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In Vivo Evaluation of Anti-diabetic Activity of Methanolic Extract of *Trillium govanianum* Rhizome in Wistar Albino Rats

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Abstract

Background: Diabetes prevalence and related mortality continue to rise globally, as reported by the World Health Organisation, underscoring the need for safer therapies. *Trillium govanianum,* a native plant of the Himalayas, was found to exhibit *in vitro* enzyme inhibitory activity relevant to glycemic control; therefore, this study was planned to evaluate the anti-diabetic effect of its methanolic rhizome extract in streptozotocin-induced diabetic rats. **Aim:** The study aimed to evaluate the *in vivo* anti-diabetic activity of the methanolic extract of *T. govanianum* rhizome. **Methods:** 24 male Wistar albino rats were divided into four groups (n=6). Diabetes was induced using STZ (45 mg/kg, i.p.) on day 0. Group 1 served as diabetic control; Group 2 received Glibenclamide (10 mg/kg); Groups 3 and 4 received *T. govanianum* extract at 100 mg/kg and 200 mg/kg, respectively. Treatments were administered orally for 14 days from day 3 (72 hours post-STZ injection) to the day 17. FBS and body weight were recorded at baseline and at subsequent intervals. At baseline and on day 17, Complete Blood Count (CBC), liver, and renal function tests were performed. On day 17, vital organs were evaluated histologically. **Results:** Both doses of *T. govanianum* extract significantly reduced FBS levels, comparable to Glibenclamide. Body weight reduction was similar across all groups. Histopathological findings indicated protective effects in the liver and pancreas. **Conclusion:** The extract demonstrated effective glycemic control and organ protection in diabetic rats, warranting further pharmacological and clinical evaluation.

Major Findings: The methanolic extract of *T. govanianum* reduced the blood sugar levels in STZ-induced diabetic Wistar rats with a favourable safety profile. Further, the extract demonstrated protective effects in the liver and pancreas of the diabetic rats as observed in the histopathological analysis.

Keywords: Diabetes, Herbal, *In Vivo*, Preclinical, *Trillium*

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1. Introduction

As per the World Health Organisation (WHO) report, the global population affected by diabetes increased from 200 million in 1990 to 830 million in 2022, with prevalence rising more rapidly in low- and middle-income countries compared to high-income nations. There was a 3 per cent increase in the mortality rate due to diabetes from 2000 to 2019. Diabetes contributes to severe health complications, including blindness, renal failure, myocardial infarction, strokes, and lower limb amputations. In 2021, diabetes and diabetes-related kidney disease accounted for more than 2 million deaths, while elevated blood glucose levels were responsible for approximately 11% of cardiovascular fatalities. Hence, early diagnosis and treatment are essential to avoid the complications due to long-term hyperglycemia[1].

Diabetes can be managed with lifestyle modifications, oral anti-diabetic drugs and insulin replacement therapy. Though several drugs are available for diabetes, most of them are associated with some unwarranted adverse effects, including gastrointestinal disturbances. Plants have historically served as a source of pharmaceuticals for humans. The Indian traditional medicinal system extensively employs botanicals for the treatment of diabetes. WHO reports that up to 90% of the population in poor nations utilises plants and their derivatives as traditional medicine for primary health care. Approximately 800 plant species have been documented to exhibit antidiabetic properties[2].

Trillium govanianum, an herb belonging to the Melanthiaceae family, is the native plant of the Himalayan region. The plant is known for its various pharmacological activities, including analgesic, anti-inflammatory, anti-cancer, anti-fungal and wound healing activities[3,4].

Suresh PS *et al.*, reported that the steroidal saponins present in the rhizome of *T. govanianum* are found to have *in vitro* inhibitory activity against the enzymes α-amylase, α-glucosidase, and dipeptidyl peptidase IV. Further, the docking studies showed that there were significant molecular interactions between the steroidal saponins of *T. govanianum* and these enzymes. The authors concluded that *T. govanianum* could have potential anti-diabetic activity based on the above results[5].

