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Research Article

Evaluation of the nephroprotective potential of Trapa natans L, silver nanoparticles on streptozotocin-induced diabetic rats

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ABSTRACT
Background and Objective: Diabetes Mellitus (DM) is a significant global health issue, characterized by chronic hyperglycemia due to impaired insulin function. It often leads to severe complications such as nephropathy and cardiovascular diseases. This study aimed to evaluate the anti-diabetic and nephroprotective effects of *Trapa natans* L. extract and its silver nanoparticles (AgNPs) in a high-fat diet and streptozotocin-induced diabetic rat model.
Method: Forty-two male albino Wistar rats were divided into seven groups: normal control, diabetic control, Glibenclamide-treated, and groups treated with different doses of *T. natans* extract and its AgNPs. Evaluation included fasting blood glucose, lipid profiles, liver enzyme activities (ALT, AST), and histopathological analysis of kidney and pancreas tissues.
Results: The higher dose of *T. natans* AgNPs demonstrated significant anti-diabetic and nephroprotective effects, reducing fasting blood glucose levels, improving lipid profiles, and normalizing liver enzyme activities compared to the diabetic control. Histopathological assessments revealed improved pancreatic and kidney structure, indicating tissue protection against diabetes-induced damage.
Conclusion: *Trapa natans* L. extract and its AgNPs exhibited potent anti-diabetic and nephroprotective properties, highlighting their potential as promising candidates for managing diabetes and its complications.

Keywords
Diabetes mellitus, *Trapa natans*, silver nanoparticles, nephroprotective, streptozotocin.

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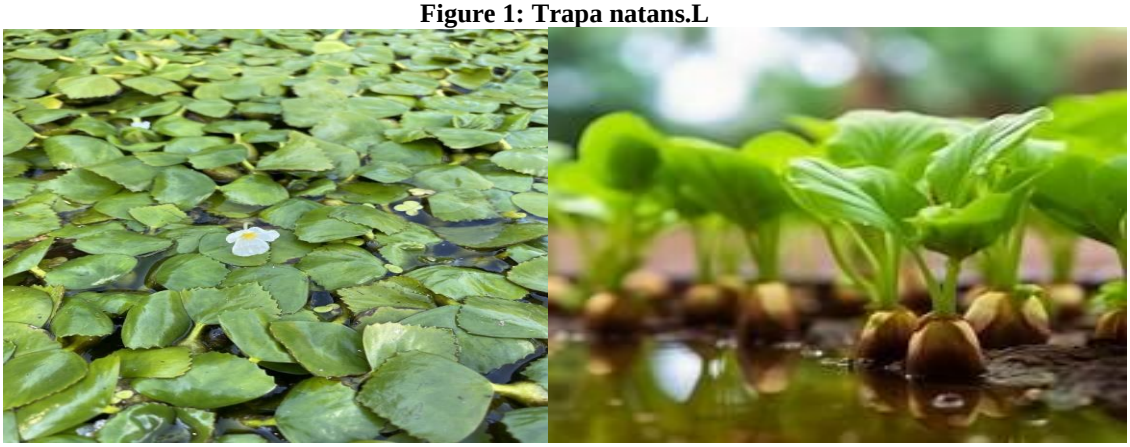
1. INTRODUCTION:

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipemia, and sometimes ketonemia(K.D.Tripati.2003,). Pathological changes such as thickened capillary membranes and vessel narrowing lead to complications like atherosclerosis, glomerular sclerosis, retinopathy, neuropathy, and peripheral vascular disease. The global diabetes epidemic, driven by industrialization and obesity, is projected to increase adult prevalence from 4% in 1995 to 6.4% by 2025, with 422 million people currently affected, primarily in low- and middle-income countries. Type 2 diabetes, linked to insulin resistance, has surged,

while Type 1 diabetes involves minimal insulin production, typically developing in early life. HbA1c levels are commonly used to monitor long-term blood sugar control.

1.1 Plant Profile

Figure 1: Trapa natans.L



In this study, *Trapa natans* L. (water chestnut or Singhara) was evaluated for its anti-diabetic activity related to nephropathic complications(Garg S,2020). This aquatic plant, native to Asia and belonging to the *Trapaceae* family, is widely cultivated and known for its rich nutrient profile, including vitamins, minerals, dietary fiber, and proteins. The plant's fruit and hulls, particularly rich in antioxidants, offer various health benefits, such as protection against cancer, heart disease, and neurological disorders. Phytochemical analysis reveals key compounds like saponins, alkaloids, ascorbic acid, cycloeucalenol, and ursolic acid, contributing to its antibacterial, anti-inflammatory, and anti-diabetic properties. *T. natans*'s potential for promoting health, particularly in managing diabetes and related complications, is well-supported by its nutritional and bioactive content(Khare CP.,2007).

2. METHODS

2.1 Preparation of Extracts

Hydroalcoholic extracts of ***Trapa natans L.*** will be prepared using a Soxhlet apparatus. Dried coarse powder of the plant will undergo hot extraction using ethanol and water successively. The extracts will be filtered, dried under reduced pressure, and stored for further analysis.

