Nephroprotective effect of *Bryophyllum pinnatum*-mediated silver nanoparticles in ethylene glycol-induced urolithiasis in rat

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Abstract
A large population is suffering from multifactorial urolithiasis worldwide with a reoccurrence rate of almost 70%–80% in males and 47%–60% in females. In the present study, the nephroprotective effect of silver nanoparticles (AgNPs) synthesised by *Bryophyllum pinnatum* was evaluated in ethylene glycol-induced urolithiasis in rat. *B. pinnatum*-mediated AgNPs which were found to be spherical and polydispersed particles with an average size of 32.65 nm determined by transmission electron microscopy analysis, and showing an absorption peak at 432 nm by the UV-Vis spectrophotometric analysis, revealing the role of hydroxyl group in the synthesis by Fourier Transformed Infrared Spectroscopy analysis, with a zeta potential value of $-15.7$ mV. The crystalline nature and fcc structure was demonstrated based on X-ray diffraction analysis. Animal study was performed on 36 male Wistar rats divided into six equal groups, which demonstrated significant increase in serum total protein, albumin and globulin and significant decrease in AST, ALT, creatinine, BUN, calcium and phosphorus in group V and VI when compared with group II and IV. No crystalluria was observed in rats given *B. pinnatum* AgNPs. Histopathological observations in group V and VI showed mild degenerative changes and restoration or maintenance of kidney parenchyma when compared with group II and IV rats. Thus, the authors conclude with the beneficial preventive and therapeutic nephroprotective effect of *B. pinnatum*-mediated AgNPs against ethylene glycol-induced urolithiasis in rats.

1 | INTRODUCTION
A renal stone or urinary calculi, commonly known as ‘urolithiasis’, is a process of development of growth of hard non-metallic mineral calcifications that are formed in the urinary system. A large number of people are suffering from urinary stones and it is prevalent in approximately 12% of the world population with a reoccurrence rate of 70%–80% in males and 47%–60% in females [1]. Similar to humans, animals also suffer from urolithiasis and males are found to be more susceptible than females due to the long urethral size [2]. The formation of the urolith involves various multifactorial a etiopathogenesis viz., bacterial infection, anatomic and feeding habits [1, 3, 4]. High levels of calcium in diet, insufficient drinking water required for body metabolism, gout, hyperparathyroidism, obesity, and continuous calcium supplement intake are some of the high risk factors responsible for urinary calculi. However, literature revealed that calcium oxalate (CaOx) is the predominant component of most stones accounting for more than 80% urolithiasis.

In order to overcome multifactorial urolithiasis many surgical as well as medicinal treatments are commonly used. Surgical removal is done if a ureteric or intrarenal obstruction occurs. If the condition cannot be managed through medicines then the lithotripsy, an extracorporeal shock wave therapy, is most widely used for treating urolithiasis, but this shock therapy if used continuously for recurrent stone formation may damage renal function [5]. The treatment of urolithiasis mainly aims with the dissolution of stones preventing its reoccurrence. However, currently using therapies for prevention as well as for cure is not effective in all cases and is highly expensive with common reoccurrence and potential side effects [5, 6]. Hence, more engrossment is given towards medicinal plants.

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Ancient literature on Ayurveda described the traditional use of *Bryophyllum pinnatum* in the treatment of urolithiasis. In Ayurveda, *Pasanabheda* is the name for *B. pinnatum* which means dissolver of stone [5]. This plant is also widely used in the ethnomedical practices for urinary disorders and urinary stones. Though many plants have been evaluated for the anti-urolithic effect, literature revealed prominent effect of *B. pinnatum* leaves during natural or ethylene glycol-induced urolithiasis minimising the chances of reoccurrence in different species [7–9].

