ORIGINAL ARTICLE

Anti-diabetic activity of dried extract of *Tionspora cordifolia* (*Guduchi ghana*) and honey in streptozotacin induced diabetic rats

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Abstract

Objective: *Guduchi* (*Tinospora cordifolia* (Willd.) Hook.f. and Thomson) is used abundantly for Madhumeha (~diabetes mellitus) in traditional Ayurvedic medicines. The different dosage forms are mentioned in Ayurvedic classics such as *Churna*, *Kwatha* and *Ghana*. Hence, present study is planned to assess its anti-diabetic activity. **Materials and Methods:** In this study, aqueous extract of *T. cordifolia* was prepared by a traditional procedure of Ayurveda and assessed for its anti-diabetic activity. Diabetes was induced in Wistar strain albino rats by injecting streptozotocin in dose 40 mg/kg body weight. Aqueous extract of *T. cordifolia* Linn in a dose of 42.34 mg/kg was mixed with honey and administered orally. Different biochemical parameters such as blood glucose, cholesterol, triglyceride, high density lipoprotein, blood urea, creatinine, serum glutamate pyruvate transaminase, serum glutamic oxaloacetic transaminase, total protein, albumin, and globumin were assessed. **Results:** 24.93% reduction in blood glucose level and 28.96% reduction in glycated hemoglobin were observed in test drug treated group in comparison to diabetic control rats. **Conclusion:** Mild reduction of blood glucose level was observed in the analysis.

Key words: Anti-diabetic activity, dried extract of *Tionspora cordifolia, Guduchi ghana,* honey

INTRODUCTION

Guduchi (*Tionspora cordifolia* (Willd.) Hook. f. and Thomson) is distributed throughout the tropical Indian subcontinent, ascending to an altitude of 300 m. It is a common wild plant of deciduous and dry forests of most distracts growing over hedges and small trees. Traditionally, its properties were reported as *Deepaniya* (Increases appetite), *Trishnanigrahan* (Quenches thirst), Rasayana (Rejuvenator), *Balya* (Increases strength), *Ayushprada* (Promotes life), *Medhya* (Brain tonic), *Jwarahara* (Reduces fever), *Dahaprashaman* (Reduces burning sensations), *Amanashaka* (Destroys toxins), *Kushtaghna* (effective in skin disorders), *Amavataghna* (Reliever of arthritis), *RaktaShodhana* (Blood purifier), and *Pramehaghna* (anti-diabetic), etc.[1] Its chemical composition was reported that varieties of constituents have been isolated from *T. cordifolia* plant, and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides. Leaves of this plant are rich in protein (11.2%) and are rich in calcium and phosphorus.[2,3] As it is a popular drug, its various medicinal properties were assessed previously like anti-cancer or anti-tumor,[4] anti-diabetic and anti-hyperglycaemic,[5] anti-inflammatory,[6] antioxidant,[7] anti-stress,[8] anti-ulcer,[9] digestive,[10] hypolipidemic,[11] immunobiological of water soluble,[12] ethanol soluble and methanol soluble extracts of *Guduchi*. Literature survey revealed that its anti-diabetic activity of traditionally prepared aqueous extract, i.e., *Ghana* was not assessed hence this activity has been investigated in the present study.

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MATERIALS AND METHODS

Stems of *T. cordifolia* were collected from the herbal garden of Gujarat Ayurved University, Jamnagar. Its botanical identification was carried out in the Pharmacognosy Laboratory of IPGT & RA, Gujarat Ayurved University, Jamnagar.

Preparation of Extract

Aqueous extract of the drug was prepared by the traditional system of medicine preparation in Ayurveda, i.e., *Ghana Kalpana*[13] in the Departmental Laboratory of Rasashastra and Baishajya Kalpana, IPGT and RA, Gujarat Ayurved University, Jamnagar. 20 kg stems of *T. cordifolia* were chopped and crushed. Its decoction was prepared by adding 4 times of water weight by volume, i.e., 80 L; by evaporating reduced up to 20 L. The decoction again subjected to heat for evaporation of the remaining amount of water up to semisolid mass. This semisolid mass was dried in hot air oven at temperature 45°C. Total 1042 g dried extract was prepared. Dried extract powdered and mixed with honey, was used for the experiment.