2.2 Animals

Male Wistar Albino rats (170-200 g) will be procured from the institution's Central Animal House and maintained under controlled conditions (12-hour light/dark cycle, 25±5°C, 40-60% humidity) with free access to food and water. The experimental protocol has received approval from the Institutional Animal Ethics Committee, adhering to the guidelines set forth by the "Guide for the Care and Use of Laboratory Animals."

2.3 Preparation of Silver Nanoparticles from Trapa natans L. Extract

A 1 mM silver nitrate solution will be prepared for biosynthesis. Five milliliters of the plant extract will be added to 95 mL of the silver nitrate solution and heated to a range of 30-95°C. The resulting hydrosol will be centrifuged at 10,000 rpm, and the resulting pellet will be suspended in Millipore water and dried for further characterization.

Characterization Techniques:

- **UV-Visible Spectroscopy:** Used to monitor the reduction of silver ions over time.
- **Particle Size Analysis:** Conducted using a Malvern Zetasizer.
- **FTIR Analysis:** Identified active biomolecules in the extract.
- **SEM:** Revealed nanoparticle morphology.
- **Zeta Potential:** Assessed charge and stability of nanoparticles.

2.4 Induction of Diabetes

Seven-week-old rats will be fed a high-fat diet (HFD) for 2 weeks, which will induce significant increases in body weight and glucose levels. A subset of these rats will receive an intraperitoneal injection of streptozotocin (35 mg/kg). Diabetes will be confirmed based on fasting blood glucose levels exceeding 200 mg/dL.

2.5 Procedure

Forty Wistar Albino rats (8-10 weeks old) will be randomly divided into seven treatment groups for four weeks, as outlined below:

Table.1: Experimental designA total of 42 animals are randomly divided into 7 groups and are treated as follows:

TREATMENT	DRUG	DOSE
Group-I	Control receives vehicle only	10 ml/kg body weight daily for 3weeks, *p.o*
Group-II	(HFD+STZ)	35 mg/kg i.p (once)
Group-III	(HFD+STZ)+standard (Glibenclamide)	150 mg/kg i.p. (once) + 10 mg/kg daily for 3 weeks *p.o*
Group-IV	Hydroalcoholic extract of *Trapa natans.L* in diabetes rats	150 mg/kg i.p. (once) + Low dose *p.o* for 3 weeks
Group-V	Hydroalcoholic extract of *Trapa natans.L* in diabetes rats	150 mg/kg i.p. (once) + High dose p.o for 3 weeks
Group-VI	Silver nanoparticles of Hydroalcoholic extract of *Trapa natans.L* in diabetes rats	150 mg/kg i.p. (once) + Low dose *p.o* for 3 weeks
Group-VII	Silver nanoparticles of Hydroalcoholic extract of *Trapa natans.L* in diabetes rats	150 mg/kg i.p. (once) + High dose p.o for 3 weeks

2.6 Evaluation Parameters

The study will evaluate anti-diabetic and nephroprotective effects based on the following parameters:

- **Blood Glucose Levels**
- **Serum Biochemical Parameters:**
  - Lipid Profile: Triglycerides, Total Cholesterol, HDL, LDL
  - Liver Enzyme Activity: AST, ALT
  - Kidney Parameters: Creatinine, Urea, Sodium, Potassium
- **Histopathological Analysis** of liver, pancreas, and kidneys
- **Statistical Analysis** using GraphPad InStat for data comparison.
- **Blood Glucose Test:** Conducted on overnight-starved rats, with blood samples collected at specified intervals following the administration of extracts and glibenclamide.
- **Organ Weight Analysis:** Body weight and organ weights will be measured throughout the treatment period, with organ-to-body weight ratios calculated for histopathological evaluation.
- **Serum Analysis:** Blood collected at the end of treatment will be analyzed for lipid and liver enzyme levels, along with urinary parameters.

2.7 Statistical Analysis

All results will be expressed as Mean ± SEM. Statistical analyses will be conducted using one-way ANOVA followed by Dunnett's test, with significance defined at $p<0.001$, $p<0.01$, and $p<0.05$.

