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Nephroprotective Effect of *Tinospora cordifolia* and Metformin on Alloxan Induced Diabetic Mice

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Abstract

Diabetes mellitus is a metabolic disease marked by hyperglycemia. The chronic hyperglycemia of diabetes is related with long-term damage, dysfunction, and failure of various organs like, the eyes, kidneys, nerves, heart, and blood vessels. Presence of high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. It is predicted that by 2030, India's diabetes burden will be almost 87 million people. Present study reports nephroprotective study of *T. cordifolia* and metformin on histology of kidney and KFT of diabetic mice. Four groups of mice were prepared for comparative study which includes control, diabetic, *Tinospora cordifolia* and Metformin administered group. Diabetic models were prepared in mice by intraperitoneal administration of single dose of alloxan @120 mg/kg b.w. Alcoholic extract of *Tinospora cordifolia* was administered @200 mg/kg b.w/day for eight weeks. Metformin were administered @50 mg/kg b.w/day for eight weeks. In diabetic group of mice glucose, urea, uric acid and creatinine in serum were increased. Effective restoration was observed in *T. cordifolia* administered group in comparison to metformin administered diabetic group of mice. Changes observed in glomerulus, Bowman's capsule, PCT and DCT were also restored effectively in *Tinospora cordifolia* administered group. Thus, it was concluded from the study that alcoholic extract of *T. cordifolia* restores glucose level, KFT and changes in renal corpuscles effectively in comparison to metformin administered group *Tinospora cordifolia* acts effectively on diabetes mice as good as metformin on biochemical parameters.

Keywords: Alloxan; Metformin; *Tinospora cordifolia;* Nephroprotective

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Introduction

Diabetes is a chronic metabolic disorder that poses a major challenge worldwide. This disease is characterized by differences in carbohydrate, protein and fat metabolism caused by the entire or relative insufficiency of insulin secretion and insulin action.

Plant's seeds, berries, roots, leaves, bark, or flowers are used as herbal medicines [1]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2,500 species are in India, out of which 150 species are used commercially on a large scale. Active ingredients of various plant species are isolated for direct use as drugs or pharmacological agents [2]. Traditional medicines or herbal plant extracts used as a natural key to unfold diabetic complications [3].

Metformin is anti-diabetic drug that is used for the therapy for patients with type II diabetes worldwide. The most common adverse effects related to the use of metformin are gastrointestinal upset, lactic acidosis and vitamin B12 deficiency. Metformin treatment increases insulin sensitivity and reduces insulin levels in patients with the polycystic ovary syndrome [4-6]. Metformin therapy may increase blood lactate levels and is occasionally connected with development of lactic acidosis [7,8]. Lactic acidosis is a serious complication of metformin therapy that carries a mortality rate of 30% to 50% [9]. Thus it is necessary to have alternate to this important anti-diabetic drug.

Tinospora cordifolia (Willd.) Miers: Commonly known as guduchi is an herbaceous plant, which is found in tropical areas such as India, Myanmar and Sri Lanka. It is widely used in

indigenous systems of medicine [10,11]. Arabinogalactan is an active compound, which is present in the aqueous extract of *T. cordifolia* stem, has shown to produce immunological activity. The plant possesses antispasmodic, antipyretic, antineoplastic, hypolipidemic, hypoglycemic, immune potentiating and other activities. It is also used in general debility, digestive disturbances, loss of appetite and fever in children, dysentery, gonorrhea, urinary diseases, viral hepatitis, and anemia [12,13].

Present study aims to illustrate nephroprotective effect of *Tinospora cordifolia* and metformin against diabetes of mice.

Materials and Methods

Animals

The mice (*Mus musculus,* BALB/c) were used. The age group of mice were selected for the study was 12 weeks old with 30 ± 2 g body weight (b.w). The mice were housed at controlled environmental conditions 20 ± 2°C, relative humidity 50 ± 10%, and 12 h dark-light cycle. All experiment was conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Permission for animal experimentation was obtained from Institutional Animal Ethics Committee of Mahavir Cancer Institute and Research Centre, Patna (IAEC No. 1129/bc/07/CPCSEA).