Nanotechnology plays an important role because of its strikingly different properties as compared to the bulk; at nanoscale, there is no alteration in the property of drugs, the duration of drug release and is specific about its targeted site [10]. The functionalized nanoproducts prepared with metal nanoparticles in general, silver nanoparticles (AgNPs) particularly, are now gaining keen interest in the field of nanomedicine because of their unique therapeutic potential and ability to cure various diseases. Green synthesis of nanoparticle is gaining importance due to its eco-friendly nature [11–13], and is found to overcome the routine use of organic solvents and toxic reducing agents. There are reports revealing potential therapeutic activity of sunlight-induced green synthesis of AgNPs from the *Kalanchoe pinnata* leaf extract [12] and, *Phlogacanthus thyrsiformis* flower extract [14], where the effect was against ethylene glycol along with ammonium chloride-induced urolithiasis in male Wistar rats. However, there are no reports on effect of AgNPs synthesised by *B. pinnatum*, against urolithiasis in rats or any other animals. Considering the importance of AgNPs and beneficial effect of *B. pinnatum*, the present study was planned to evaluate the effect of AgNPs synthesised by *B. pinnatum* AgNPs on ethylene glycol-induced urolithiasis in rat.

## MATERIAL AND METHODS

### 2.1 Biosynthesis of silver nanoparticles using *B. pinnatum* leaf extract

The fresh leaves of *B. pinnatum* were washed thoroughly with tap water to remove dust particles and then washed with double distilled water. Twenty-five grams chopped leaves were added to 100 ml of distilled water and boiled at 80°C till it turns light green [15]. The extract was filtered twice with 0.2 µm size of vacuum filter. From 100 ml, 10 ml of pure plant extract was diluted in 90 ml of distilled water and boiled at 80°C till it turns light green for 10 min and the subsequent displacement of the supernatant with sterile distilled water was performed, so that the unreacted plant metabolites, nitrate and silver ions could be removed and nanoparticles were made to settle at the bottom forming the precipitate [18]. Furthermore, the precipitate was collected and oven dried, and the powdered AgNPs obtained were stored at room temperature and used for further study.

### 2.2 Characterisation of AgNPs UV-Vis spectra analysis

The AgNPs powder was dissolved in sterile water and subjected to UV-Visible analysis [19]. Preliminary confirmation of synthesised AgNPs was done with the help of UV-Visible double beam spectrophotometer (Shimadzu- UV 1700, Japan) operated at a resolution of 1 nm by scanning the absorbance spectra from 200 to 800 nm of the colloidal sample.

### 2.3 Nanoparticle tracking analysis

The average size of the synthesised nanoparticles was characterised by the nanoparticle tracking analysis (NTA) system [18, 20]. NTA is a laser-based light-scattering technique (LM-20, NanoSight Pvt. Ltd., UK), in which particles suspended in the liquid medium were injected into the LM viewing unit and visualised under the optical element. At controlled conditions, the sample preparations and measurements were carried out and analysed by NanoSight LM 20 using a beta version of NTA 2.3 software.

### 2.4 Zeta potential measurement

Zeta potential of AgNPs was measured by using a Zetasizer (3000 Harmonized system; Zetasizer Nano-ZS 90, Malvern Instruments, Malvern, UK) with a zeta dip cell. For the sample analysis 10 µl of AgNPs colloidal solution was diluted in 1000 µl of distilled water. The surface charge of nanoparticles was measured using Zetasizer. A potential distribution around the particles was obtained as a result of surface charges. Zeta potential usually falls in the range of −70 mV to +70 mV and is expressed in millivolts (mV) [18, 21, 22].

### 2.5 X-ray diffraction analysis

The physical and chemical nature of AgNPs were elucidated by XRD (X-ray diffraction) analysis using the Rigaku diffractometer. The samples were analysed by powder diffraction using finely ground powder of AgNPs.

### 2.6 Fourier transformed infrared spectroscopy

The instrument Alpha-T FTIR, Bruker optik GmbH, Germany, equipped with a room temperature DTGS detector and supported with the software OPUS was used for recording the Fourier Transformed Infrared Spectroscopy (FTIR) spectrum of synthesised AgNPs. The scans recorded were the average of 100 scans on the attenuated total reflection (ATR) diamond crystal of the Alpha-T FTIR instrument unit. Each sample was scanned in 500 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹.
2.7 Transmission electron microscopy

The shape and size of the biosynthesised *B. pinnatum* AgNPs were determined by transmission electron microscopy (TEM). Colloidal sample was sonicated (Vibronics VS 80) for approximately 15 min. A drop of this solution was then loaded on a copper grid coated with carbon and evaporated by means of the infrared light. The TEM was measured using the JEOL model JEM 2100 instrument operated at an accelerating voltage of 200 kV [23].