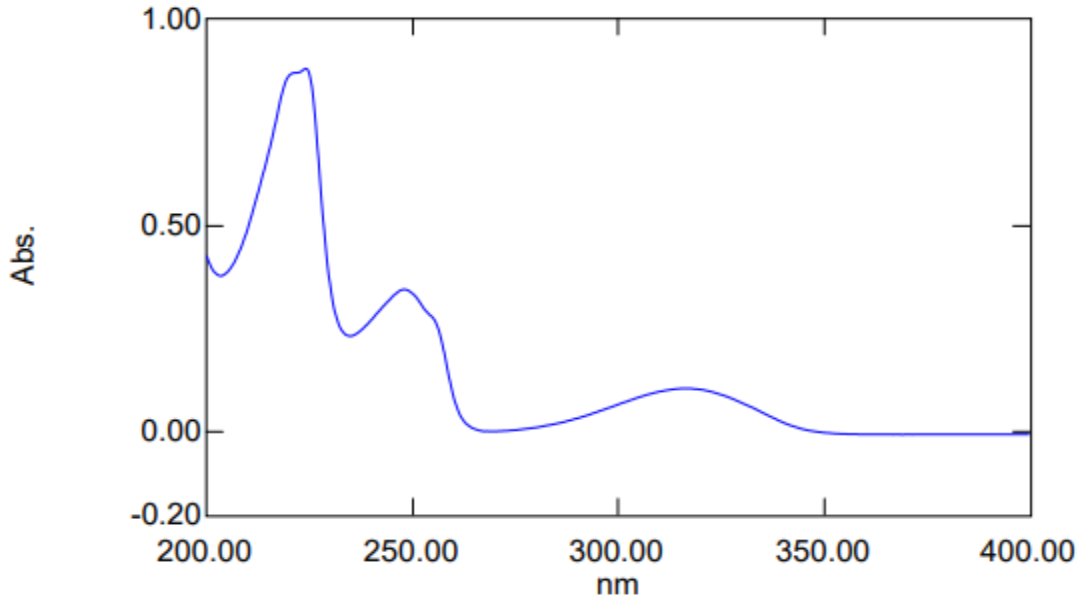
3. RESULTS:

3.1 Characterization of Silver Nanoparticles from T. natans

3.1.1 UV-Visible Spectroscopy

The formation of silver nanoparticles (Ag NPs) was confirmed by a color change from green to yellow, indicating surface plasmon resonance (SPR), likely facilitated by phytochemicals from *T. natans* in the reduction of Ag^{2+} to Ag^{0}. Compared to conventional methods, phytogenic synthesis offers cost-effectiveness and environmental sustainability. UV-Vis spectroscopy in the 250–500 nm range showed a prominent absorbance peak at [specific wavelength] nm, confirming the formation of Ag NPs.

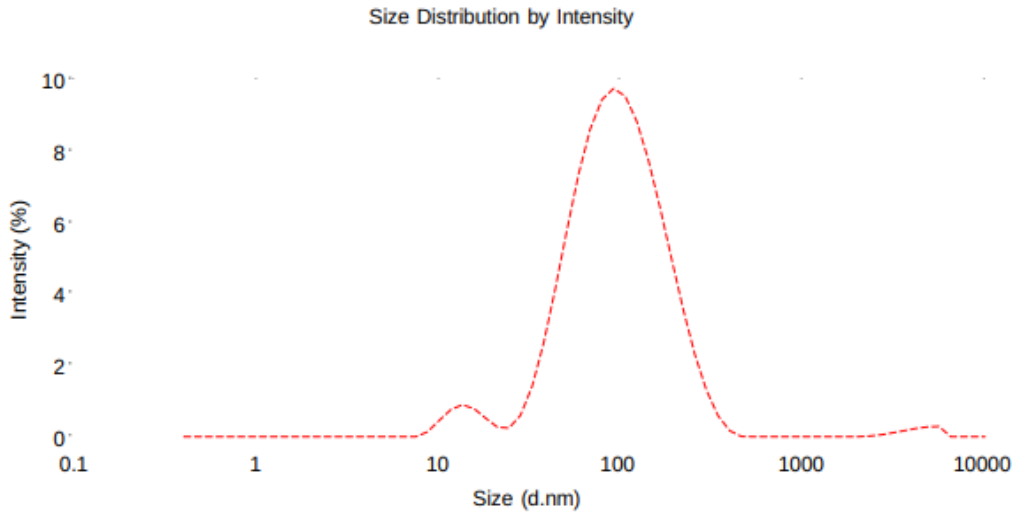
Figure 2: UV-visible spectrum of *T.natans* Ag NPs



3.1.2 Particle Size Analysis

Particle size analysis revealed an optimal nanoparticle size of approximately 110.2 nm, with a Z-average of 77.32 nm and a polydispersity index (PdI) of 0.284, indicating a narrow size distribution. The dominant peak, with 94.9% intensity, reflects the uniformity of the nanoparticles, making them suitable for applications that require consistent performance. Overall, the results are of high quality, supporting effective formulation use.

