Original Research Article

Comprehensive Evaluation of the Anti-hyperglycemic Efficacy of *Tinospora cordifolia* in Rodent Models Coupled with a Detailed Assessment of Its Adverse Effect Profile

Abstract:

A prevalent endocrine condition, diabetes mellitus (DM) is generally characterized by diminished insulin function, which results in hyperglycemia and complications such as neuropathy and retinopathy. Bigunaides, sulfonylureas, thiazolidinediones (TZDs) and SGLT2 inhibitors are common oral antidiabetic drugs while each has unique adverse effects. A medicinal herb called *Tinospora cordifolia* has the potential to control blood sugar levels by lowering oxidative stress and increasing insulin secretion. In the experimental rat model, diabetes was induced using alloxan. 50 rats were split up into ten groups for the study in order to examine how *Tinospora cordifolia* extract affected blood glucose levels. Following alloxan-induced diabetes, the rats were given metformin, different dosages (such as 300mg, 600 mg and 900 mg) of *Tinospora cordifolia* extract or plain water and food from day 7 to day 42. Elevated blood glucose, liver damage (increased SGPT/SGOT), kidney dysfunction (high creatinine/urea), and changed lipid profiles were all consequences of alloxan-induced diabetes. The therapeutic potential of the plant extract in managing diabetes and its complications is suggested by the significant reduction in blood glucose, improvement in liver and kidney markers, and correction of lipid imbalances, especially at medium (600mg) and high doses (900 mg).

Keyword: *Tinospora cordifolia*, Alloxan, antidiabetic, neuropathy, rat model

Introduction:

Diabetes mellitus represents the most prevalent disorder of the endocrine system characterized by impairments in insulin secretion, insulin function, or a combination of these factors. Consequently, a lack of insulin causes persistent hyperglycemia along with inefficiencies in the metabolism of proteins, lipids, and carbohydrates. Major diabetes complications, such as retinopathy, neuropathy, nephropathy, cardiovascular diseases, and ulceration, arise from tissue or vascular damage that happens as the disease progresses. As a result, diabetes encompasses a wide range of different conditions. [1]. Biguanides, sulfonylureas, thiazolidinediones (TZDs), dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter-2 (SGLT2) inhibitors, and alpha-glucosidase inhibitors are the primary classes of oral antidiabetic medications [2].

Among biguanides, metformin is the most commonly prescribed first-line oral drug for the treatment of type 2 diabetes mellitus in people of all ages. Nevertheless, it may result in an acute medical condition called lactic acidosis, which is characterized by symptoms like dizziness, extreme sleepiness, fatigue, chills, bluish or cold skin, muscle soreness, a slow or irregular heartbeat, and abdominal pain that is accompanied by nausea, vomiting, or diarrhea [3]. The primary side effect of all all sulfonylureas is hypoglycemia, while other common side effects include

The primary side effect of all sulfonylureas is hypoglycemia, while other common side effects include headache, dizziness, nausea, hypersensitivity reactions, and weight gain. Sulfonylureas should not be used in patients with liver or kidney diseases, nor in pregnant women, as they may cause prolonged hyperglycemia in new born [2]. Thiazoli-dinediones (Rosiglitazone) is a new class of insulin-sensitizing antidiabetic drugs that attach strongly to the peroxisome proliferator-activated receptor (PPAR-γ). However, Studies reported a notable rise in total cholesterol and triglyceride levels in patients treated with rosiglitazone [4]

SGLT2 inhibitors are a class of medications that help lower blood sugar levels by blocking the kidney's ability to reabsorb glucose, without the need for insulin. While these drugs can be effective, they also come with some potential side effects, such as acute kidney injury, infections, diabetic ketoacidosis, and genital or urinary tract complications [5]. DPP-4 inhibitors are a crucial class of oral diabetes medications that are often prescribed as a second-line treatment when metformin alone isn't enough. These drugs work by boosting insulin production, and they've proven to be safe and well-tolerated in clinical trials. However, the most common side effects reported were nasopharyngitis and skin irritation [6]. Complex carbohydrates are broken down into absorbable monosaccharide units by the intestinal alpha-glucosidase enzyme, which is reversibly inhibited by the α-glucosidase inhibitors such as acarbose. Blood glucose levels rise less sharply and more slowly after a meal as a result of this action. With acarbose, the most frequent adverse drug responses are flatulence, diarrhea, and abdominal pain [7].