With this background, we wanted to evaluate the anti-diabetic activity of the methanolic extract of *T. govanianum* rhizomes in Streptozotocin-induced diabetes in Wistar albino rats. The primary objective was to evaluate the efficacy of the methanolic extract of *T. govanianum* rhizome in terms of reduction in fasting blood sugar and improvement in body weight. The secondary objective was to evaluate the safety of the extract using the haematological and biochemical parameters, mortality and histopathological changes in the vital organs of rats.

2. Materials and Methods

The study was initiated after getting approval from the Institutional Animal Ethics Committee (IAEC) (Approval No: IAEC 1/Proposal:75/A. Lr:55/ Dt:11.05.2022). 24 male Wistar albino rats weighing 200-250 g were selected and used in the experiment. The rats were divided into 4 groups with 6 rats in each group.

In all the 4 groups, diabetes was induced in all the animals using Streptozotocin (procedure explained in detail in the subsequent section).

After induction of diabetes (72 hours after STZ injection), the rats in groups 1-4 received the interventions as follows:

- Group 1 did not receive any treatment and served as diabetic control.
- Group 2 received standard anti-diabetic treatment (Glibenclamide 10 mg/kg).
- Groups 3 and 4 received 100 mg/kg and 200 mg/ kg of the methanolic extract of *T. govanianum* rhizome, respectively.

2.1 Induction of Diabetes

Diabetes was induced in all the animals (Groups 1-4) using Streptozotocin (STZ) 45 mg/kg body weight. The drug was freshly prepared by dissolving STZ in 0.1M citrate buffer (pH 4.5). After an overnight fasting of 8 hours, FBS levels were measured, and STZ was injected intraperitoneally (IP) into the rats. After 72 hours of STZ injection, all the animals in groups 1 to 4 were screened, and the rats that had fasting blood sugar levels of more than 200 mg/dl were included in the study in each group.

2.2 Treatment

The animals in all the 4 groups were treated as follows (Table 1).

2.3 Study Assessments

The following assessments were done on all the animals in Groups 1 to 4.

- FBS- at baseline (day 0), 72 hours post STZ injection (day 3), day 10 and day 17.
- Body weight- at baseline (day 0), 72 hours post STZ injection (day 3) and day 17.
- CBC, RFT, LFT- at baseline (day 0) and day 17.
- Histopathology of vital organs- on day 17 after sacrificing the animals.

2.4 Preparation of Extract

The plant (*Trillium govanianum*) was identified and certified by the authorised botanist before starting the experiment. *T. govanianum* roots (2.5 kg) were shade-dried and then finely ground. Later, the dried powder was macerated using a single maceration technique in 5 litres of methanol for 10 days at room temperature. After that, the extracted material was filtered, and the solvent was evaporated under vacuum using a rotary evaporator (below 45 °C) to obtain the extract.

2.5 Dose Selection

The doses of STZ and Glibenclamide were selected based on a few previous anti-diabetic studies done in Wistar rats[6,7].

The doses of *T. govanianum* rhizome extract (100 mg/kg and 200 mg/kg) were selected based on the study done by Ur Rahman S *et al.*, where the authors evaluated the analgesic and anti- inflammatory activities of methanolic extract of *T. govanianum* at these doses. Both the doses were found to be safe, and the LD50 (Lethal dose 50) of the methanolic extract of *T. govanianum*, evaluated in that experiment, was 2030.4 mg/kg[4].

2.6 Statistical Analysis

Statistical analysis was done using GraphPad Prism version 8.0.2. All the continuous variables (body weight, haematological parameters, blood sugar and other biochemical parameters) were summarised as mean ± SD and statistically compared using paired t-test and repeated measures ANOVA (within groups) and unpaired t-test and one-way ANOVA (between groups).