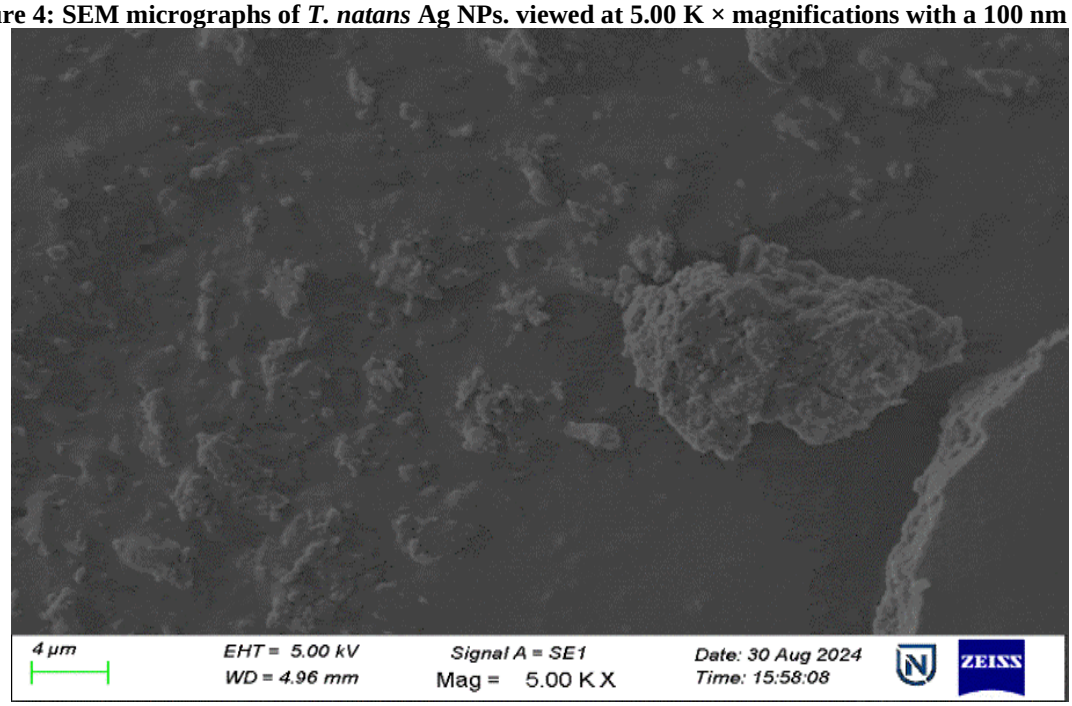
Figure 3 : Particle size analysis of *T.natans* Ag NPs



3.1.3 SEM analysis

To improve, surface morphology of the optimized *T. natans* Ag NPs was investigated using SEM. The information showed that the NPs had a smooth surface and a definite spherical shape (Figure 6). SEM images of Ag NPs . examined at 5.00 K × magnifications using a scale of 100 nm

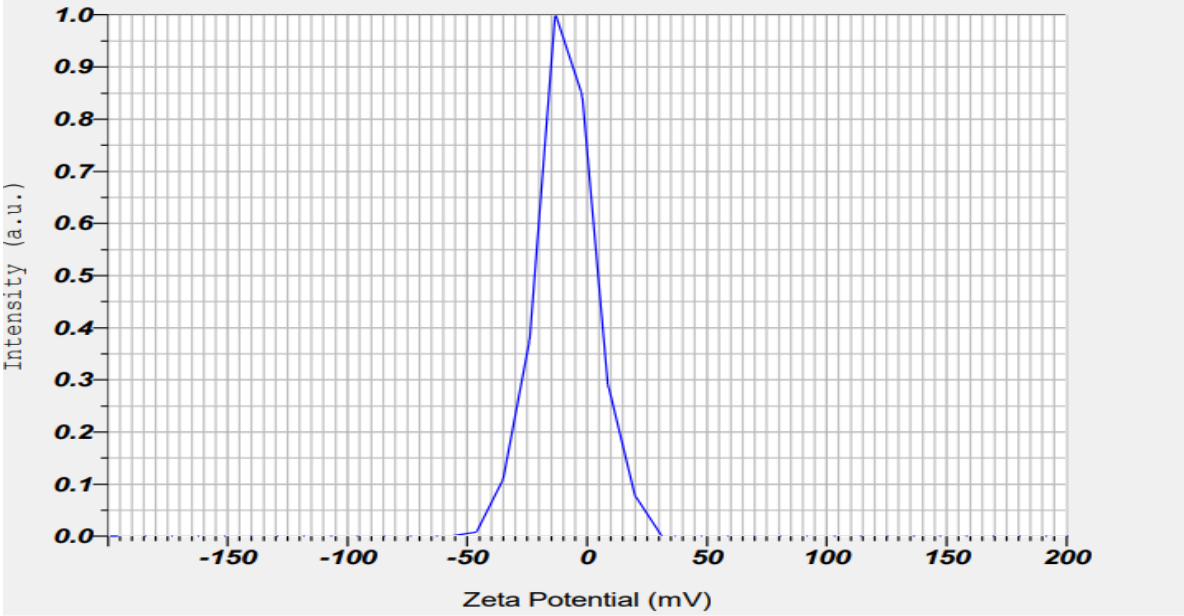
Figure 4: SEM micrographs of *T. natans* Ag NPs. viewed at 5.00 K × magnifications with a 100 nm scale



3.1.4 Zeta Potential

Zeta potential (ZP) is key to predicting nanoparticle stability, with high values (>+50 mV or <-50 mV) indicating strong resistance to aggregation. In this study, silver nanoparticles (Ag NPs) had a ZP of −8.9 mV, suggesting uniform distribution and sufficient stability to prevent self-aggregation, enhancing their potential for various formulations.