3 EXPERIMENTAL ANIMALS

The experiment was approved by the Institutional Animal Ethics committee (IAEC) and was executed as per the national guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) [24]. Male Wistar rats weighing about 150–200 g were procured from the CPCSEA registered breeder. The animals were acclimatised for 7 days before commencement of the experiment. The rats were accommodated in polypropylene cages layered with corn cob as the bedding material under standard management, hygienic and stress-free conditions in the laboratory animal house. The animals were fed with a standard commercial diet and with tap water ad libitum and were maintained in an air conditioned room at 23 ± 2°C with 12 h light/dark cycle.

4 EXPERIMENTAL PROTOCOL

A total of 36 male Wistar rats weighing about 150–200 g were randomly allocated to six groups of six animals in each, the groups were labelled as follows.

- Group I: Normal control group had 0.9% saline in drinking water for 28 days.
- Group II: 0.75% ethylene glycol in drinking water for 28 days.
- Group III: Administered with AgNPs suspended in 0.9% saline (@10 mg/kg bwt) orally for 28 days.
- Group IV: Administered 0.75% ethylene glycol in drinking water for 14 days and withdrawal for next 14 days.
- Group V: Administered 0.75% ethylene glycol in drinking water from 0 to 28 days and treatment of AgNPs (@10 mg/kg bwt orally), from the 14th day to the 28th day of the experiment.
- Group VI: Given 0.75% ethylene glycol in drinking water + AgNPs (@10 mg/kg bwt orally) for 28 days.

4.1 Collection of blood/serum sample and euthanasia

At the end of the 28th day of the experiment, rats were anesthetised with thiopental sodium (50 mg/kg bwt) and a blood sample from each animal was collected from the retro-orbital plexus. For the biochemical assay, the blood was collected in clot activator. Serum was then separated by centrifugation in microfuge tubes at 3000 rpm for 15 min. Sacrification of animals was accomplished by the Humane method. Rats were sacrificed by inhalation through anaesthesia in a jar containing cotton wool soaked in diethyl ether.

4.2 Biochemical analysis

Serum total protein (STP), albumin, globulin, aspartate aminotransferase (AST/SGOT), alanine transaminase (ALT/SGPT), BUN, creatinine, calcium, phosphorus were estimated using an autoanalyzer (Make-AGD Biomedical, Model No. AGD 2020) with the diagnostic kit obtained from M/s. AGD Biomedical (P) Ltd. Andheri (E), Mumbai, India.

4.3 Urine analysis

The urine samples were directly collected from bladder of all sacrificed animals, centrifuged and the supernatant was discarded. A small drop of the sediment was observed under the microscope at ×10 and ×40 for the detection as well as identification of urinary crystals [25].

4.4 Histopathology of kidney

After scarification of animal the kidney tissues, they were tissue collected in 10% formal saline solution for histopathological examination. After fixation, tissues were processed manually through graded ethanol, cleared in xylene, and impregnated and embedded in paraffin wax (58–60°C). Thin sections of 4–6 microns were cut on rotary microtome, stained by routine haematoxylin and eosin stain [26] and observed for pathological changes.

4.5 Statistical analysis

The data was analysed statistically by employing Completely Randomised Design using ICAR WASP programme version 2.0. *p* < 0.05 was considered significant.

5 RESULT

5.1 Characterisation of *B. pinnatum*-mediated AgNPs

UV-Vis spectrum of synthesised AgNPs from the extract of *B. pinnatum* showed an absorbance peak at around 423 nm (Figure 1) which is the characteristic surface plasmon absorbance peak of AgNPs.

NTA analysis was performed by using Nanosight LM-20 to determine the size and size distribution of nanoparticles depending on the Brownian motion in suspension [20]. The mean size of the synthesised AgNPs from NTA analysis was
found to be 44.7 nm, whereas the mode of the particle distribution was 14.3 nm, SD of 39.4 nm and concentration of the particles in the colloidal suspension was found to be 1.25 \times 10^9 \text{ particles/ml} (Figure 2).

The zeta potential of AgNPs was found to be +3.0 mV, which provided the evidence that the fabricated nanoparticles are less stable and likely to form the aggregates (Figure 3(a)).