3. Results

3.1 Fasting Blood Sugar

The rats in all the groups developed diabetes at 72 hours post STZ injection as evidenced by FBS >200 mg/dl, which was significantly higher compared to the values at baseline. Group 1, the diabetic control group, had elevated FBS on the 10th day and at the end of the study (17th day). The Groups 2,3 and 4 treated with Glibenclamide, 100 and 200 mg/kg of the extract, respectively, showed significant reduction in the FBS values on day 10 and end of treatment compared to 72 hours post STZ injection ($P<0.001$). Further, the reduction in FBS on day 17 was similar with Glibenclamide and both doses of the extract ($P>0.05$).

The details are shown in Figure 1.

3.2 Body Weight

The body weight of the animals compared at three different time points ie., Baseline, day 3 (72 hours after STZ injection) and at the end of the experiment (day 17), showed that there was significant reduction in the

Table 1. Treatment of rats group-wise

Groups		Baseline (Day 0)	Day 3 to Day 17
Group 1	Diabetic control	STZ (45 mg/ kg) single IP dose	Distilled water 0.5 ml orally once daily
Group 2	Standard treatment	STZ (45 mg/ kg) single IP dose	Glibenclamide 10 mg/kg (in 0.5 ml distilled water) orally once daily
Group 3	Test group 1	STZ (45 mg/ kg) single IP dose	Methanolic extract of *Trillium govanianum* rhizome (100 mg /kg) orally once daily
Group 4	Test group 2	STZ (45 mg/ kg) single IP dose	Methanolic extract of *Trillium govanianum* rhizome (200 mg /kg) orally once daily

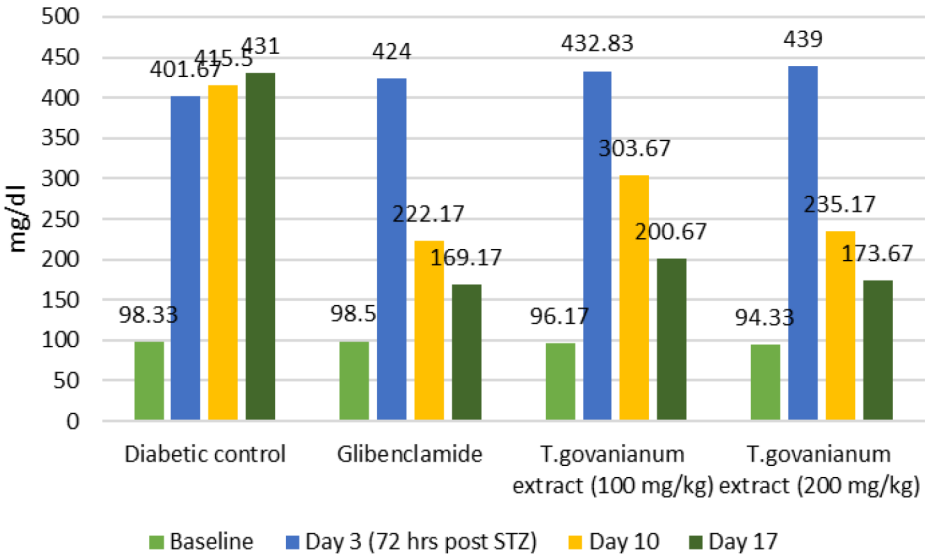


Figure 1. Fasting blood sugar.