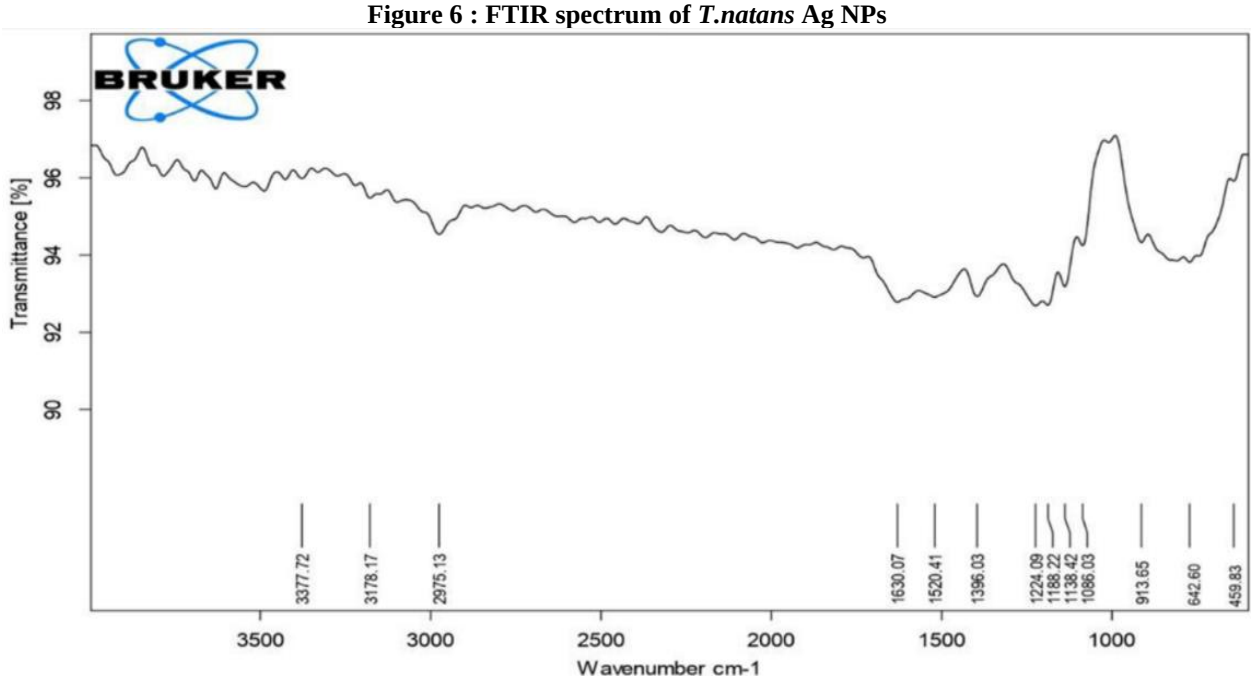
Figure 5 : Zeta potential of *T.natans* Ag NPs



3.1.5 FTIR Analysis

The FTIR spectrum of silver nanoparticles (AgNPs) shows key functional groups within the 4000–400 cm^{-1} range, indicating O-H at 3377.72 cm^{-1}, C-H stretching of alkanes at 2975 cm^{-1}, primary amines at 1630.07 cm^{-1}, and nitro compounds at 1520 cm^{-1}. Peaks at 1224 cm^{-1} and 1138 cm^{-1} suggest the presence of alcohols, carboxylic acids, ethers, and esters, while peaks between 400–600 cm^{-1} indicate metal-oxygen groups, with Ag binding evident at 459 cm^{-1}. These results imply that phytochemicals such as proteins, alkaloids, phenolics, and flavonoids are involved in the biosynthesis of AgNPs from *Trapa natans* L.

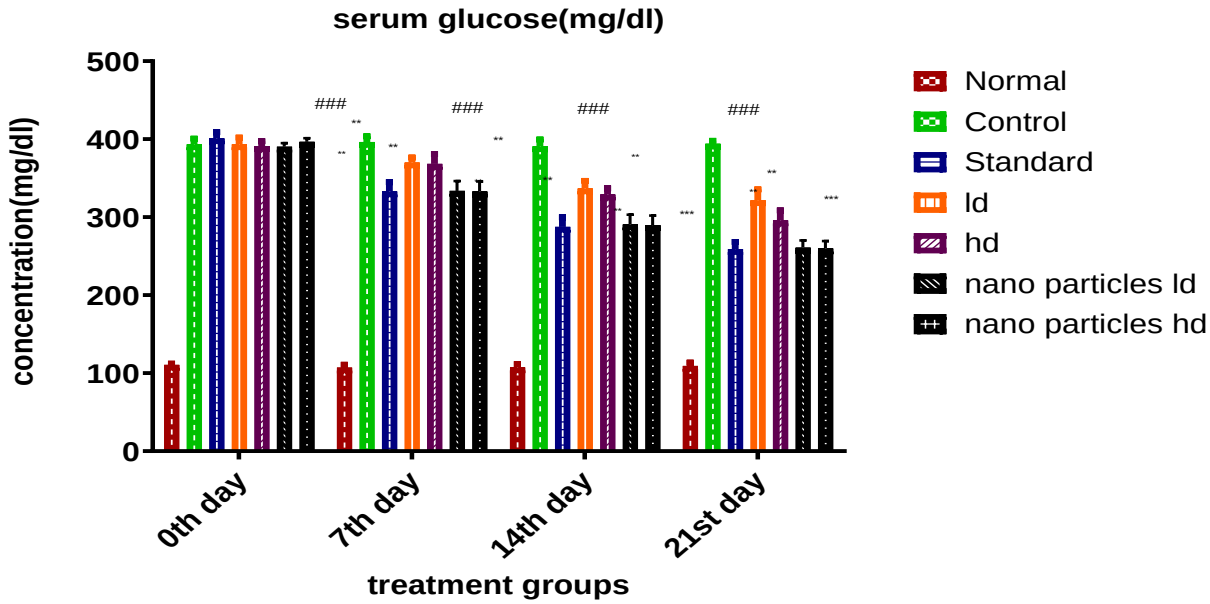
Figure 6 : FTIR spectrum of *T.natans* Ag NPs