The common dividing line between stable and unstable suspensions is generally taken at either +30 or −30 mV for naked nanoparticles. The particles with the zeta potential values more positive than +30 mV or are more negative than −30 mV are usually considered stable and the zeta potential was more positive than −15 mV representing a suspension at the urge of agglomeration. The crystalline nature of AgNPs was elucidated on the basis of XRD data analysis. The B. pinnatum-mediated AgNPs were confirmed by characteristic peaks observed in the XRD image (Figure 3(b)). The distinct diffraction peaks at the 2theta value of 38.14°, 46.27°, 67.52° and 77.25° can be assigned to the plane of (111), (200), (220) and (311), respectively which indicates that the AgNPs are crystalline and FCC (face centred cubic) in nature (JCPDS file no. 84-0713 and 04-0783) [27].
The synthesised AgNPs were also further characterised by the FTIR analysis to determine the functional group responsible for capping or stabilisation of the nanoparticles. In the FTIR analysis, control spectra of the silver nitrate (precursor salt) show prominent peaks (Figure 4) at 3353, 2379 and 1644 cm\(^{-1}\), and the plant extract revealed major peaks at 3354, 2939, 1632 and 1045 cm\(^{-1}\) which are comparatively similar to the previous study [27].

However, AgNPs demonstrate the absorbance at 3354, 2379, 1330 and 809 cm\(^{-1}\). For the AgNPs, the broad band appearing at 3354 cm\(^{-1}\) is assigned for O–H stretching vibration indicating the presence of hydroxyl groups in the capping agent [28, 29]. The minor peak at 2379 cm\(^{-1}\) is assigned to the C-H group [30]. The strong intense peaks at 1330 cm\(^{-1}\) correspond to C=C and C=N stretch vibrations [31]. The absorption peaks located around 809 cm\(^{-1}\) are assigned to CO stretching vibrations [28, 32]. The TEM analysis of the synthesised AgNPs revealed the presence of polydispersed, spherical nanoparticles with a mean size of 32.65 nm and SD of 1.84 nm (Figure 5).

### 5.2 Clinical observations

Rats from group II, IV and V showed abnormal behaviour such as lethargy and dullness. Increased urine output was
prominently observed in group II and IV. These clinical symptoms were more prominent in group II as compared to group IV indicating the restitution effect. Group V showed comparatively mild dullness till the first 14 days and the symptoms were missing after 14 days and animals were found to be normal and healthy to that of control animals. Group VI animals also behave normally to that of the control rats. No single mortality was recorded in any of the groups [14].

5.3 | Urine examination

Microscopic observations of urine samples of control and group III rats showed clear and transparent sample. While the rats from group II showed large enormous and group IV showed mild CaOx crystals of different shape and size (Figure 6). However, group V and VI showed no crystals or only one or two crystals per animal smear.
5.4 | Biochemical observations

The biochemical estimations were carried out at the end of the 28th day of the experiment and are presented in Table 1. Serum biochemical parameters revealed significant decrease in total protein, albumin and globulin in group II followed by group IV but were found to be significantly increased in group V and VI. Though the values of total protein, albumin and globulin in group V and VI were not comparable with group I and III, were towards normalization. The withdrawal of ethylene glycol also showed some self-curative effect in group IV. Significant elevated values of serum AST, ALT, creatinine, BUN, calcium and phosphorus were observed in group II followed by group IV when compared with the control group suggested nephrotoxicity and hepatotoxicity caused by ethylene glycol. Conversely progressive decline was found in group V and VI due to the abolishment of the toxicity effect as a result of administration of *B. pinnatum*-mediated AgNPs [14].

5.5 | Histopathology of kidney

The kidney tissue of rats from control and group III exhibited normal renal tubules along with normal renal pyramidal structure of renal cortex and medulla (Figure 7(a)). The tissue sections of kidney from group II given ethylene glycol only showed blood venous congestion, inter-tubular haemorrhages and varying degrees of granular and vacuolar degenerative changes in renal tubules and glomeruli (Figure 7(b)). Foci showed detachment of tubular epithelium from the basement membrane, tubular necrosis, desquamation of tubular epithelium in the lumen and interstitial nephritis.