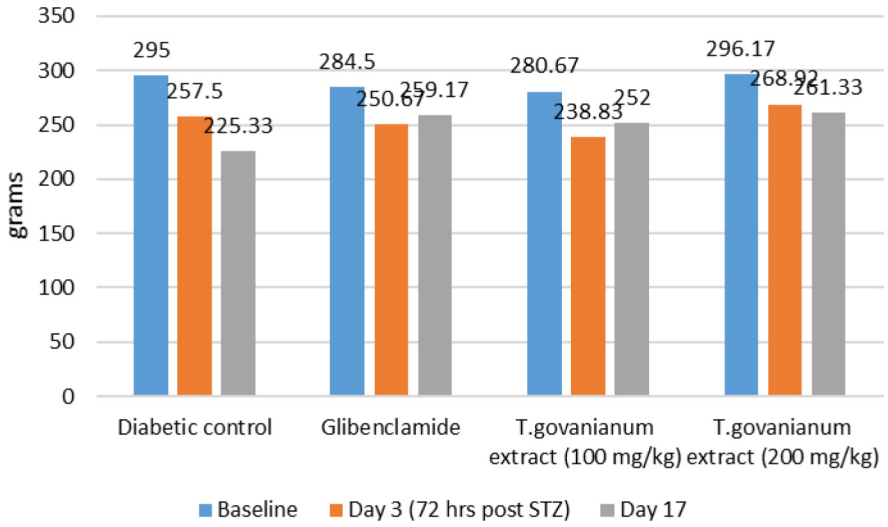


Figure 2. Body weight.

body weight on day 17 when compared to baseline in all the 4 Groups (Repeated measures ANOVA $p<0.05$ in all the Groups). When the reduction in body weight was compared among the 4 groups, the reduction in body weight was similar in all the groups (one-way ANOVA, $p>0.05$). The data is shown in Figure 2.

3.3 Complete Blood Count

There was no significant difference noted in the Haemoglobin (Hb) levels from baseline to the end of the study in all the groups except in the Diabetes control group, where Hb showed a statistically significant decline at the end. However, it does not have any clinical relevance as the mean value was within the normal physiological limits. Total WBC count was significantly elevated in the diabetic control and 100 mg/kg extract group ($P<0.05$, paired t-test), whereas the difference was not seen with Glibenclamide and 200 mg/kg extract treatment. Platelet levels did not show any significant difference within the groups from baseline to end of the study ($P>0.05$, paired t-test) and among the groups ($P>0.05$, one-way ANOVA). The data of CBC parameters are shown in Figure 3.

3.4 Renal Function Tests

Induction of diabetes with STZ resulted in elevation of urea and creatinine in all Groups, and the difference

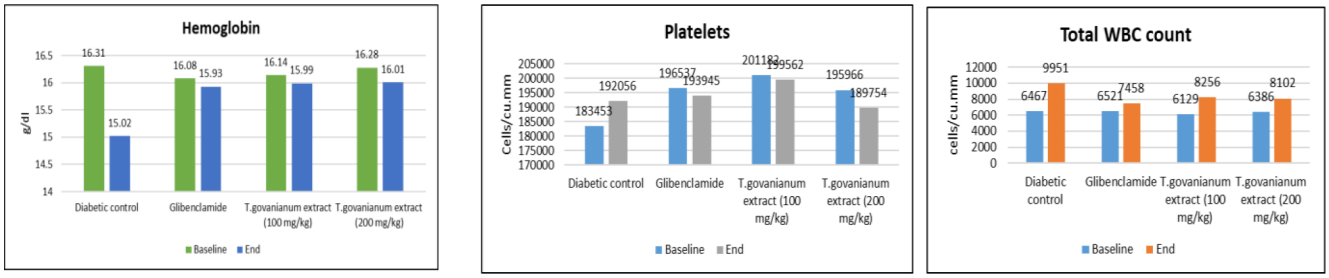


Figure 3. Complete blood count.