Chemicals

Alloxan, manufactured by Loba Chem Pvt Ltd, Mumbai was utilized for the experimental design. Alloxan was administered at the rate of 120 mg/kg b.w. intraperitoneally for induction of diabetes.

Drug used

Metformin was orally administered to the diabetic group of mice at the rate of 50 mg/kg b.w. for 8 weeks.

Medicinal plant used

Alcoholic stem extract of *Tinospora cordifolia* (Willd.) Miers: was orally administered to diabetic group of mice at the rate of 200 mg/kg b.w for 8 weeks. LD50 and Maximum Permissible Dose (MPD) were calculated and 200 mg/kg b.w was selected as low dose of this medicinal plant. Fresh stem of *Tinospora cordifolia* was purchased from herbal store in Patna, India.

Study groups & sampling

The control group of six mice received distilled water orally. The 'treatment' groups (n=6) received alloxan 120 mg/kg b.w by intra-peritoneal route for diabetic model preparation. *Tinospora cordifolia* (200 mg/kg/b. w/day) stem extract administered to diabetic mice orally through gavage. After 96 h of alloxan administration glucose level was analyzed to confirm diabetes induction. These mice were kept untreated for two weeks and glucose level was monitored every week to observe persistence of diabetes. Then after confirmation of diabetes in mice, it was treated with stem extract of *T. Cordifolia* and metformin for eight weeks. Mortality was not observed in any group of experimental animal. Mice were sacrificed after the scheduled treatment. Serum was collected for glucose (GOD/POD method), urea (Mod. Berthelot method), uric acid (Uricase/PAP method) and creatinine (Alkaline Picrate method) estimation.

Statistical analysis of data

The effect of 3 independent experiments were quantified and the data collected from all experiments analyzed by one-way ANOVA (analysis of variance) using statistical software SPSS version 20 (SPSS Inc., Chicago, USA). The purpose of analysis of variance is to test differences in means (for groups or variables) for statistical significance. Significant differences among means were analyzed using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

Results

Change in blood biochemistry

In control group of mice, level of glucose was 99.00 ± 2.30 mg/dl. In diabetic group of mice it was 201.3 ± 12.55 mg/dl. 119.4 ± 1.184 mg/dl metformin 8 weeks administered group of mice. While in *Tinospora cordifolia* 8 weeks administered group it was 106.5 ± 9.695 mg/dl (Text Figure 1 and Table 1).

In control group of mice, urea level was 20.33 ± 2.33 mg/dl. While in diabetic group of mice was 46.73 ± 0.079 mg/dl. Urea level of metformin 8 weeks administered group of mice was 38.52 ± 1.092 mg/dl. While in *T. cordifolia* 8 weeks administered group it was 28.60 ± 0.9299 mg/dl (Text Figure 2 and Table 1).

Uric acid level in control group of mice was 4.34 ± 0.20 mg/dl. In diabetic group of mice was 9.26 ± 0.26 mg/dl. While uric acid level of metformin 8 weeks administered group of mice was 7.817 ± 0.02028 mg/dl. Uric acid in *T. cordifolia* 8 weeks administered group was 6.047 ± 0.01764 mg/dl (Text Figure 3 and Table 1).

Creatinine in control group of mice was 0.74 ± 0.06 mg/dl. In diabetic mice creatinine level was 1.97 ± 0.04 mg/dl. Creatinine level was 0.5547 ± 0.003480 mg/dl in metformin 8 weeks administered group of mice. It was 0.5180 ± 0.0005773 mg/dl in *T. cordifolia* 8 weeks administered group of mice (Text Figure 4 and Table 1).