Experimental Animals

Albino rats (160-220 g) of either sex were used. Animals were procured from the Animal house after taking the permission of the Animal Ethical Committee (IAEC-06/09-11/02) of the same institute. The animals were fed on a pelleted diet and water. All these facilities were provided by the Pharmacology Laboratory of IPGT & RA. All the studies were conducted in accordance with the National Institute of Health's guide for the Care and Use of Laboratory Animals.[14]

Collection of Blood Samples

The blood samples were collected from the rats by tail venipuncture. In the unanesthetized animals, lateral or dorsal veins were dilated by rubbing with xylol and then cleaning the part with spirit. The tail was grasped between the thumb and index finger. A needle (25-27 gauge and 0.5-1 inch long) was introduced near the distal portion of the tail with bevel up. The drop of blood accumulated at the tip of the tail after the puncture was collected at the tip of Glucometer strip and reading was noted down. This procedure was carried out for the estimation of Blood glucose level before injection of streptozotocin (STZ), 3rd day after injection, 5th day, 10th day, 15th, and 21st day during trials.

On the 21st day during sacrifice, animals were anesthetized followed by stroked over tiles. The blood samples were collected by the dissection of jugular veins of animals. The samples were collected in the test tubes and sealed. The sealed test tubes were sent to Pathology Laboratory of IPGT and RA, Jamnagar for the biochemical investigations.

Anti-diabetic effect was evaluated by the effect of test drug on the body weight and different body organs weight, biochemical variables like blood glucose,[15] serum total cholesterol,[16] serum triglyceride,[17] serum urea,[18] serum creatinine[19] were estimated using an autoanalyzer (ERBA CHEM-5, Trans Asia) using standard kit available at the end of the study. At the end of the treatment, all the animals (as per the advice of Ethics Committee) were sacrificed. Gross and histological appearances of vital organs (heart, liver, pancreas and kidney) were examined.[20]

Experimental Induction of Diabetes

Diabetes was induced in the rats by single intra peritoneal injection of STZ (40 mg/kg). STZ was the first weighed individually for each animal, according to its weight, and solubilized with 0.2 mL saline (154 mM NaCl) just prior to injection. After 72 h after STZ injection,[21] rats with diabetes hyperglycemia with blood glucose more or equal to 250 mg/dL were used for this experiment. All the animals (albino Wistar rats) used in this study developed diabetes mellitus after STZ injection.

Treatment with *T. cordifolia* dried extract with honey in suspension form was started after 72 h of STZ injection. The blood glucose level was investigated by every 5th, 10th, 15th, and 21st days by one touch strip *(Ez Smart)* method.

Experimental Design

Animals were divided into four groups, six rats in each. Food and water were provided to the animals.

The dose was calculated by extrapolating the human dose to animal based on the body surface area ratio by referring to the table of Paget and Barnes (1969).[22,23] The grouping is as follows.

- Group 1 (NC): Normal control water control
- Group 2 (DC): Diabetic control
- Group 3 (GG): Diabetic control + *Guduchi Ghana* and honey suspension (42.34 mg/kg)
- Group 4 (RS): Diabetic control + reference standard Glibenclamide (0.45 mg/kg body wt.)

Statistical Analysis

All the values of fasting blood glucose were expressed as mean±standard error mean (SEM) and analyzed using Student's *t*-test.

Observations and Results

Non-significant loss of body weight was in Group GG and RS in comparison to diabetic control rats [Table 1].