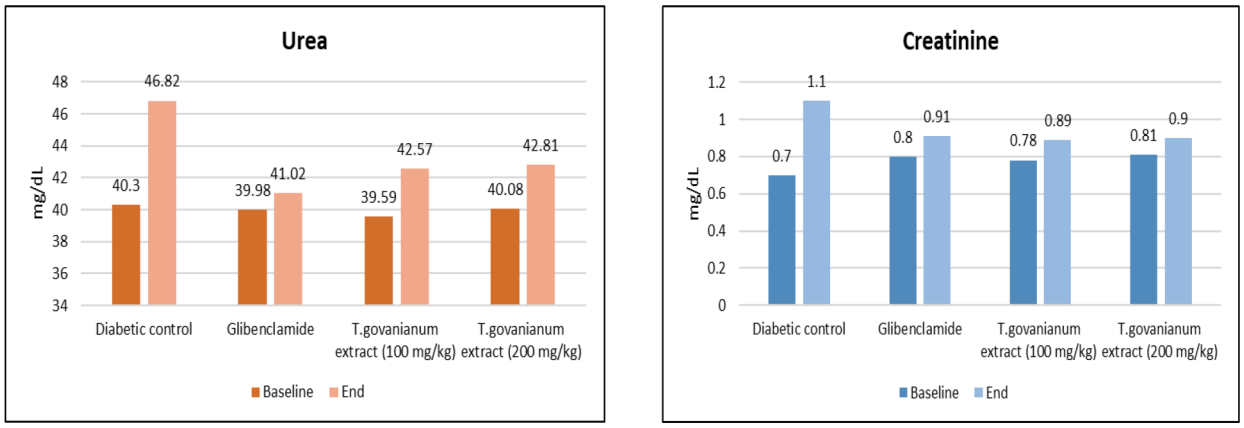


Figure 4. Renal function tests.

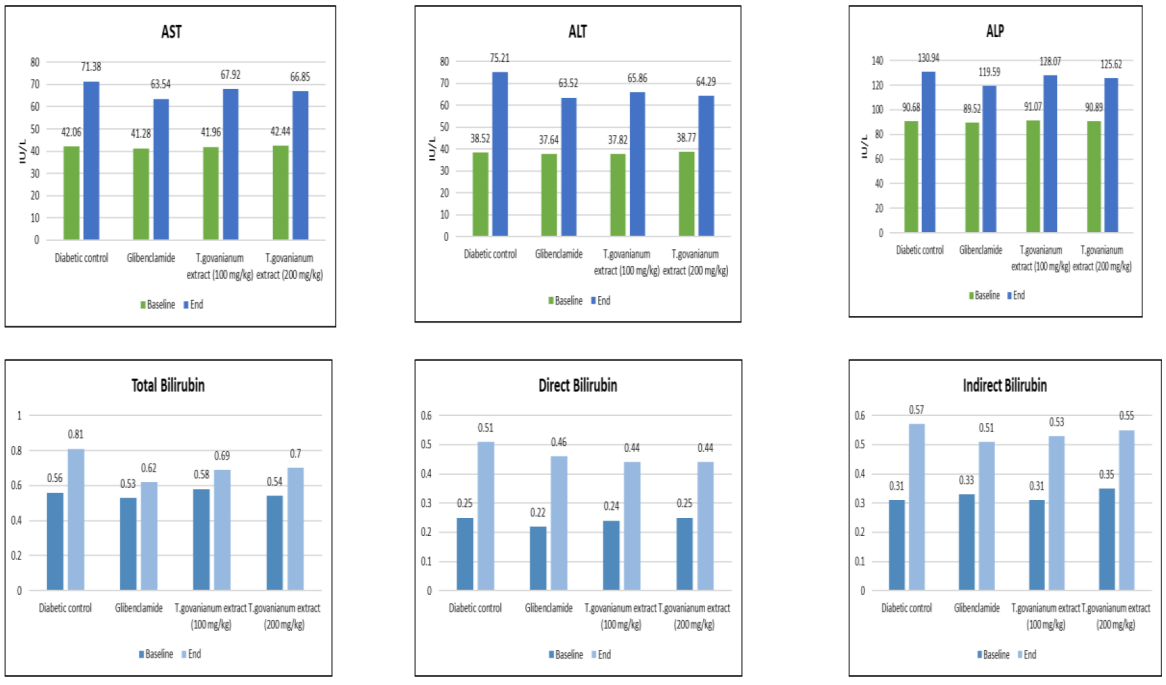


Figure 5. Liver function tests.