The most distinguishable finding was the presence of enormous oxalate crystals having various shapes and sizes and huge proteinous mass accumulation mainly in the kidney tubules (Figure 7(c)) which ultimately leads to dilatation of the renal tubules. Thus, lesions observed in group II confirmed that ethylene glycol causes crystal formation in the renal tubules leading to urolithiasis. The kidney sections from group IV also showed foci of accumulation of CaOx crystals and proteinous mass in renal tubules along with mild to moderate degenerative and inflammatory changes in the tubules and glomeruli (Figure 7(d)). Sections from group V showed restoration of tubular and glomerular epithelium in the form of mild mononuclear cell infiltration and mild granular and vacuolar changes in the tubular and glomerular epithelium suggesting beneficial therapeutic effect of *B. pinnatum* AgNPs (Figure 8(a)). There was a minimum proteinous mass accumulation and no crystal accumulation was observed in

![Image of urine examination showing crystals](https://example.com/image)

**Figure 6** Urine examination from group II showing enormously sized crystals of different shapes (×100)

| Table 1 | Serum biochemical parameters in control and different treatment groups |
|---------|-------------------------------------------------|
| Groups  | Total Protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | AST (IU/L) | ALT (IU/L) | Creatinine (mg/dl) | BUN (mg/dl) | Calcium (mg/dl) | Phosphorus (mg/dl) |
| I       | 8.43 ± 0.39 <sup>a</sup> | 3.96 ± 0.07 <sup>b</sup> | 4.47 ± 0.42 <sup>c</sup> | 127.68 ± 1.13 <sup>d</sup> | 26.86 ± 2.33 <sup>e</sup> | 0.59 ± 0.04 <sup>f</sup> | 37.10 ± 2.14 <sup>g</sup> | 9.44 ± 0.44 <sup>h</sup> | 4.22 ± 0.22 <sup>i</sup> |
| II      | 5.58 ± 0.17 <sup>a</sup> | 2.95 ± 0.10 <sup>b</sup> | 2.89 ± 0.12 <sup>c</sup> | 148.53 ± 2.01 <sup>d</sup> | 49.37 ± 2.33 <sup>e</sup> | 1.04 ± 0.15 <sup>f</sup> | 49.53 ± 2.30 <sup>g</sup> | 15.72 ± 0.38 <sup>h</sup> | 7.36 ± 0.19 <sup>i</sup> |
| III     | 7.98 ± 0.26 <sup>a</sup> | 3.74 ± 0.27 <sup>b</sup> | 4.24 ± 0.19 <sup>c</sup> | 126.92 ± 2.05 <sup>d</sup> | 24.42 ± 1.04 <sup>e</sup> | 0.65 ± 0.06 <sup>f</sup> | 36.27 ± 2.94 <sup>g</sup> | 9.56 ± 0.12 <sup>h</sup> | 4.28 ± 0.06 <sup>i</sup> |
| IV      | 6.06 ± 0.11 <sup>a</sup> | 2.82 ± 0.13 <sup>b</sup> | 3.24 ± 0.06 <sup>c</sup> | 140.13 ± 1.57 <sup>d</sup> | 45.95 ± 1.33 <sup>e</sup> | 1.02 ± 0.05 <sup>f</sup> | 44.80 ± 1.66 <sup>g</sup> | 13.86 ± 0.26 <sup>h</sup> | 6.51 ± 0.16 <sup>i</sup> |
| V       | 6.95 ± 0.38 <sup>a</sup> | 3.20 ± 0.34 <sup>b</sup> | 3.75 ± 0.23 <sup>c</sup> | 137.28 ± 2.01 <sup>d</sup> | 33.78 ± 1.35 <sup>eb</sup> | 0.93 ± 0.03 <sup>f</sup> | 40.02 ± 1.34 <sup>g</sup> | 10.68 ± 0.61 <sup>h</sup> | 4.42 ± 0.26 <sup>i</sup> |
| VI      | 6.56 ± 0.16 <sup>a</sup> | 3.20 ± 0.04 <sup>b</sup> | 3.36 ± 0.14 <sup>c</sup> | 134.52 ± 1.46 <sup>d</sup> | 29.15 ± 1.28 <sup>e</sup> | 0.79 ± 0.08 <sup>f</sup> | 35.22 ± 1.94 <sup>g</sup> | 11.03 ± 0.53 <sup>h</sup> | 4.52 ± 0.26 <sup>i</sup> |
| Level of significance | * | * | * | * | * | * | * | * | * |

CD (0.05) | 0.752 | 0.551 | 0.657 | 5.029 | 4.898 | 0.229 | 5.947 | 0.368 | 0.571

Note: Values indicate mean ± SE. Mean values with common alphabet as superscript do not differ significantly.