Herbal medicine has achieved exponential growth in recent years and is becoming increasingly popular in both developed and developing nations due to its natural origins and fewer adverse effects [8]. Medicinal herbs have been used empirically as antidiabetic treatments and have been reported to be helpful in the treatment of diabetes worldwide. Diabetes and its associated consequences remained a significant medical issue even with the availability of recognized antidiabetic medications on the pharmaceutical market. These plants are thought to have anti-hyperglycemic properties because they can help the pancreatic tissues work again by increasing insulin production, blocking intestinal glucose absorption, or facilitating metabolites in insulin-dependent processes. Although there are over 400 plant species with hypoglycemic activity documented in the literature, it is still appealing to look for novel antidiabetic medications made from natural plants since they contain compounds that have safe and different effects on diabetes mellitus. The glycosides, alkaloids, terpenoids, flavonoids, cartenoids, and other compounds found in the majority of plants are commonly thought to have antidiabetic properties [9].

Tinospora cordifolia, also known as "Guduchi" in Sanskrit, is a large, deciduous, climbing shrub with greenish yellow flowers that is genetically varied and found at higher elevations. It belongs to the Menispermaceae family. Alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides are among the many active ingredients that have been extracted from the plant's various parts, including the root, stem, and entire plant. Because of its reported medicinal properties, including anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, antioxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic activities, the plant has recently attracted the attention of researchers worldwide [10].

By controlling blood glucose, the stem of *Tinospora cordifolia* is frequently utilized in diabetes treatment. It has been shown to mediate its anti-diabetic potential and control blood glucose by reducing oxidative stress (OS), increasing insulin secretion, and blocking gluconeogenesis and glycogenolysis [11]. The stem's isoquinoline alkaloid-rich fraction, comprising palmatine, jatrorrhizine, and magnoflorine, has been shown to exhibit both in vitro and in vivo insulin-mimicking and insulin-releasing properties [12]. According to reports, the root extract lowers the levels of ceruloplasmin, hydroperoxides, plasma thiobarbituric acid reactive compounds,

glycosylated hemoglobin, and vitamin E in diabetic rats [13]. In diabetic rats, *T. cardifolia* root extract (TCE) has been shown to have a hypoglycemic and hypolipidemic effect by decreasing hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) [14]. An α-glucosidase inhibitor known as saponarin (apigenin-6-C-glucosyl-7-O-glucoside) was found in the leaf extract of *Tinospora cordifolia*. Different-origin sucrase and α-glucosidase activity were both competitively inhibited by saponarin [15].

Method and Materials

We purchased ethanol, alloxan, normal saline, chemicals, and kits from Sigma Aldrich in Germany. Metformin, a common antidiabetic medication that we used in this study, was a privilege from Incepta Pharmaceutical Limited. Blood serum analysis kits for total cholesterol, HDL, LDL, triglycerides, SGOT, SGPT, and creatinine were offered by Plasmatic Laboratory Product Ltd. in the UK. A Humalyzer 3000, a semi-automated clinical chemistry analyzer manufactured by Medigroup Asia Limited in Cambodia, was used to evaluate the biochemical parameters. The Alere GI glucometer, manufactured by Alere Inc. in the United States, was acquired from Shahbag in Dhaka, Bangladesh.