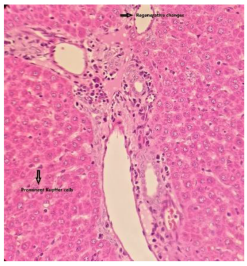
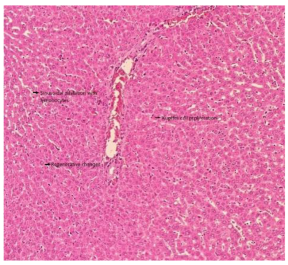
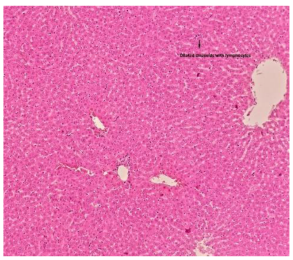
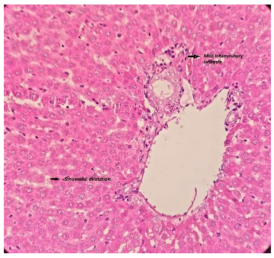
G1	G2	G3	G4
			
Hepatocytes show variation in size, nuclei with open chromatin and prominent nucleoli. Dilatation of sinusoids with neutrophils and RBCs. Dilatation of the central vein and prominent Kupffer cells are also seen.	Regenerative changes of hepatocytes (nucleomegaly, prominent nucleoli, binucleation), some show hydropic change, and Kupffer cell proliferation. Sinusoidal dilatation and congestion, periportal and sinusoidal lymphocytic infiltrate.	Regenerative changes in hepatocytes, Sinusoidal dilatation and moderate inflammatory infiltrate in the periportal region composed of lymphocytes, few histiocytes and plasma cells. Clustering of lymphocytes in sinusoids and the central vein. Dilatation and congestion of the central vein	Minimal periportal inflammatory infiltrate, mild sinusoidal dilatation with lymphocytes

Figure 6. Histopathology of the liver.

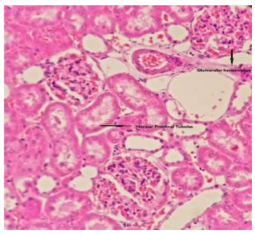
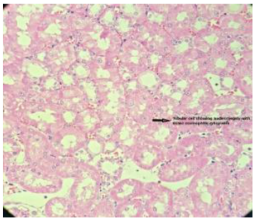
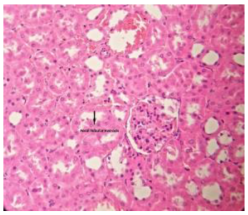
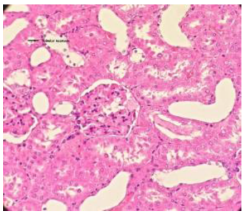
G1	G2	G3	G4
			
Glomerular haemorrhage, normal tubules and interstitium	Tubular cells show nucleomegaly with eosinophilic cytoplasm	Mild congestion within the glomeruli, focal tubular necrosis. Few glomeruli show an increase in size and mesangial cellularity	Tubular necrosis, glomerular congestion

Figure 7. Histopathology of the kidney.

in the mean values of creatinine and urea was similar across all the Groups (Figure 4).

3.5 Liver Function Tests

STZ elevated the liver enzymes and bilirubin levels in all the Groups, but the increase in enzyme levels and bilirubin was significantly less in Glibenclamide and extract (both doses) treated rats compared to the diabetes control group. The change in mean enzyme values and bilirubin was similar between the Glibenclamide and extract-treated groups (Figure 5).

3.6 Mortality

Mortality was not observed in any of the groups throughout the experiment period.