Table 1: The effect of Group GG on body weight of STZ induced diabetic Wistar strain albino rats at various intervals

Group	0 day (g)	5th day (g)	10th day (g)	15th day (g)	21st day (g)	% change in comparison to 0 day
NC	187.00±10.95	192.00±11.27	196.33±11.03	205.67±10.28	213.00±10.57	13.90
DC+STZ	170.67±11.73	165.33±12.49	165.67±14.00	167.00±13.61	166.67±15.85$^{\alpha}$	02.34
GG+STZ	159.20±04.59	142.40±08.61	144.00±10.33	146.00±10.69	139.20±18.42	12.56
RS+STZ	172.67±04.15	162.00±09.62	158.33±14.04	161.17±14.38	161.33±17.15	06.57

Data: Mean±SEM, $^{\alpha}P$<0.05, (comparison to normal control group, unpaired *t*-test). STZ: Streptozotocin, SEM: Standard error of mean

Increased weight of kidney and liver in diabetic control rats was not reversed by test drugs at significant extent [Table 2].

Elevated blood glucose levels were decreased by Group GG and RS 24.93% and 44.04% respectively in comparison with diabetic control [Table 3]. Treatment with GG and RS significantly decreased the glycated hemoglobin content in comparison to diabetic control group [Table 4].

Serum cholesterol, triglyceride, and high-density lipoprotein (HDL) were non-significantly increased in the diabetic control group, which are non-significantly decreased by the administration of GG and standard anti-diabetic drug. Blood urea levels were significantly increased in the diabetic control group in comparison to normal control group while moderately decreased in the reference standard group but showing an increase in GG. Serum creatinine levels were significantly increased in the diabetic control group, which were non-significantly decreased by the administration of GG while non-significantly increased in reference standard group in comparison to diabetic control group. Serum glutamic oxaloacetic transaminase (SGOT) activity was significantly decreased in the diabetic control group in comparison to a normal control group. Treatment with GG and standard anti-diabetic drugs significantly attenuated this parameter. Serum glutamate pyruvate transaminase (SGPT) activity was significantly increased in the diabetic control group in comparison to a normal control group. Group GG and standard anti-diabetic drugs significantly attenuated this parameter, respectively, in comparison to diabetic control rats. Further total protein level was significantly decreased in the diabetic control group in comparison to a normal control group. Group GG and standard anti-diabetic drugs failed to attenuate total protein level to a significant extent [Table 5].

DISCUSSION

Honey was described in the classical text of Ayurveda for the treatment of diabetes.[24] Along with this, its anti-diabetic property was reported.[25] The role of adjuvant was well described in Ayurveda for the treatment purpose.[26] So here, it was decided to use honey as an adjuvant with *T. cordifolia* dried extract to increase its efficacy and assess its role as adjuvant and anti-diabetic effect.[27]

Table 2: The effect Group GG on weight of liver and kidney in STZ induced diabetic Wistar strain albino rats

Organs	Kidney (g/100 g)	Liver (g/100 g)
NC	0.65±0.05	2.38±0.08
DC+STZ	0.99±0.07$^{\alpha\alpha}$	3.49±0.19$^{\alpha\alpha\alpha}$
Percentage change in comparison to NC	52.31	46.64
GG+STZ	0.95±0.04	3.35±0.25
Percentage change in comparison to DC	04.40	04.01
RS+STZ	0.92±0.08	3.36±0.17
Percentage change in comparison to DC	07.07	03.73

Data: Mean±SEM, $^{\alpha\alpha}P$<0.01, $^{\alpha\alpha\alpha}P$<0.001 (comparison to normal control group, Unpaired *t*-test). STZ: Streptozotocin, SEM: Standard error of mean

The blood glucose levels were increased as expected in STZ-injected animals since STZ causes a massive destruction of β-cells of Islets of Langerhans thereby inducing hyperglycemia.[28-31]

An increase in the liver weight was observed in diabetic animals might be due to decreased breakdown of glycogen. It was reported in previous studies that 15% rise in kidney weight within 72 h of induction of STZ in experimental diabetic rats. This may be due to the glomerular cell proliferation accompanying glomerular enlargement in the early phase of STZ-induced diabetes in rats. Treatment with GG and RS were failed to reverse STZ induced change in the weight of liver and kidney to a significant extent.