3.2 Effect of *T. natans* on Serum Glucose Levels in Diabetic Rats

Diabetic control rats exhibited significantly elevated blood glucose levels compared to normal control rats. Treatment with Glibenclamide (10 mg/kg), high-dose *T. natans* (400 mg/kg), and high-dose nanoparticles (400 mg/kg) resulted in a significant reduction in blood glucose levels ($p < 0.001$) compared to the diabetic control group. In contrast, diabetic rats receiving a low dose of *T. natans* (200 mg/kg) demonstrated a less significant decrease in blood glucose levels.

Figure 7 : Effect of *T.natans* on serum glucose levels in diabetic rats

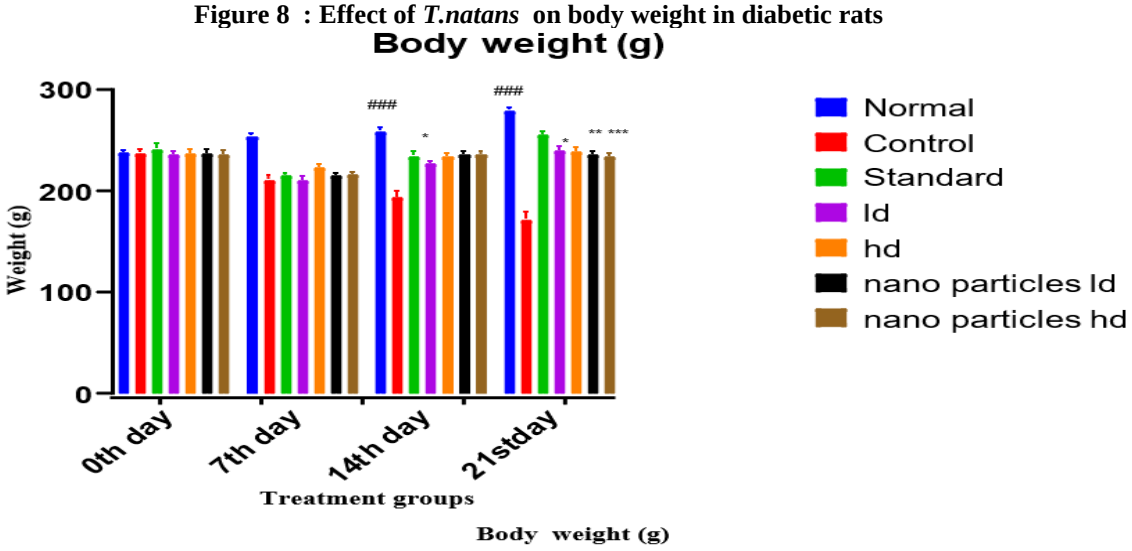


All values are expressed in mean ± SEM, n=6 $^{####}p < 0.001$ vs normal control $****p < 0.0001$, $***p < 0.001$, $**p < 0.01$, $^{ns}p > 0.05$ vs diabetic control

3.3 Effect of *T.natans* on Body weight in diabetic rats

The body weight of the diabetic control rats was significantly lower than in normal rats. Treatment with *T.natans* (400 mg/kg) and high dose of nano particles (400mg/kg) ($p < 0.001$) significantly increased the body weight compared to diabetic control rats. At the lower dose (200 mg/kg) ($p < 0.01$) *T. natans* exhibit a less significant effect compared to diabetic control.

Figure 8 : Effect of *T.natans* on body weight in diabetic rats

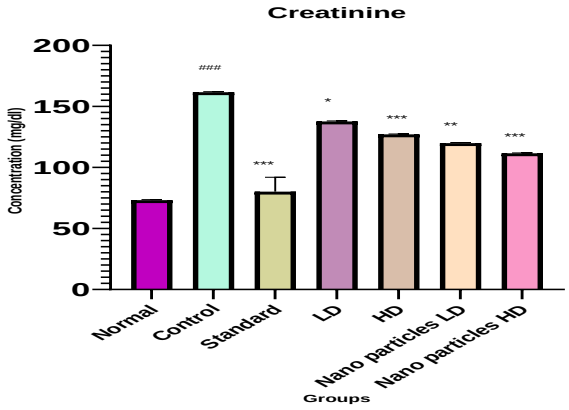


All values are expressed as mean ± SEM, n=6, $^{\#\#\#\#}p < 0.0001$ when compared to normal control, $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{ns}p > 0.05$ when compared to diabetic control by using One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test.