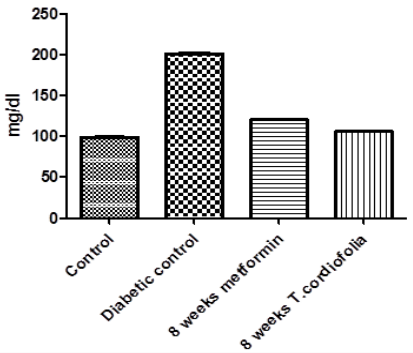
Change in histopathology

Microphotograph of kidney of control mice show normal glomerulus and Bowman's capsule proximal convoluted tubule and distal convoluted tubule are normal in structure. Renal cortex is also normal (Figure 1). Kidney of diabetic mice shows degeneration of cytoplasmic material in glomerulus with clustered nuclei. Degenerated cytoplasmic material with vacuolated spaces was observed. Dilated PCT and DCT were observed with clustered nuclei (Figure 2). Metformin shows restoration in Bowman's capsule. Glomerulus was restored with normal cytoplasmic and nuclear

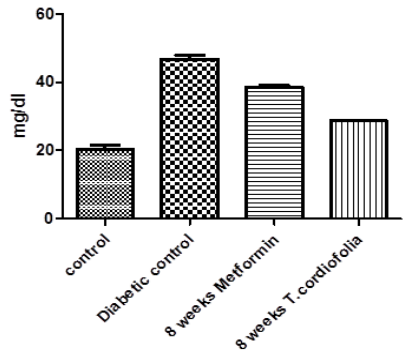
Table 1: Showing glucose level and kidney function test in different group of mice.

	Kidney Function Test in different group of mice			
	Glucose Level (mg/dl)	Urea Level (mg/dl)	Uric acid Level (mg/dl)	Creatinine level (mg/dl)
Control	99.00 ± 0.577	20.33 ± 1.154	4.34 ± 1.154	0. .74 ± 0.127
Diabetic	201.00 ± 1.154	46.73 ± 1.154	9.33 ± 0.365	1.65 ± 0.550
Metformin 8 weeks	120.40 ± 1.249	38.52 ± 0.577	7.28 ± 0.962	0.55 ± 0.190
T. cordifolia 8 weeks	105.66 ± 1.892	28.60 ± 0.250	6.71 ± 0.347	0.51± 0.176

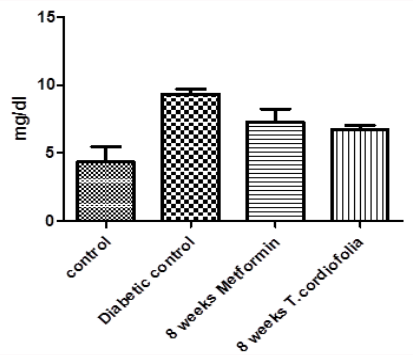
*Values are mean ± SE from 3 independent experiments; Mean within a column followed by common letters is not significantly different at $P \leq 0.05$, according to Duncan Multiple Range Test (DMRT).



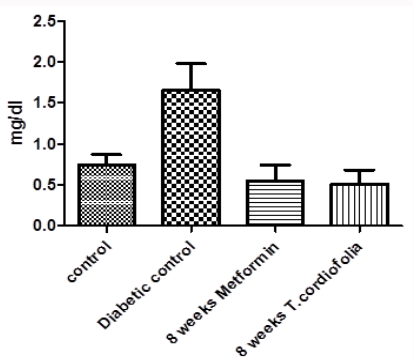
Text Figure 1: Glucose level in different group of mice.



Text Figure 2: Urea level in different group of mice.



Text Figure 3: Uric acid level in different group of mice.



Text Figure 4: Creatine level in different group of mice.

material. PCT and DCT were observed in restored condition. Few clustered nuclei were observed in PCT (Figure 3). Enlarged view of

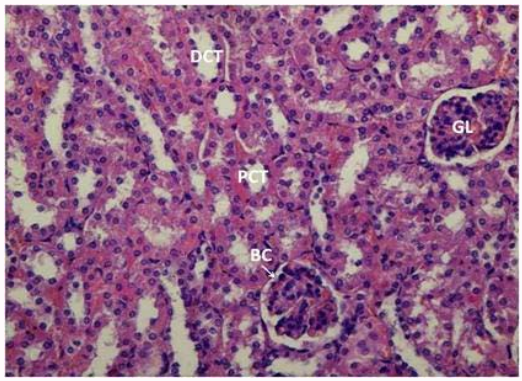


Figure 1: Microphotograph of kidney of control mice showing normal glomerulus and Bowman's capsule. Proximal convoluted tubule and distal convoluted tubule are normal in structure. Renal cortex is also normal.