Abbreviation: NS, non-significant.

Significance levels *p ≤ 0.05.
the tubules. Group VI also revealed mild granular and vacuolar degenerative changes in the tubular epithelium with foci of mononuclear cell infiltrations. However, no crystal formation was observed in the renal tubules (Figure 8(b)). Foci of proteinous mass cast was observed in few sections only [14].
6 | DISCUSSION

Urolithiasis or kidney stone is nothing but formation of urinary calculi in the urinary tract. It is one of the oldest and most wide-spread diseases in humans as well as in animals and is considered as the third most common urinary tract infection. Since ancient times, quiescent origin of medicine is from indigenous plants with anti-urolithic effect [33, 34]. The biosynthesis of AgNPs is eco-friendly, nontoxic and its size plays a vital role by increasing the surface area of reactivity and decreasing the quantity and size with high bioavailability [35].

Thus, nanotechnology is found to overcome the routine uses of ethanolic or methanolic extract of various plants. The present study elaborated the use of B. pinnatum-mediated AgNPs during ethylene glycol-induced urolithiasis in rat.

UV-Vis spectroscopy proves to be one of the important and simple ways to confirm the formation of nanoparticles. The absorbance peak at around 423 nm is the characteristic surface plasmon absorbance peak of AgNPs [36, 37]. Shifting of the absorption peak towards higher energy wavelengths indicates decrease in the size of the nanoparticles and vice versa [38, 39]. The presence of a single absorption peak in the present study reveals the presence of spherical nanoparticles which corroborates with the transmission electron microscopic analysis. The NTA analysis showed similarity with earlier results [40] for determining the size of AgNPs. NTA analysis can be used to understand the aggregation in the samples, which cannot be seen in other methods such as differential centrifugation sedimentation and dynamic light-scattering technique [7]. In the present study, the large difference between mean and mode of the particle size of AgNPs can be attributed to the aggregation of nanoparticles which might have formed the aggregates and would have resulted in the higher average size of the AgNPs. Despite lower zeta potential value, which is approaching almost zero, the nanoparticles were found to be almost stable due to capping of biomolecules and the zeta potential value of the particles is due to the potential contributed by nanoparticles and by the capping agent together. Moreover, the presence of the capping agent prevents the aggregation of nanoparticles hereby imparting them stability. The size and the stability of nanoparticles are the functions of diffusion and attachment of the capping material that forms the surface chemistry. The stability of nanoparticles can be characterised by surface coverage or concentration of the capping material in the plant extract [14, 18, 41, 42]. The electrostatic stability provided by biomolecules in the capping layer of AgNPs that either have formed a complex with Ca^{2+} and Mg^{2+} making them unavailable for crystal formation or AgNPs adsorbed on crystals may have prevented the crystal growth by inhibiting the nucleation process [35, 43, 44].

On comparing FTIR spectra of control and AgNPs, it was found that few bands present in the precursor salt and plant extract disappeared at the time of AgNPs synthesis; this clearly indicates that few molecules are responsible for the capping and stabilisation of AgNPs. The average size evaluated by TEM analysis was smaller than the average size predicted by NTA [45]. These two methods rely on different physical principles and/or detection methods and might have the reason for difference in the results. In addition, TEM is a number-based method that has a bias towards the smaller sized particles compared to NTA, which is a number-based method but fails to probe the weakly scattering particles (below 10–20 nm) and is thus mostly measuring the nanoparticle aggregates which may clarify some of the differentiation in size measurements.