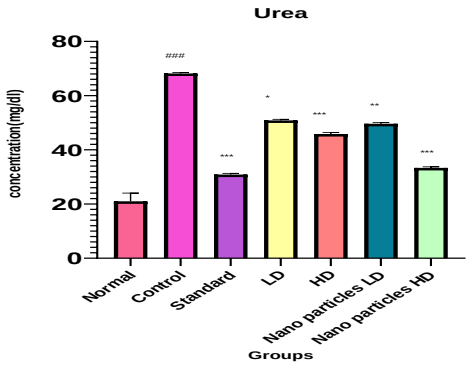
3.4 Effect of *T.natans* on kidney functions in High fat diet and low dose STZ induced diabetic rats

Diabetic control rats exhibited significantly high level of creatinine, urea, sodium, potassium level when compared to that of normal rats. Creatinine, urea, sodium, potassium level treated with *T. natans (*400 mg/kg) was significantly lower when compared to diabetic control rats.

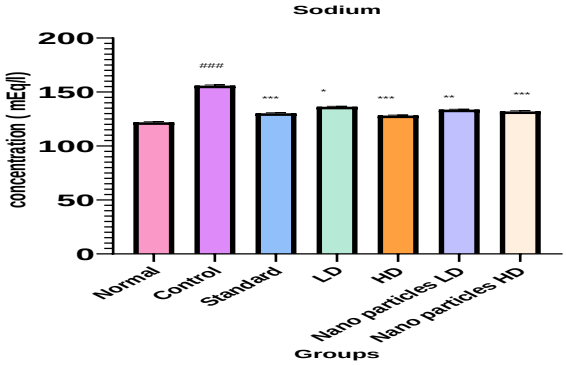
Figure 9: Effect of *T.natans* on kidney functions in High fat diet and low dose STZ induced diabetic rats



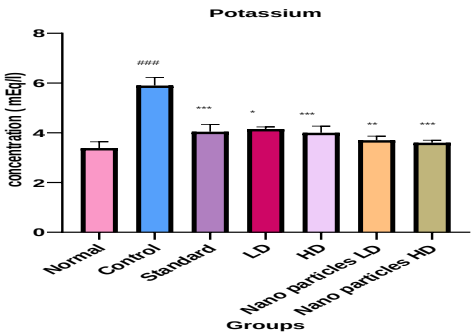
a. Effect of *T.natans* on Creatinine



b. Effect of *T.natans* on Urea



c.Effect of *T.natans* on Sodium



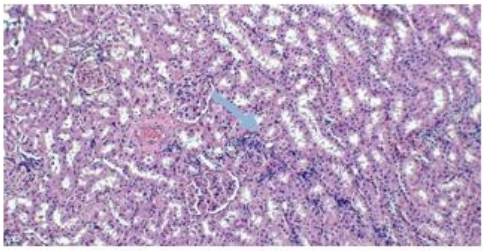
d. Effect of *T.natans* on Potassium

All values are expressed as mean ± SEM, n=6, $^{\#\#\#\#}p < 0.0001$ when compared to normal control, $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, $^{ns}p > 0.05$ when compared to diabetic control by using One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test.

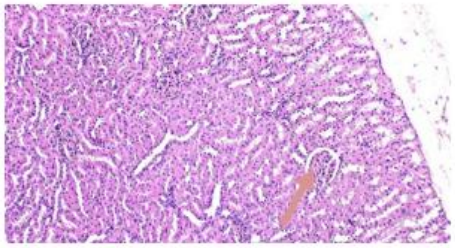
3.6 Histopathological studies of Kidney in normal and High fat diet and low dose of STZ induced diabetic rats

3.6.1 Histopathological examination of Kidney

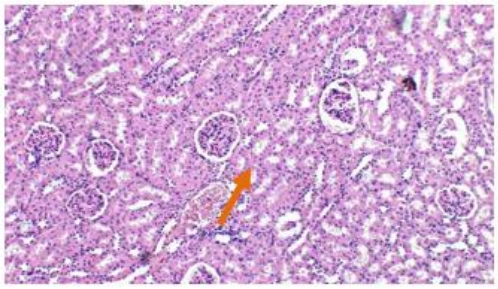
Figure 10 : Histopathological examination of Kidney



