means ± SD for groups of six animals each. Statistical analyses were performed by Student's t-test. The values are statistically significant at three levels, ***p<0.001, **p<0.01, *p<0.05. But NS if p>0.05.

RESULTS:

Phytochemical Constituents: The phytochemical analysis of ethanolic extracts of leaf and fruit of *T. dioica* revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids and tannins whereas leaf of *C. ternatea* comprised a wide range of active chemical constituents such as flavonoids, glycosides, phenols, steroids, tannins and triterpenoids.

Acute Oral Toxicity Study: The extracts were safe or non-toxic up to a dose of 2000 mg/kg body weight. Behaviour of the individual animal was observed at least twice daily during the experimental period of 14 d. The extracts did not cause mortality on rats during 14 d observation. Thus, it was concluded that ethanolic extracts of leaf and fruit of *T. dioica* and leaf of *C. ternatea* were safe at 2000 mg/kg body weight.

Blood Glucose Level and other Biochemical Parameters: Table 1 and **2** shown the levels of blood glucose, serum insulin, glycosylated hemoglobin, urea and creatinine of normal, diabetic control and drug treated rats. There was a significant increased in blood glucose ($p<0.001$), glycosylated hemoglobin ($p<0.01$), urea ($p<0.01$) and creatinine ($p<0.05$) and decreased serum insulin ($p<0.01$) in STZ-induced diabetic rats (Group II) when compared with normal control rats (Group I). The ethanolic extracts of individual, combined extracts and known drug treated groups showed a significant ($p<0.05$; $p<0.01$) decrease in the blood glucose, urea, creatinine and glycosylated hemoglobin levels along with increased serum insulin level in diabetic treated rats.

TABLE 1: EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T. DIOICA* AND LEAF OF *C. TERNATEA* ON BLOOD GLUCOSE, SERUM INSULIN, GLYCOSYLATED HEMOGLOBIN, UREA AND CREATININE LEVEL IN NORMAL, DIABETIC INDUCED AND DRUGS TREATED RATS

Treatment groups	Dose (mg/kg bw)	Blood glucose (mg/dl)	Insulin (µU/ml)	Glycosylated hemoglobin (%)
I Normal control	Normal saline	79.56 ± 3.21	13.56 ± 1.21	3.68 ± 0.11
II DC	Normal saline	214.55 ± 16.59***	4.31 ± 0.93**	10.16 ± 1.14**
III D + TDL	200	131.56 ± 3.93*	8.56 ± 1.14*	5.31 ± 0.34^{NS}
IV D+ TDL	400	121.33 ± 4.16^{a}	11.21 ± 1.39^{a}	4.23 ± 0.14^{a}
V D + TDF	200	108.16 ± 3.26^{a}	12.03 ± 1.88^{a}	4.98 ± 0.11^{a}
VI D + TDF	400	92.11 ± 2.86^{aa}	12.16 ± 1.23^{a}	4.03 ± 0.24^{a}
VII D + CTL	200	112.16 ± 2.92^{a}	10.11 ± 1.08	5.28 ± 0.19^{NS}
VIII D + CTL	400	81.53 ± 2.17^{aa}	13.46 ± 1.19^{aa}	3.96 ± 0.06^{aa}
IX D+ TDL + CTL	200 +200	92.11 ± 1.94^{aa}	12.86 ± 1.56^{a}	4.14 ± 0.12^{a}
X D + TDF + CTL	200+200	73.28 ± 1.84^{a}	13.08 ± 1.34^{aa}	3.74 ± 0.08^{aa}
XI D + Glibenclamide	0.6	79.16 ± 1.93^{a}	12.98 ± 1.13^{a}	3.93 ± 0.07^{aa}

DC- Diabetic control; D-Diabetic; TDL - *T. dioica* leaf; TDF - *T. dioica* fruit; CTL - *C. ternatea* leaf

Values are reported as mean ± SD for six animals in each group. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significance between normal control *vs.* diabetic control and drug treated groups; $^{a}p<0.05$, $^{aa}p<0.01$ significance between diabetic control *vs* drug treated groups; NS: Not significant

TABLE 2: EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T. DIOICA* AND LEAF OF *C. TERNATEA* ON BLOOD GLUCOSE, SERUM INSULIN, GLYCOSYLATED HEMOGLOBIN, UREA AND CREATININE LEVEL IN NORMAL, DIABETIC INDUCED AND DRUGS TREATED RATS