The diabetic control group showed marked elevation in blood glucose levels during all the time intervals. Glibenclamide is a standard hypoglycemic drug that stimulates insulin secretion from β-cells of islets of Langerhans. The observed BSL reducing the effect of test drugs may be due to similar mechanisms.

Previous studies have reported that *T. cordifolia* may act by increasing hepatic glycogen synthase and decreasing glycogen phosphorylase activity.[32] Glucose-6-phosphatase and fructose 1, 6-bisphosphatase are the important regulatory enzymes in the gluconeogenic pathway. Glucose-6-phosphatase is one of

Table 3: The effect of Group GG on blood glucose level in STZ induced diabetic rats at various intervals (actual data recorded during study)

Group	Blood glucose level (mg/dl)					
	0 day	5th day	10th day	15th day	21st day	% change in comparison to 0 day
DC+STZ	383.67±51.44	380.33±37.26	355.33±28.09	372.83±34.49	328.17±30.67	14.67
GG+STZ	413.20±36.69	309.00±82.52	531.67±67.33	338.50±113.68	310.2±16.38	24.93
RS+STZ	512.83±21.43	374.33±22.16	334.83±32.03	314.83±29.68	287.00±33.78*	44.04

Data: Mean±SEM, *P<0.05 (comparison to diabetic control, unpaired t-test). STZ: Streptozotocin, SEM: Standard error of mean

Table 4: The effect of test drugs on glycated haemoglobin

Parameter	HbA1c
NC	5.62±0.06
DC	12.12±0.70^{aaa}
Percentage change in comparison to NC	115.66
GG+STZ	08.61±0.98*
Percentage change in comparison to DC	28.96
RS+STZ	7.68±0.92**
Percentage change in comparison to DC	36.63

Data: Mean±SEM, ^{aaa}P<0.001, (comparison to normal control group, unpaired t-test). *P<0.01, **P<0.01 (comparison to DC group, unpaired t-test). STZ: Streptozotocin, SEM: Standard error of mean, HbA1c: Glycated hemoglobin

the key enzymes in the homeostatic regulation of blood glucose levels. It catalyzes the terminal step in both gluconeogenesis and glycogenolysis, converting glucose-6-phosphate to glucose. This enzyme is mainly found in the gluconeogenic tissues liver and kidneys, where it plays a major role in the glucose production.[33] Fructose 1,6-bis phosphatase catalyzes one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of this process,[34] catalyzing the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate. Activities of these two enzymes are found to be increased in diabetes. It was reported that the activity of glucose-6-phosphatase was increased 2-3 fold, and the activity of fructose1, 6-bisphosphatase in diabetes was increased 2-5 fold.[35] Activities of these two enzymes in the kidneys were increased in diabetic rats. Treatment with *T. cordifolia* found significantly decreases their levels in kidneys. This may be possible that *T. cordifolia* could decrease the blood glucose levels in treated animals.[36]

Another study revealed that antidiabetic activity of methanolic extract of *T. cordifolia* stem may be due to increased serum C-peptide levels in the blood which directly suggest that regeneration of β-cells of Langerhans. It was also observed in the same study that induction of methanol extract of *T. cordifolia* increased the glucokinase activity and decreased the glucose-6-phosphatase.[37]

Some studies reported that isoquinoline alkaloid reach fraction from stem, including pamatine, jatrorrhizine, and magnoflorine of *T. cordifolia* have been reported for insulin mimicking and insulin releasing effect both *in vitro* and *in vivo*.[38] Oral treatments of root extracts have been reported to regulate blood glucose levels, enhance insulin secretion and suppress OS markers. Initiation and restoration of cellular defense antioxidant markers including superoxide dismutase, glutathione (GSH) peroxidase and GSH, inhibition of glucose 6-phosphatase and fructose 1, 6-diaphosphatase, restoration of glycogen content in liver were reported *in vitro* studies.[38] The crude stem ethyl acetate, dichloromethane, chloroforms and hexane extracts of *T. cordifolia* inhibited the enzymes salivary and pancreatic amylase and glucosidase[39] thus increase the postprandial glucose level and finds potential application in the treatment of diabetes mellitus.[40]