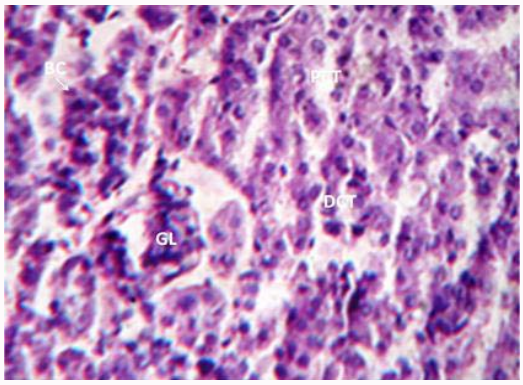


Figure 2: Kidney of diabetic mice shows degeneration cytoplasmic material in glomerulus with clustered nuclei. Degenerated cytoplasmic material with vacuolated spaces. Dilated PCT and DCT were observed with clustered nuclei.

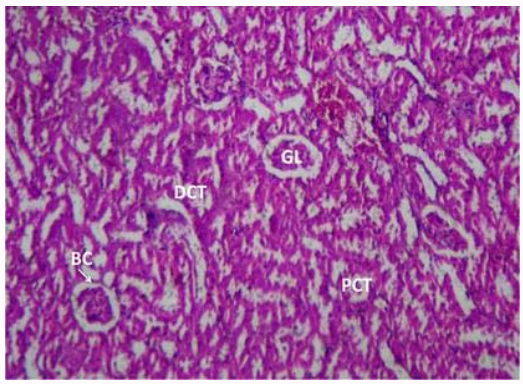


Figure 3: Metformin shows restoration in Bowman's capsule. Glomeruli were restored with normal cytoplasmic and nuclear material. PCT and DCT were observed in restored condition. Few clustered nuclei were observed in PCT.

glomerulus shows well organized nuclear material and cytoplasmic material in glomerulus. Bowman's capsule is continuous with distinct podocyte cells. Restoration was also observed in DCT effectively. Few degenerated cytoplasmic materials were observed in PCT (Figure 4). *Tinospora cordifolia* shows effective restoration in glomerulus and Bowman's capsule. PCT and DCT were also restored like normal one. Few vacuolated spaces were observed (Figure 5). *T. cordifolia* shows effective restored glomerulus with almost normal Bowman's capsule. PCT and DCT were restored effectively with normal cytoplasmic and

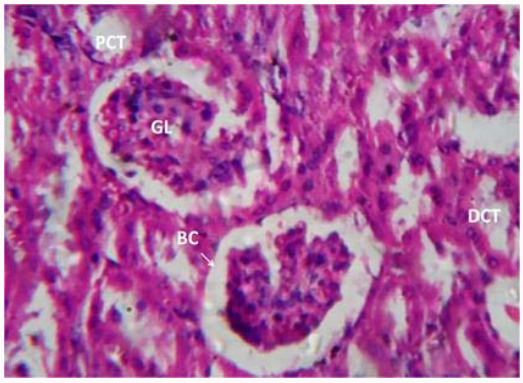


Figure 4: Enlarged view of glomerulus shows well organized nuclear material and cytoplasmic material in glomerulus. Bowman's capsule is continuous with distinct podocyte cells. Restoration was also observed in DCT effectively. Few degenerated cytoplasmic materials were observed in PCT.