Treatment groups	Dose (mg/kg bw)	Urea (mg/dl)	Creatinine (mg/dl)
I Normal control	Normal saline	16.31 ± 0.83	0.58 ± 0.03
II DC	Normal saline	38.56 ± 1.21**	1.56 ± 0.14*
III D + TDL	200	21.16 ± 0.93	1.53 ± 0.15
IV D+ TDL	400	23.69 ± 1.19	1.38 ± 0.17
V D + TDF	200	20.12 ± 1.05	1.21 ± 0.11
VI D + TDF	400	18.13 ± 1.13^{a}	0.81 ± 0.02^{a}
VII D + CTL	200	19.96 ± 1.24^{NS}	1.02 ± 0.09^{NS}
VIII D + CTL	400	17.91 ± 1.03^{a}	0.94 ± 0.05^{a}
IX D+ TDL+ CTL	200 +200	18.16 ± 0.84^{a}	0.89 ± 0.06^{a}
X D + TDF + CTL	200+200	14.36 ± 0.73^{aa}	0.81 ± 0.06^{a}
XI D+ Glibenclamide	0.6	15.84 ± 0.84^{aa}	0.77 ± 0.03^{a}

DC- Diabetic control; D-Diabetic; TDL - *T. dioica* leaf; TDF - *T. dioica* fruit; CTL - *C. ternatea* leaf

Values are reported as mean ± SD for six animals in each group. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significance between normal control *vs.* diabetic control and drug treated groups; $^{a}p<0.05$, $^{aa}p<0.01$ significance between diabetic control *vs* drug treated groups; NS: Not significant.

Liver Marker Enzymes: The level of liver marker enzymes such as SGOT, SGPT, LDH and ALP in the serum of diabetic rats were given in **Table 3**. The level of liver marker enzymes in serum were increased significantly ($p<0.05$; $p<0.01$) in diabetic rats (Group II) when compared to normal control group (Group I). Administration of ethanolic extracts of test plants restored the activities of the liver marker enzymes. Among these, the combined extracts (Group IX and X) and Glibenclamide (Group XI) treated groups showed a significant decrease in the levels of SGOT ($p<0.05$), SGPT ($p<0.01$), and ALP ($p<0.05$). The levels of LDH was significantly ($p<0.01$) diminished in higher dose (400 mg/kg body weight) in all the individual and combined extracts treated experimental groups.

TABLE 3: EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T. DIOICA* AND LEAF OF *C. TERNATEA* ON THE ACTIVITY OF SGOT, SGPT, LDH AND ALP LEVELS IN SERUM OF NORMAL, DIABETIC INDUCED AND DRUGS TREATED RATS

Treatment groups	Dose (mg/kg bw)	SGOT[#]	SGPT[#]	LDH[#]	ALP[##]
I Normal control	Normal saline	21.51 ± 0.36	13.61 ± 0.14	228.54 ± 11.38	119.27 ± 4.31
II DC	Normal saline	49.31 ± 1.31*	41.31 ± 0.74*	394.17 ± 24.61**	184.51 ± 5.26*
III D + TDL	200	31.56 ± 1.08	28.14 ± 0.54	246.38 ± 14.28[a]	143.54 ± 4.56
IV D + TDL	400	26.11 ± 1.26[a]	23.11 ± 0.39	234.66 ± 15.88[aa]	137.31 ± 4.81
V D + TDF	200	27.39 ± 1.13[NS]	17.56 ± 0.74[a]	263.92 ± 16.84[a]	129.36 ± 3.92
VI D + TDF	400	27.14 ± 1.72[NS]	15.21 ± 0.39[a]	229.14 ± 19.24[aa]	121.16 ± 3.17
VII D + CTL	200	23.16 ± 1.56[a]	18.56 ± 0.14[a]	246.34 ± 16.98[a]	126.56 ± 2.84
VIII D + CTL	400	21.11 ± 1.07[a]	14.11 ± 0.73[a]	213.35 ± 14.62[aa]	123.33 ± 2.17
IX D + TDL + CTL	200 + 200	21.16 ± 1.23[a]	13.56 ± 0.14[aa]	234.91 ± 19.36[aa]	118.49 ± 2.56[a]
X D + TDF + CTL	200 + 200	19.16 ± 1.56[a]	13.14 ± 0.31[aa]	219.35 ± 18.13[aa]	109.56 ± 2.19[a]
XI D + Glibenclamide	0.6	20.70 ± 1.34[a]	13.26 ± 0.27[aa]	236.63 ± 12.86[a]	124.51 ± 1.98[a]

DC- Diabetic control; D- Diabetic; TDL - *T. dioica* leaf; TDF - *T. dioica* fruit; CTL - *C. ternatea* leaf

Values are reported as mean ± SD for six animals in each group. *p<0.05, **p<0.01, ***p<0.001 significance between normal control *vs.* diabetic control and drug treated groups; [a]p<0.05, [aa]p<0.01 significance between diabetic control *vs* drug treated groups; NS: Not significant. SGOT- Serum glutamate oxalate transaminase; SGPT- Serum glutamate pyruvate transaminase; LDH-Lactate dehydrogenase; ALP-Alkaline phosphatase; [#]µmoles of pyruvate liberated/ L; [##]µmoles of phenol liberated/ L.