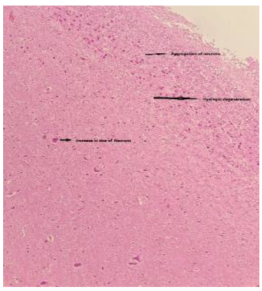
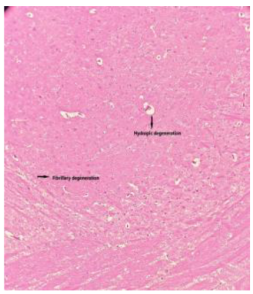
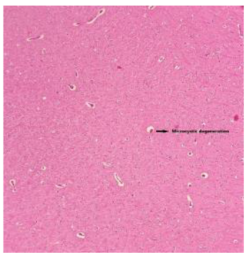
G1	G2	G3	G4
			
Hydropic degeneration, fibrillary degeneration, and focal aggregation of neurons with an increase in the size of neurons	Fibrillary degeneration, hydropic degeneration, aggregation of neurons, with an increase in size, anisonucleosis and prominent nucleoli. The cerebellum also shows an increase in the size of neurons and degenerative changes	Microcystic change, fibrillary degeneration, clustering of neurons, reactive changes in neurons, mild lymphocytic infiltrate	Fibrillary degeneration ++, inflammatory infiltrate ++, reactive changes in neurons/ ganglion cells

Figure 8. Histopathology of the brain.

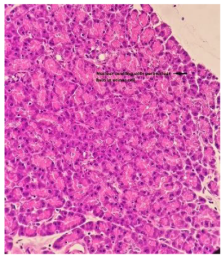
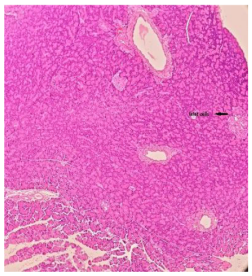
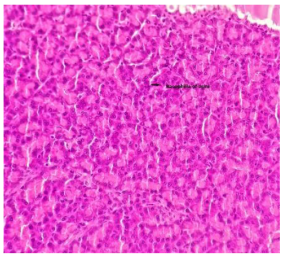
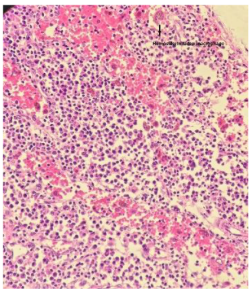
G1	**G2**	**G3**	**G4**
			
A few scattered islets are seen. Nuclear swelling of the acinar cells with perinuclear clearing. Occasional acinar cells show increased basophilia of the cytoplasm	Increase in the number of acinar cells, with some lymphocytic infiltrate. Basophilic cytoplasm and perinuclear clearing of acinar cells.	Basophilia of acini noted. Absence of islet cells	Presence of islet cells ++. The adjacent lymph node shows a focal collection of hemosiderin-laden macrophages

Figure 9. Histology of the pancreas.

3.7 Histopathology of Vital Organs

The histopathological changes noted in the vital organs (liver, kidney, brain and pancreas) are illustrated in the following Figures 6-9.

4. Discussion

The present study investigated the anti-diabetic potential of the methanolic extract of *T. govanianum* rhizome in STZ-induced diabetic rats. The findings indicate that both tested doses of the extract (100 and 200 mg/kg) significantly lowered Fasting Blood Sugar (FBS) levels, demonstrating efficacy comparable to the standard anti-diabetic drug, Glibenclamide. This finding correlates with the *in vitro* anti-diabetic efficacy of *Trillium* reported by Suresh PS *et al.*[5].

Additionally, as expected, STZ administration led to a reduction in body weight across all groups, a common consequence of diabetes. Decrease in body weight with STZ has been supported by other studies in literature[8-10]. Although there was no statistically significant difference in body weight between untreated, Glibenclamide-treated, and extract-treated groups, the numerical increase in body weight observed at the end of treatment in both extract- and Glibenclamide-treated rats may hold clinical relevance.

The changes observed in the haematological and biochemical parameters, including a slight decline in haemoglobin in the diabetes control group, elevated WBC count and deranged liver function parameters, match the literature reports from multiple STZ models[11,12]. Partial restoration of a few values, including WBC count and liver enzymes in the treatment groups (Groups 2-4), indicates the antioxidant and organ-protective effect of Glibenclamide and *T. govanianum* extract.