Plant Collection and Extraction

We procured seeds of *Tinospora cardofolia* from neighborhood market. The process of taxonomic identification and authentication was then carried out. The plant sample was kept at Bangladesh's National Herbarium after their prerequisites. Accession number DACB 94814 was issued by the herbarium authorities on April 4, 2024, for future use. Before being ground into a fine powder, cardifolia seeds were allowed to dry in the shade for seven to ten days. For 96 hours, the ground seeds were vigorously shaken while submerged in 70% ethanol. The extract was filtered and the filtrate was gathered after it had completed soaking. A rotary evaporator was used to further concentrate the filtrate. The dried extract was then meticulously gathered and preserved for later use.

Ethical Review: The ethical Review Committee of the Chandpur Medical College , Chandpur , has given the ethical approval for this work (Ref. no. MIU/SEST/ERC/2024003).

Expermental Animal: In this study, Healthy adult male wistar rats weighing 125–200 grams were acquired from the pharmacy department of Jahangirnagar University in Dhaka, Bangladesh. The rats were acclimatized at the Institute of Nutrition and Food Science, University of Dhaka, in a temperature-controlled, well-ventilated space with a constant seven-day cycle of light and dark. The animals were routinely given clean water and fed a classic pellet diet. Prior to the experiment, the rats were placed there for adaptation purposes. The Institutional Animal Ethics Committee's (IEAC) guidelines were followed during all animal procedures. Animals were handled and treated in accordance with the standards set by the Swiss Academy of Sciences (SCNAT) and the Swiss Academy of Medical Sciences (SAMS).

Experimental Guidelines: All studies were carried out in accordance with the 2013 Declaration of Helsinki's ethical guidelines. The "3R" principles, which are recognized as a fundamental element of Swiss and international standards governing animal research, were strictly followed in every aspect of the investigation. The initial "R" stands for "replacement," which can be absolute (such as substituting computer-simulated models for animal models) or relative (such as substituting vertebrates for invertebrates or living animals for tissue or cell cultures). As part of the "replacement" concept, we started our investigation with an in-silico analysis. Regretfully, this model was unable to generate enough documentation. Consequently, an animal model was employed for additional research. Mammalian vertebrates, such as rats, were selected over invertebrates for anti - hyperglycemic investigation because these animals had specialized pancreatic and beta cells. "Reduction" is the second "R" in this principle, signifying a method that employs fewer animals to gather adequate evidence to address research questions or maximize the information gleaned from each animal. The "power analysis method to meet this condition" was used to determine the sample size, and there were five rats in each group for this study. The third "R" stands for "refinement," which suggests lowering the experimental animals' pain threshold by alleviating their discomfort. Before and after every blood glucose test, the tips of the rats' tails were rubbed with isopropyl alcohol to make the process more comfortable and less unpleasant. The rats were given a healthful meal throughout the experiment, and in accordance with the 2013 edition of the Guidelines for the Euthanasia of Animals, they were put to sleep under general anesthesia without causing any pain.

Induction of diabetes: Alloxan was used to cause hyperglycemia in the experimental rat model. Initially, a cold citrate buffer (0.1 M; pH=4.5) was used to dissolve Alloxan. The rats were subsequently given 150 mg/kg body weight of alloxan intraperitoneally [16]. Blood glucose levels were checked four times a day at six-hour intervals following the administration of alloxan. All experimental rats given alloxan had a mean blood glucose level of greater than 12.5 mmol/L within 72 hours, which is indicative of hyperglycemia or diabetes [17]. *T. cardifolia* extract and metformin were administered orally to rats.

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Evaluation of Anti-hyperglycemic Activity: To determine how the extract affected blood glucose levels, 50 rats were split up into 10 groups. Rats in groups 2–6 were first given injections of alloxan after their blood glucose levels were assessed. Over the following three days, alloxan-induced blood glucose levels were regularly checked. All of the rats developed diabetes within three days, and they were then left untreated for seven days. Beginning on the seventh day, each group received the particular medications shown in the graph. Treatment lasted until day forty-two. Interestingly, three groups were given metformin, low, medium and high doses of the T. Cardifolia extraxct, respectively, in place of alloxan. From day 14 to day 42, the rats received their prescribed doses. Furthermore, rats in group 1 received water and standard food [18].