Total Protein, Albumin and Globulin Levels in Serum: Table 4 depicted the levels of total protein, albumin and globulin levels in normal control, diabetic control, plant extracts treated groups and known drug treated groups. Total protein, albumin and globulin levels were significantly decreased (p<0.05) in diabetic rats (Group II) when compared to normal control rats (Group I) and Glibenclamide treated rats (Group XI). Treatment with ethanolic extracts of individual, combined extracts and standard drug increased the levels of total protein, albumin and globulin near to the normal values.

TABLE 4: EFFECT OF ETHANOLIC EXTRACTS OF LEAF AND FRUIT OF *T. DIOICA* AND LEAF OF *C. TERNATEA* ON SERUM PROTEIN, ALBUMIN AND GLOBULIN LEVEL IN NORMAL, DIABETIC INDUCED AND DRUGS TREATED RATS

Treatment groups	Dose (mg/kg b.w.)	Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
I Normal control	Normal saline	8.11 ± 0.13	4.61 ± 0.11	3.68 ± 0.11
II DC	Normal saline	5.36 ± 0.14*	3.20 ± 0.63	2.16 ± 0.15
III D + TDL	200	7.12 ± 0.11	4.02 ± 0.51	3.10 ± 0.71
IV D + TDL	400	7.84 ± 0.20	4.26 ± 0.34	3.58 ± 0.22
V D + TDF	200	7.26 ± 0.11	4.56 ± 0.21	2.70 ± 0.14
VI D + TDF	400	8.02 ± 0.14[a]	4.81 ± 0.17	3.21 ± 0.32
VII D + CTL	200	7.94 ± 0.26	4.11 ± 0.26	3.83 ± 0.41
VIII D + CTL	400	8.18 ± 0.19[a]	4.21 ± 0.19	3.99 ± 0.32
IX D + TDL + CTL	200+200	8.06 ± 0.21[a]	4.12 ± 0.13	3.94 ± 0.23
X D + TDF + CTL	200 + 200	7.98 ± 0.14	4.06 ± 0.23	3.92 ± 0.12
XI D + Glibenclamide	0.6	7.89 ± 0.21	4.31 ± 0.11	3.58 ± 0.33

DC- Diabetic control; D-Diabetic; TDL - *T. dioica* leaf; TDF - *T. dioica* fruit; CTL - *C. ternatea* leaf

Values are reported as mean ± SD for six animals in each group. *p<0.05, **p<0.01, ***p<0.001 significance between normal control *vs.* diabetic control and drug treated groups; [a]p<0.05, [aa]p<0.01 significance between diabetic control *vs* drug treated groups; NS: Not significant.

DISCUSSION: Insulin is a peptide hormone which controls the level of blood glucose. In addition to its role of regulating glucose metabolism, stimulates lipogenesis, increases amino acid transport into cells, altering transcription and the cell content of numerous mRNAs, stimulates growth, DNA synthesis and cell replication [23].

In the present study, the STZ-induced diabetic rats (Group II) elicited significant rise in blood glucose to a level of 62.92% and decreased serum insulin to 68.21% compared to normal control rats (Group I). On the contrary, diabetic rats treated with ethanolic extracts of individual and combined extracts of leaf and fruit of *T. dioica* and leaf of *C. ternatea* and standard drug glibenclamide for 28 d, exhibited decrease in blood glucose and increase in serum insulin levels. It was observed that ethanolic extracts reversed these effects in diabetic animals.

STZ-induced diabetes exhibit the diabetic complications mediated through oxidative stress involving in pancreatic *β*-cells destruction [24, 25]. The ethanolic extracts contain phytochemicals such as flavonoids, total phenolics, tannins, glycosides and alkaloids which might have played an important role in reduction of oxidative stress of pancreatic *β*-cells. This might have lead to increased glucose metabolism. Another possible mechanism may be by potentiation of the insulin from *β*-cells of Islets of Langerhans or its responsiveness [26].

Glycosylated hemoglobin or HbA_{1C} is formed by abnormal attachment of sugar to hemoglobin. The amount of this increase is directly proportional to the fasting blood sugar level [27]. It is produced progressively and irreversibly over a period of time and is stable over the life span of the red blood cells. It is unaffected by diet, insulin or exercise, even on the day of test. Therefore, glycosylated hemoglobin can be used as an excellent marker of overall glycemic control [28].