Further, there was no mortality observed in any of the groups, which indicates that the *T. govanianum* extract is safe in diabetic rats. The safety of the methanolic extract of *T. govanianum* rhizome was evaluated by Ur Rahman S *et al.*, in an *in vivo* toxicity study and found the LD_{50} value as 2030.4 mg/kg, reaffirming the fact that the extract is very safe in rats[4].

The bioactive molecules identified in *T. govanianum* are phenolics, flavonoids, saponins, terpenoids and sulfur-containing compounds[13]. The presence of phenolic and flavonoid compounds, which have strong antioxidant and anti-diabetic properties and Hydroxyl-rich polyphenols like flavonoids can inhibit α-amylase and α-glucosidase enzymes, reducing postprandial glucose levels. Flavonoids present in the extract modulate glucose metabolism by acting on GLUT-4 transporters or PPAR-γ receptors. The inhibitory effect of many flavonoids, leading to antioxidant and antihyperglycemic effects have been reported in the literature[14,15].

Saponins from *T. govanianum* might mimic insulin action, improving glucose uptake by cells. Further, saponins could attenuate the oxidative stress in diabetes. In addition, sulfur-containing compounds present in the extract have the potential to modulate oxidative stress in diabetes[16].

Since the present study focused on the *in vivo* evaluation of the antidiabetic activity of *T. govanianum,* a dedicated phytochemical analysis was not done. Nevertheless, the presence of various phytochemicals, including flavonoids in *T. govanianum* rhizome, has been extensively reported in the literature[5,13,17,18].

The histopathological evaluation of vital organs, including the liver, kidney, brain, pancreas, and heart, provides key insights into the biological impact of the *T. govanianum* extract and its potential role in diabetes management. The observed histological changes across different groups indicate varying degrees of cellular response, inflammation, regeneration, and degenerative alterations. The liver showed regenerative changes in hepatocytes, sinusoidal dilatation, Kupffer cell proliferation, and periportal inflammatory infiltrates. These findings suggest hepatoprotective properties, potentially linked to the presence of flavonoids and phenolics, which are known for their antioxidant and anti-inflammatory effects[19]. The reduction in periportal inflammation in the 200 mg/kg extract group may indicate ameliorative effects of the plant extract against liver damage caused by metabolic stress, which is a key concern in diabetes[20].

In the kidney, observations included glomerular congestion, nucleomegaly in tubular cells, and focal tubular necrosis. The presence of mild congestion and glomerular changes suggests that the plant extract may influence renal protection in diabetic nephropathy. Given the role of polyphenols and flavonoids in modulating oxidative stress, their presence in *T.*

govanianum extract could contribute to renal protection against hyperglycemia-induced damage[21].

Brain examination revealed fibrillary degeneration, hydropic changes, and reactive neuronal alterations, with inflammatory infiltrates, which suggest potential neuroprotective effects, possibly due to antioxidant and anti-inflammatory compounds present in the plant extract. In Group 4, islet cell presence was markedly improved, suggesting a possible β-cell regenerative effect. This observation is crucial as islet cell depletion is a hallmark of diabetes, and the presence of bioactive saponins and flavonoids may stimulate β-cell regeneration and insulin secretion[22].

The histopathological findings support the hypothesis that *T. govanianum* extract may exhibit protective effects on vital organs, particularly in the liver, kidneys, pancreas, and brain, which are significantly impacted in diabetes. The presence of flavonoids, saponins, and phenolics in the extract aligns with its anti-inflammatory, antioxidant, and possible β-cell regenerative properties, reinforcing its potential as a natural anti-diabetic agent. Further studies, including biochemical and pharmacological validation, are necessary to establish its efficacy in diabetes management.

5. Conclusion

Overall, *T. govanianum* rhizome extract is shown to be an effective and safe treatment option for diabetes in terms of reduction in fasting blood sugar and protection of vital organs against diabetes induced damage. However, further preclinical studies are necessary to validate the pharmacological activity of the extract, as well as clinical studies to establish its efficacy in diabetes management.

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