Similar symptoms of dullness and depression in ethylene glycol-treated group with non-significant differences in behaviour were observed in earlier studies in rats [46, 47]. Moreover, the finding of increased urine output in the lithic control group is in agreement with a similar study in rats [48]. As ethylene glycol is sweet in test it may elevate the palatability and increase the water consumption thereby increase the urine output [49]. Contrary to the present findings, a previous study [8] reported decreased urinary output rats administered with ethylene glycol and suggested a decline in urinary output in rats treated with ethylene glycol that occurred as a result of formation of CaOx crystals along with its retention and results in decreased glomerular filtration rate, which promotes decreased seepage of Ca^{2+}, Cl− and Na+ ions and promotes stone formation.

In the present study, formation of crystals in urine indicated that ethylene glycol administered at 0.75% in water causes urolithiasis in rats. Similar findings of marked crystal formation treated with ethylene glycol and decrease in urinary crystal deposition on administration of aqueous extract of B. pinnatum AgNPs was recorded in rats [9, 25]. B. pinnatum contains antioxidant phytochemicals such as flavonoids, polyphenols and saponins which might have contributed for dissolving stones formed during urolithiasis and might be the reason for observation of decreased amount of crystals in urine in group V and VI [8].

The significant decrease in serum total protein in group II and IV might be due to a significant decrease in serum albumin. This might be due to the degenerative changes in liver and kidney which may lead to alteration in protein and free amino acid metabolism in the liver. The findings in groups III, V and VI suggested that the plant helps in the regeneration of hepatic cells by elevating the serum albumin and total protein levels [50]. Increased serum levels of AST and ALT are known indicators for hepatic degeneration or damage. The damage in hepatocytes leads to increase in cell membrane permeability which ultimately leads to the leakage of enzymes in to blood circulation. In a similar study, increased serum AST and ALT levels in the mercuric chloride-treated rats were found to be decreased by the treatment of B. pinnatum and suggested a potent antioxidant, hepatoprotective and nephroprotective activity of the plant against mercuric chloride [51].

Elevated levels of serum creatinine is observed in kidney disease due to muscle degeneration and due to the effect of some drugs or chemicals involved in disrupting the renal architecture [52]. The degenerative changes observed in group II and IV and restoration of renal parenchyma in group V and VI might be the reason for the increase and decrease in serum creatinine levels, respectively, in the present study. Administration of ethylene glycol results in calculi formation which obstructs the normal renal outflow and causes decline in
glomerular filtration or obstruction of renal outflow which leads to accumulation of serum creatinine and blood urea nitrogen [9, 14, 35]. Decreased serum creatinine level in group V and VI corroborates with the earlier finding in which restoration of serum creatinine level after treatment with B. pinnatum plant in the form of extract or nanoparticles against various toxicities, was recorded [51–53]. The plant B. pinnatum constitutes the anti-oxidant property which averts the oxidative stress caused due to lipid peroxidation thereby helping in normalising the serum creatinine and BUN level [9]. Saponins and other phyto-chemicals present in B. pinnatum might have played a role in decreasing the calcium level in groups treated with AgNPs synthesised by B. pinnatum extract [8].

Ethylene glycol administered for 28 days caused severely disrupted renal parenchyma with vacular degeneration, focal calcification and inflammatory changes in glomerulo-tubular structures. This can be directly correlated with histological findings in the kidney in group II rats administered with ethylene glycol at 0.75% for 28 days. The massive tubular cell death exposes the basement membrane to the urinary stream, thus increasing the opportunity for the degraded structure to act as a seed for CaOx crystal formation or deposition, which in turn causes the gradual disruption of the tubular structure and lumen dilatation [54]. Histopathological section of group V and VI showed restoration of tubular and glomerular epithelium in the form of mild mononuclear cell infiltration and mild granular and vacuolar changes in the tubular and glomerular epithelium suggesting beneficial therapeutic effect of AgNPs synthesised by B. pinnatum. The nephroprotective activity of B. pinnatum might be due to the phytochemicals quercetin and kaemferol present in plant which acts as an antioxidant and prevents renal damage [55].

7 | CONCLUSION

Overall, observations are suggestive of protective as well as therapeutic effect of B. pinnatum-mediated AgNPs administered along with ethylene glycol in rats. The present findings thus suggested the potential effect of AgNPs synthesised by B. pinnatum in dissolving the CaOx crystals and promoting regeneration of the tissue towards normalcy.

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