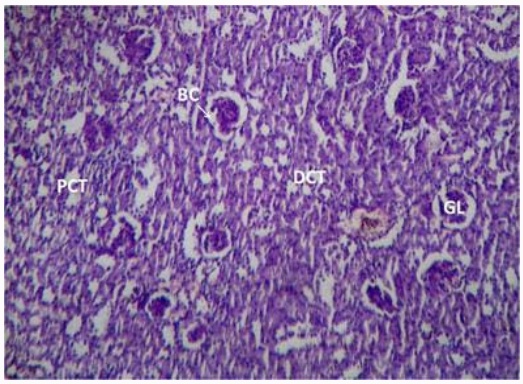


Figure 5: *Tinospora cordifolia* shows effective restoration in glomerulus and Bowman's capsule. PCT and DCT were also restored like normal one. Few vacuolated spaces were observed.

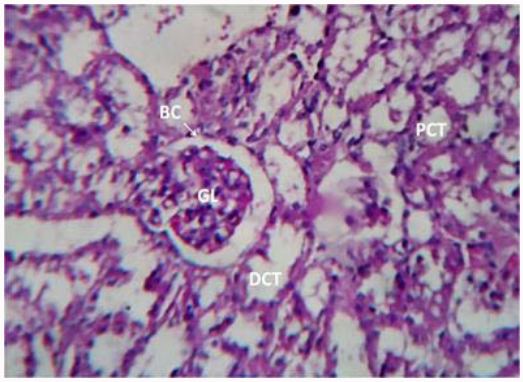


Figure 6: *T. cordifolia* shows effective restored glomerulus with almost normal Bowman's capsule. PCT and DCT were restored effectively with normal cytoplasmic and nuclear material. Few clustered nuclei were observed on DCT.

nuclear material. Few clustered nuclei were observed on DCT (Figure 6).

Discussion

The diabetes associated complications include retinopathy, neuropathy, nephropathy and atherosclerosis [14]. Atherosclerosis consists of coronary artery disease, leading to myocardial infarction or angina, stroke and intermittent claudication as well as diabetic foot [15]. Diabetic nephropathy is the most common micro vascular problems of diabetes [16]. Kidney maintains optimum chemical composition of body fluid by acidification of urine and exclusion of metabolic wastes such as urea, uric acid, and creatinine. During renal diseases, the concentration of these metabolites rises in blood [17]. Diabetic nephropathy is a long standing common problem of diabetes, which is a leading cause of end stage renal disease and cause of diabetes mellitus linked with morbidity and mortality [18]. There are proofs that subclinical chronic inflammation is complex in the pathogenesis of diabetic nephropathy [19-21]. In our present study we have also observed degeneration in renal corpuscles.

Metformin is contraindicated in cases where there is conjoined tissue perfusion such as cardiovascular, pulmonary, renal and liver failure, and this limits its use [22]. Gastrointestinal side effects, i.e. diarrhea, nausea, bloating and metallic taste in the palate are not rare when treatment with metformin is started, affecting 1% to 30% of patients [23]. The risk of hypoglycemia was low, exactly the same as in the placebo group. Lactic acidosis is the most risky side effect, luckily rare, with an incidence of 0 to 0.084 cases/1000 patient/year [2]. In our study we have observed restoration in glucose level after 8 weeks metformin administration while urea, uric acid and creatinine are not restored effectively.

Tinospora cordifolia stem phytochemical alkaloids are heterocyclic indole compounds, which have showed pharmacological properties [25]. The presence of flavonoids and tannins in all the plants expected responsible for the free radical scavenging effects. Flavonoids and tannins are phenolic compounds and plant phenolics are main group of compounds that are as primary antioxidant or free radical scavengers [26]. In our study we have observed effective restoration in urea, uric acid, creatinine and glucose in *Tinospora cordifolia* administered group. Renal corpuscles are also maintained effectively [27].

Conclusion

It is concluded from entire study that *Tinospora cordifolia* effectively restores glucose level in diabetic mice. It also restores diabetes induced changes in urea, uric acid and creatinine very effectively in comparison to metformin. Glomerulus and Bowman's capsule are restored more in stem extract of *Tinospora cordifolia* administered group in comparison to metformin administered group. Alloxan induced diabetes causes insulin deficiency due to degeneration in beta cells of islets of Langerhans. It is evident from the study that *Tinospora cordifolia* maintains glucose level and renal profile very effectively in comparison to metformin.

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