Table 1: Anti-hyperglycemic activity assessment

Group Number	**Group Status**	**Treatment Specimen**	**Dose of Treatment specimen**	**Group Abbreviation**
01	Control	Physiological Saline	10 ml/kg	C
02	Alloxan Control	Alloxan	N/A	A
03	Alloxan+ *T.Cardifolia*	*T.Cardifolia*	300 mg/kg	A+ T_{300}
04	Alloxan+	*T.Cardifolia*	600 mg/kg	A+T_{600}

	T.Cardifolia			
05	Alloxan+ *T.Cardifolia*	*T.Cardifolia*	900 mg/kg	A+ T_{900}
06	Metformin	Metformin	100 mg/ 60 kg	M
07	*T.Cardifolia*	*T.Cardifolia*	300 mg/kg	T_{300}
08	*T.Cardifolia*	*T.Cardifolia*	600 mg/kg	T_{600}
09	*T.Cardifolia*	*T.Cardifolia*	900 mg/kg	T_{900}

Biological Sample Collection: Rat's tail tips were punctured to obtain blood samples in order to measure blood glucose levels. Following the sacrifice of the rats, blood was promptly extracted by puncturing the heart and transferring it to a micro centrifuge tube. The collected specimens were centrifuged for five minutes at 5,000 rpm in order to obtain the supernatant fluid. The fluid was transferred to a different microcentrifuge tube for biochemical analysis. The kidneys, livers, and pancreas were immediately removed from the animal's body after sacrifice and meticulously cleaned with ice-cold saline in order to perform histopathological tests and enzymatic activity.

Estimation of Biochemical Parameters: Blood glucose levels were measured using a glucometer. Renal, liver function, and cardiac profile tests were performed in addition to the Humaluzer 3000 [19].

Statistical Analysis: The study's parameters are all shown as mean±SD for each group. By analyzing intergroup heterogeneously in terms of multiple biological traits, the "One-way ANOVA test" was utilized to determine statistical significance. The "SPSS 16" software was used to conduct the study. The result was deemed statistically significant when the "p" value was less than 0.05 ($p < 0.05$), and highly significant when the value was less than 0.01 ($p < 0.01$) instead [20].

Result and Discussion

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In this research, we evaluated the antidiabetic properties of *Tinospora Cardifolia* using recognized benchmarks. By performing the change in blood glucose test, it was possible to assess how the *T. cardifolia* extract affected blood glucose levels. Markers like SGPT and SGOT were evaluated as part of the liver function assessment, whereas creatinine and urea were the main focus of the kidney function evaluation. The measurement of total cholesterol, triglycerides, LDL, and HDL was another aspect of the lipid profile analysis.

Determination the change in blood glucose level:

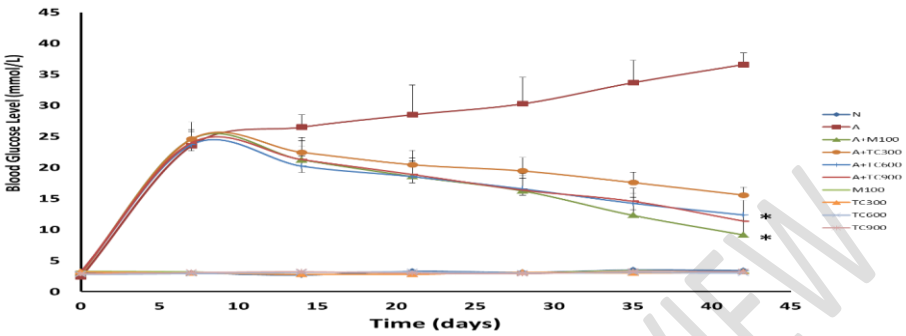

Figure 1: Blood glucose level of rats belonging to 10 groups throughout receiving respective treatments. Values were expressed as mean ± SD (n = 10/group). ∗p< 0.05 indicate significant difference from the disease group (NC = control group, AC= alloxan-treated group, M = metformin, A + M = alloxan + metformin, A +TC = alloxan + *T. cardifolia* and TC = *T. cardifolia*)