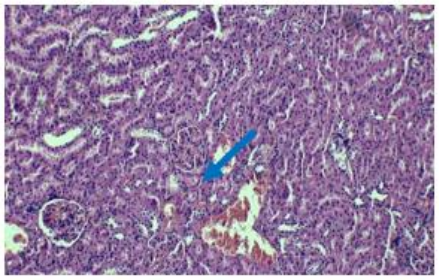
A)Normal



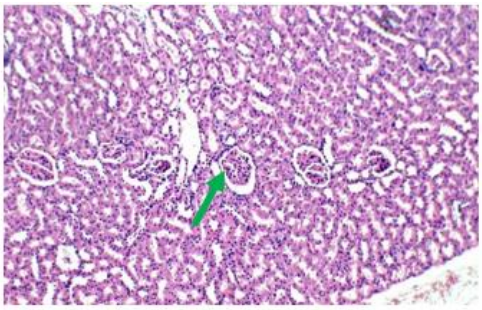
B)Control



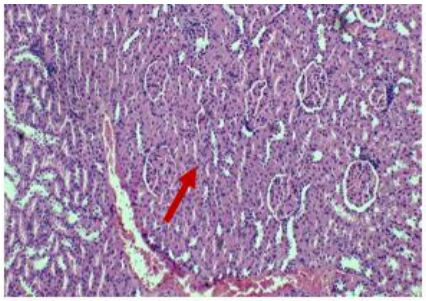
C) Standard



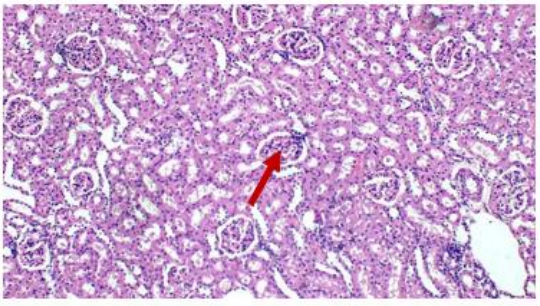
D)Low dose *T. natans*



E) High dose of *T.natans*



F) Low Dose ofAg NPs *T.natans*



G) High Dose ofAg NPs *T.natans*

Normal Group: Kidney architecture is normal with well-preserved glomeruli and intact tubules, showing no signs of damage, inflammation, fibrosis, or necrosis(which is shown in blue colored arrow).

1. **Induced Group**: Significant kidney damage with shrunken, sclerotic glomeruli and marked tubular degeneration and necrosis. Interstitial inflammation and fibrosis are prominent, indicating severe diabetic

nephropathy(which is shown in brown colored arrow).

2. **Standard Group**: Moderate structural improvement with better-preserved glomeruli, reduced sclerosis, and less pronounced tubular degeneration. Mild inflammation and early regeneration are observed, reflecting the therapeutic effect of glibenclamide(which is shown in light orange colored arrow).
3. **Low Dose T. natans**: Largely preserved glomeruli and tubules with minimal damage and early signs of tubular regeneration. Minimal inflammation is present, indicating protective effects against nephropathy(which is shown in blue colored arrow).
4. **High Dose T. natans**: Well-preserved kidney tissue with significant tubular regeneration and no necrosis, inflammation, or fibrosis, demonstrating strong nephroprotective effects(which is shown in green colored arrow).
5. **Low Dose Nanoparticles of T. natans**: Preserved glomeruli and tubules with clear tubular regeneration and minimal necrosis. Inflammation is negligible, with no fibrosis, indicating enhanced nephroprotective effects compared to non-nano formulations(which is shown in red colored arrow).
6. **High Dose Nanoparticles of T. natans**: Excellent preservation of glomeruli and tubules with robust tubular regeneration and no signs of necrosis, inflammation, or fibrosis, demonstrating superior nephroprotective effects and near-normal kidney function(which is shown in dark red colored arrow).

4. DISCUSSION:

Diabetes mellitus, marked by chronic hyperglycemia due to insulin deficiency or resistance, is a growing global health concern, especially type 2 diabetes. This study assessed the anti-diabetic and nephroprotective effects of *Trapa natans* L. extract and its silver nanoparticles (AgNPs) in high-fat diet-induced diabetic rats. Both the crude extract and AgNPs significantly reduced fasting blood glucose, likely due to phytochemicals like flavonoids and alkaloids that enhance insulin sensitivity and glucose uptake. They also demonstrated nephroprotective effects by lowering serum creatinine, blood urea nitrogen, and improving kidney histopathology. These benefits, driven by the antioxidant properties of *T. natans*, were comparable or superior to glibenclamide, particularly in renal protection. Further research should isolate active compounds and explore clinical trials to confirm their therapeutic potential in diabetes management.

5. CONCLUSION:

The present study evaluated the anti-diabetic and nephroprotective effects of *Trapa natans* L. extract and its silver nanoparticles (AgNPs) in high-fat diet-induced diabetic rats, utilizing 42 male Wistar Albino rats divided into seven groups, including normal control, diabetic control, and treatment groups receiving the standard drug (Glibenclamide) or various doses of *T. natans* extract and its nanoparticles. Key parameters, including blood glucose levels, body weight, lipid profiles, and renal function markers, were monitored over a specified period, alongside histopathological analyses of the pancreas, kidneys, and liver to assess the structural impact of the treatments. Oral administration of *T. natans* extract and AgNPs resulted in a significant reduction in blood glucose levels, improvements in lipid profiles, and enhanced kidney function markers, with effects being dose-dependent and most pronounced in higher-dose groups. Histological analysis indicated preservation of pancreatic beta cells and improved kidney and liver tissue structure, further supporting the therapeutic potential of *T. natans* in managing diabetes and its complications, particularly in preventing diabetic nephropathy. These findings suggest that *T. natans* could serve as a promising natural remedy for diabetes management.

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