A recent study observed the activity of hexokinase enzyme that it was decreased in the liver of alloxan induced diabetic rats, but the administration of *T. cordifolia* methanolic stem extract into alloxan induced rats resulted in an increased activity of liver hexokinase. The increased activity of hexokinase leads to increased glycolysis and increased utilization of glucose for energy production.[41] *T. cordifolia* methanolic stem extract was found to reduce the level of glucose in the blood. The activity of fructose 1,6 biphosphatase and glucose 6 phosphatase were found to be increased in the untreated diabetic rats liver,[42] in this study fructose 1,6 biphosphatase and glucose 6 phosphatase activity was brought to normal for treatment with *T. cordifolia* methanolic stem extract.[43]

Any of the above discussed mechanism behind the action of *T. cordifolia* dried extract may be present, but needs further detailed study. The results observed in the present study found similar to previous studies.[44]

Glycated hemoglobin (HbA1c) levels were increased in uncontrolled diabetes [Table 4]. This has shown to be associated with the induction of oxidative stress and subsequent tissue damage to major organs such as the heart and kidneys that may lead to other diabetic complications. Administration of GG and RS prevented it significantly. Decrease in HbA1c levels in diabetic rats could be due to the result of improved glycemic control proved by *T. cordifolia.*

The elevated levels of triglyceride were observed in STZ diabetes rats indicate that triglyceride metabolism was affected

Table 5: The effect of test drugs on various serum biochemical parameters

Parameters	NC	DC+STZ	GG+STZ	RS+STZ
B. glucose (mg/dl)	117.50±2.50	321±27.80$^{\alpha\alpha\alpha}$	319.80±73.66	286.33±29.52
Cholesterol (mg/dl)	57.17±4.72	62.00±4.03	059.20±05.30	58.83±5.87
Triglyceride (mg/dl)	64.33±7.28	80.83±4.10	80.80±11.37	70.83±6.02
HDL (mg/dl)	25.00±3.22	34.17±2.60	28.40±04.26	28.33±3.26
Blood urea (mg/dl)	90.33±4.92	137.17±11.66$^{\alpha\alpha}$	147.40±20.86	100.50±10.43*
Creatinine (mg/dl)	0.58±0.03	0.72±0.05$^{\alpha}$	0.66±0.07	0.75±0.07
SGPT (IU/L)	75.50±8.60	323.00±21.29$^{\alpha\alpha\alpha}$	184.20±04.75***	90.33±3.10***
SGOT (IU/L)	223.00±12.84	120.83±11.67$^{\alpha\alpha\alpha}$	386.80±84.26*	304.67±20.66***
Total protein (g/dl)	7.35±0.11	6.70±0.23$^{\alpha}$	06.91±0.13	6.08±0.31
Albumin (g/dl)	3.38±0.11	3.08±0.09	3.28±0.12	3.00±0.18
Globumin (g/dl)	3.93±0.16	3.67±0.25	3.12±0.31	3.13±0.27

Data: Mean±SEM, $^{\alpha}P<0.05$, $^{\alpha\alpha}P<0.01$, $^{\alpha\alpha\alpha}P<0.001$ (comparison to normal control group, unpaired *t*-test). *$P<0.05$, ***$P<0.001$ (comparison to DC group, Unpaired *t*-test), HDL: High-density lipoprotein, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamic oxaloacetic transaminase, STZ: Streptozotocin, SEM: Standard error of mean

with this condition. This was attenuated by RS treated group. However, if the drug itself as lipid metabolism modifying effects the observed effect may be different. Thus, the non-significant effect observed in GG may be due to the inhibitory effect of the adjuvant on triglyceride breakdown and utilization, whereas the effect observed in groups may be indicative of the presence of anti-hyperglycemic effect on them.