Alloxan is a susbstance that is frequently used in experimental studies to cause diabetes in rodents. It is known to specifically damage the beta cells in the pancreas, which causes rats to develop insulin-dependent diabetes [21,22]. As shown in above Figure, our results show that the rats in group 1 had normal blood glucose levels. In contrast to the other groups, the diabetic control group, which was characterized by beta cell destruction and no treatment, showed abnormally elevated blood glucose levels. Blood glucose levels decreased in all groups treated with metformin and the extract, although the reduction varied from group to group. Interestingly, the group that took metformin showed a more noticeable drop in blood glucose levels. Nonetheless, blood glucose levels decreased statistically significantly ($p<0.05$) in response to the medium (600 mg/kg) dose of *T.cardifolia* extract. It was noticed that both the higher and the medium dose significantly lowers blood glucose levels.

Assessment of liver function

Table 2: Effect of various treatment specimens on SGPT and SGOT levels of alloxan-treated rats.

Group Number	Group Status	SGPT	SGOT
1.	NC	43.7 ± 3.91	40.82±4.02
2.	AC	108.26 ±8.62	105.99±6.82
3.	$A+M_{100}$	64.75±6.97	58.91±3.82
4.	$A+TC_{300}$	106.99±7.91	97.58±8.69*

5.	A+TC_{600}	99.34±5.51*	93.47±7.69*
6.	A+TC_{900}	94.69±5.82*	90.09±6.73*
7.	M_{100}	42.88±4.08	40.99±3.21
8.	TC_{300}	42.69±3.91	38.69±2.62
9.	TC_{600}	46.52±4.02	40.76±3.59
10.	TC_{900}	44.94±3.26	42.42±4.01

Comparison of SGPT and SGOT levels (U/L) of rats, belonging to 10 groups just before sacrifice. (*indicates statistically significant change where p<;0.05, correlation is significant at a 95% confidence interval.)

Significant indicators, such as the test groups' SGPT and SGOT values, were compared to the disease control group in order to assess liver functioning. During the tests, there was a noticeable increase in the aforementioned markers in the disease control group. The impact of alloxan, which is known to significantly raise serum SGPT and SGOT levels, is responsible for this sharp rise. This increase, which is a sign of liver damage, is brought on by processes including oxidative stress, hepatocyte fatty degeneration, and diabetes-induced liver pathology [23–26]. The diseased rats' aforementioned markers underwent a notable, dose-dependent change after receiving *T. cardifolia* in vivo. When given at medium and high doses, the constituents of the *T. cardifolia* ethanolic extract successfully counteracted the hepatotoxic effects of alloxan on both SGOT and SGPT markers in a statistically significant way ($p<0.05$). In the case of SGPT, the high dose administered group showed a significant reduction. Besides that, the same dosage showed a decrese in SGOT. Additionally, our plant extract lowers the ALP level in a dose-dependent manner; the group that received the highest dose also showed a noticeable decrease. *Tinospora cardifolia's* diverse composition of bioactive compounds is responsible for its strong therapeutic potential.

Evaluation of kidney function

Table 3: Effect of various treatment specimens on the creatinine level of alloxan-treated rats.

Group Number	Group Status	Urea	Creatinine
1.	NC	45.96 ± 4.59	0.96±0.06

2.	AC	104.22 ±8.71	2.87±0.86
3.	A+M_{100}	55.22±5.06	1.27±0.79
4.	A+TC_{300}	100.56±7.70	2.40±0.92
5.	A+TC_{600}	95.96±5.21*	1.89±0.79
6.	A+TC_{900}	89.61±6.20*	1.33±0.63
7.	M_{100}	47.91±5.28	0.87±0.06
8.	TC_{300}	47.56±4.33	0.82±0.06
9.	TC_{600}	42.61±3.20	0.71±0.09
10.	TC_{900}	40.50±3.59	0.84±0.09

Comparison of creatinine and urea (mg/dl) level of rats, belonging to 10 groups just before sacrifice. Each group consisted of 5 rodents each with equal body mass index. The data were expressed as mean ± standard deviation (*indicates statistically significant change where $p<0.05$, correlation is significant at a 95% confidence interval.).