In present study, STZ-induced diabetic rats (Group II) showed significantly increased HbA_{1C} level when compared with normal control rats (Group I). The percentage of HbA_{1C} in normal control groups has shown 3.68 ± 0.11 and the same is elevated nearly three times in STZ-induced diabetic control animals. The significant decrease in the level of glycosylated hemoglobin in STZ-induced diabetic rats following leaf and fruit of *T. dioica*, leaf of *C. ternatea* and standard drug therapy indicated that the overall blood glucose level was controlled, probably due to improvement in insulin secretion. It is noteworthy that the serum insulin level in diabetic animals treated with leaf and fruit of *T. dioica* and leaf of *C. ternatea* also increased when compared to the diabetic control animals.

Urea is the main end product of protein metabolism. Amino acid deamination takes place in the liver, where ammonia is converted into urea and excreted through urine. Creatinine is a protein produced by muscle and released into the blood. It is also a waste product formed in muscle by creatinine metabolism. It is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. The creatinine level in the blood is determined by the rate it is being removed, which is roughly a measure of kidney function. Its retention in the blood is evidence of kidney impairment [29].

In the present study, a significant elevation in markers of renal dysfunction such as urea and creatinine were observed in STZ-induced diabetic rats (Group II) when compared to normal control rats (Group I). After treatment of STZ-diabetic rats with test drugs and glibenclamide reversed urea and creatinine level to near normal.

STZ-induced diabetes in rats have increased the activities of urea metabolizing enzymes which results in formation of urea in hepatic cells and increase the urea excretion in diabetes [30]. STZ can also cause an abnormal glomerular function, marked by reduction in the glomerular filtration rates, which was accompanied by an increase in the serum creatinine level, indicating the induction of acute renal failure. Streptozotocin has a profound effect on the activity of liver marker enzymes. The animals treated with STZ developed hepatic damage which is evident from the increase in the enzyme activities [31].

In the present investigation, when rats were exposed to STZ, the activities of the liver marker enzymes were significantly increased when compared to the control group. Also it revealed that the treatment of diabetic animals with individual and combined ethanolic extracts of leaf and fruit of *T. dioica*, leaf of *C. ternatea* and Glibenclamide treated groups resulted in reduced the level of SGOT, SGPT, LDH and ALP in the serum of STZ-induced diabetic rats. However combined extracts were found to be better than individual extracts.

The elevated levels of these marker enzymes in diabetic rats may be to increase the concentration of glucose precursors. In other words, the gluconeogenic action of SGOT and SGPT plays an important role in providing new supplies of sugar from other carbohydrate sources such as amino acids. Absence of insulin and increased availability of amino acids were responsible for the increased gluconeogenesis and ketogenesis observed in diabetes [32]. The elevated marker enzymes also indicated that diabetes may induce hepatic dysfunction. It has been found that liver was necrotized in diabetic patients [33].

Therefore, the increment in the activities of SGOT, SGPT, LDH and ALP in serum may also be due to the leakage of these enzymes from the liver cytosol into the blood stream [34], which gives an indication on the hepatotoxic effect of STZ [35]. On the other hand, treatment of the diabetic rats with ethanolic extracts of plants significantly restored liver marker enzymes level. The results suggested that test samples possess hepatoprotective capacity due to flavonoids and other components such as saponins, tannins, alkaloids *etc*. This also leads to improvement in the carbohydrate, fat and protein metabolism in drug treated groups [36].

The levels of serum protein, albumin and globulin were significantly decreased in STZ-induced diabetic rats when compared to control group. This was in agreement with hypoalbuminemia observed in diabetes [37]. On the other hand, oral administration of ethanolic extracts of leaf and fruit of *T. dioica*, leaf of *C. ternatea* and combined extracts treated diabetic rats never deviated protein metabolism from normal range.

Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. An overall reduction in serum total protein in diabetic animals and consequent albumin fall were observed in the present study. The reversal of these changes by ethanolic extracts of leaf and fruit of *T. dioica*, leaf of *C. ternatea* therapy proved that insulin deficiency has been grossly corrected. Animals, which received standard drug glibenclamide, also showed the similar result.

CONCLUSION: Considering above all the parameters, it can be clearly concluded that combined extract of *T. dioica* fruit and *C. ternatea* leaf was found to record beneficial effect than other test groups as antidiabetic agent. The added advantage is that the combination may act at multiple levels to bring about the therapeutic effects.

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CONFLICT OF INTEREST: Nil

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