In the present study, a significant increase in blood urea levels was observed in STZ control rats in comparison to normal rats. Results obtained showed that GG increased the serum urea level. RS drug which produced a good anti-hyperglycemic effect and correlating lowering of serum cholesterol, serum triglyceride, and serum HDL level reversed the elevation observed in the urea level at significant extent. This indicates that RS *per se* have the property of reversing nitrogen metabolism altering effect leading to decrease in this elevated parameter. This further gives observation that RS reversing altered nitrogen metabolism may attenuate degeneration of organs and catabolism which may reverse the stimulation of gluconeogenesis pathway.

The serum creatinine level is considered as a marker of kidney function. Multiple changes in protein metabolism occur in diabetes mellitus. Enhanced catabolism of muscle proteins in diabetes elevates the serum creatinine levels.[45] In the present study, induction of STZ diabetes led to a significant elevation in serum creatinine level. This parameter was not affected to a significant extent by RS administration in which a marginal elevation was observed. In contrast, a moderate decrease was observed in GG administered group. It can be suggested that the observed effect may have linkage with the anti-hyperglycemic effect of the test drug. From these results, it can be assumed that GG may protect the protein catabolism in muscle or it ameliorates the renal functions in diabetic rats.

The increase in the activities of plasma SGOT, SGPT indicated that diabetes may induce hepatic dysfunction. In present study, significant increase in SGPT levels were observed in STZ induced diabetic animals. It was reversed in GG and RS in a significant manner. It shows that test drug and RS attenuated the hepatic and renal dysfunction of STZ induced diabetic rats. The SGOT levels were decreased to a significant extent in STZ-induced diabetic rats. This decrease was reversed at significant extent in RS and GG group. The observations show that test drug and RS may have the property of reversing diabetes induced hepatic and renal dysfunction which correlates the anti-hyperglycemic activity of test drug and reference standard drug.

In the present study, a significant decrease was observed in serum total protein level in STZ diabetic rats. This indicates the severity of the diabetes is strong enough to produce significant changes in this protein related parameters. Administration of test drug and RS for 20 days were attenuated elevated serum total protein level but not up to significant extent. This supports the anti-diabetic activity of test drugs and glibenclamide, which may help to reduce diabetic nephropathy or diabetes induced protein catabolism.

The degenerative changes observed in the pancreas, liver and kidney in diabetic control rats can be considered as indicators of diabetes induced degenerative changes. The liver and kidney sections were studied histopathologically. As expected, the sections of liver and kidney from diabetic control groups showed severe degenerative changes. These changes were markedly reversed by test formulation (Figures 1a-d and 2a-d). This may be due to anti-oxidant activity of test formulation. However, this needs further study.

Marked degeneration of Islets of Langerhans and degranulation of β-cells of pancreas in the histo-pathological sections of diabetic control animals. Treatment with test formulations moderately reversed these changes (Figure 3a-d); the observed activity profile may be attributed to cytoprotective property of the test drug.

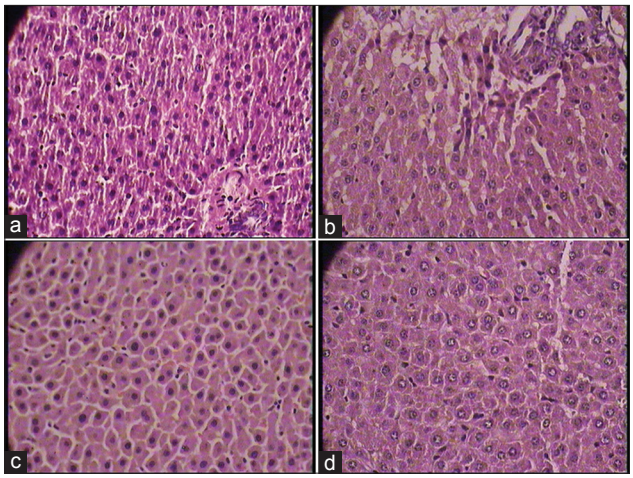

Figure 1: (a) Normal cytoarchitecture of liver in normal control group (1 × 400 magnification), (b) photomicrographs of a representative section of liver. Macro and micro fatty changes, cell infiltration in almost all the sections streptozotocin control group (1 × 400 magnification), (c) *Tinospora cordifolia* treated rats shows non-significant changes of liver in comparison with streptozotocin control group diabetic rats (1 × 400 magnification), (d) glibenclamide treated rat showed almost normal cytoarchitecture of liver sections in comparison with streptozotocin control group diabetic rats (1 × 400 magnification)