By examining the important indicator creatinine and urea, a comparison between the different groups was conducted to assess kidney function [27]. The disease control group in this study showed a marked increase in creatinine and urea levels. According to earlier studies, alloxan exposure causes tubular atrophy, dilatation, and interstitial nephritis, which impair renal function and cause nephrotoxicity [28-29]. Creatinine levels significantly decreased ($p<0.05$) when the ethanolic extract of *T. cardifolia* was administered at three different doses (low, medium, and high), with the high dose showing a 46% reduction. Conversely, only the medium and high doses of the extract resulted in a statistically significant restoration of urea levels ($p<0.05$). These result indicated that the extract may be useful as a nephrotoxicity preventive agent[30].

Table 4: Effect of various treatment specimens on the Total cholesterol, triglyceride, LDL, and HDL levels of alloxan-treated rats.

Group Number	Group Status	Total Cholesterol	Triglyceride	HDL	LDL
1.	NC	110.25± 5.53	51.67±3.90	96.18±7.17	24.23±3.01
2.	AC	203.97±9.82	121.56±8.22	51.27±5.82	137.28±7.56
3.	A+M_{100}	137.90±7.81	82.67±6.73	62.61±6.09	47.29±9.21
4.	A+TC_{300}	197.50±6.82	115.67±9.27	93.96±6.71	126.28±6.91*
5.	A+TC_{600}	190.62±7.70	110.212±8.47	88.57±5.79*	114.96±8.21*
6.	A+TC_{900}	182.40±6.89	104.91±7.18	84.83±5.71*	107.22±47.29*

7.	M_{100}	109.57±4.81	50.27±4.91	91.25±0.06	30.21±4.91
8.	TC_{300}	113.91±5.07	52.61±3.29	93.82±6.91	28.69±3.29
9.	TC_{600}	110.80±6.18	51.67±4.08	94.27±6.81	26.97±3.08
10.	TC_{900}	108.81±5.28	53.91±4.07	90.50±5.18	30.46±2.82

Comparison of total cholesterol, triglyceride, LDL, and HDL levels (mg/ dL) of rats, belonging to 10 groups just before sacrifice. Each group consisted of 5 rodents each with equal body mass index. The data were expressed as mean ± standard deviation (*indicates statistically significant change where $p < 0.05$, correlation is significant at a 95% confidence interval.)

Serum lipid levels (HDL, LDL, triglycerides, and total cholesterol) were assessed using *T.Cardifolia* ethanolic extract. Alloxan's hyperlipidemic effect was demonstrated by the fact that its administration significantly raised LDL, triglycerides, and total cholesterol levels while lowering HDL levels. It is commonly known that alloxan causes diabetes in rats, which results in metabolic abnormalities that lead to hyperlipidemia [31,32]. This condition is typified by increased serum levels of LDL, triglycerides, and total cholesterol as well as decreased HDL levels [33,34]. *T. cardifolia's* capacity to undo the changes in the lipid profile brought on by alloxan demonstrated its possible anti-hyperlipidemic effect.

Conclusion:

The study concludes that *Tinospora cordifolia* extract has a significant amount of therapeutic potential for the treatment of diabetes and its related complications. Rats with alloxan-induced diabetes showed improved liver and kidney function markers, corrected lipid profile imbalances, and a significant reduction in blood glucose levels when the extract was administered at medium (600 mg) and high (900 mg) doses. These results demonstrate *T. cordifolia's* potential as a natural supplement or substitute for traditional antidiabetic medications, providing a viable means of reducing the oxidative stress and organ damage frequently linked to diabetes. In order to verify its effectiveness and human safety profils, further clinical studies are necessary.

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