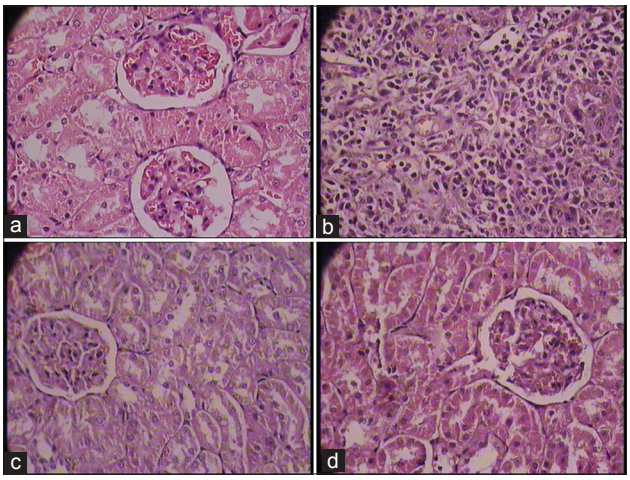

Figure 2: (a) Normal cytoarchitecture of sections of kidney in normal control group (1 × 400 magnification), (b), photomicrographs of representative section of kidney. Cell infiltration and micro-fatty changes in all the sections of streptozotocin control treated diabetic rats (1 × 400 magnification), (c) *Tinospora cordifolia* treated rat showed almost normal cytoarchitecture in comparison with streptozotocin control group diabetic rats (1 × 400 magnification), (d) glibenclamide treated rat showed Almost normal cytoarchitecture in comparison with streptozotocin control group diabetic rats (1 × 400 magnification)

By considering the antidiabetic activity of honey[25] and its adjuvant anti-diabetic effect[46,47] it may be possible that revealed results are due to *T. cordifolia* as well as the adjuvant antidiabetic action of honey too.

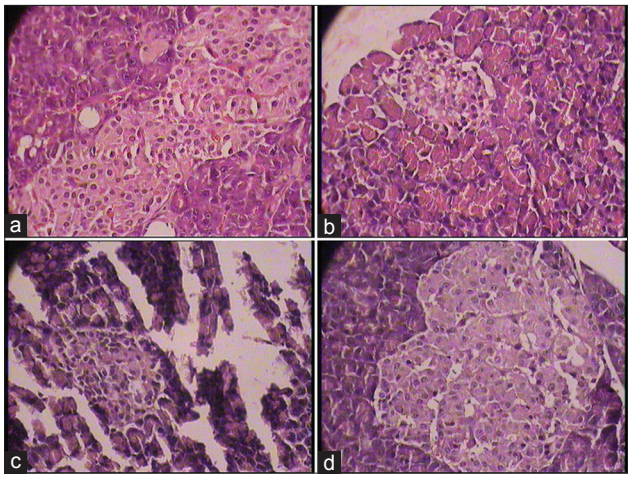

Figure 3: (a) Normal cytoarchitecture in normal control group, (b) Photomicrographs of representative section of the pancreas. Marked degeneration of Islets of Langerhans and degranulation of streptozotocin control treated diabetic rats (1 × 400 magnification), (c) *Tinospora cordifolia* treated rats shows comparatively less degeneration of Islets of Langerhans and degranulation in comparison with streptozotocin control group diabetic rats (1 × 400 magnification), (d) glibenclamide treated rat showed normal cyto-architecture with intact granules in comparison with streptozotocin control group diabetic rats (1 × 400 magnification)

CONCLUSION

From the overall analysis of data obtained during the study, it can be concluded that dried extract of *T. cordifolia* and honey has better potential in the treatment of diabetes mellitus.

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