AN EXPERIMENTAL EVALUATION OF NEPHROPROTECTIVE POTENTIAL OF *BORAGO OFFICINALIS* STEMS EXTRACT IN ALBINO RATS

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ABSTRACT
Renal dysfunction and nephrotoxicity are major concerns in modern medicine, necessitating the search for effective nephroprotective agents. *Borago officinalis* (BO), a medicinal plant with a long history of traditional use, has been attributed with antioxidant, anti-inflammatory, and anti-apoptotic properties. This experimental study aimed to evaluate the nephroprotective potential of BO extract in albino rats subjected to gentamicin-induced nephrotoxicity. The study employed a randomized, controlled experimental design, wherein albino rats were randomly divided into five groups: control, gentamicin-induced nephrotoxicity (GNT), Standard Silymarin (200mg/kg), BO extract low dose (100mg/kg), and BO extract high dose (200mg/kb). The GNT group received gentamicin to induce renal damage, while the BO groups received BO extract at low and high doses, respectively. Renal function markers, including serum creatinine, urea were assessed, along with histopathological analysis and evaluation of oxidative stress markers. The results demonstrated that BO extract significantly reduced gentamicin-induced renal dysfunction. In comparison to the GNT group, plant extract exhibited improved renal function, as evidenced by reduced levels of serum creatinine and urea. Histopathological analysis revealed lesser degrees of tubular necrosis, glomerular damage, and interstitial inflammation in the BO groups. The findings of this study suggest that BO extract may serve as a potential nephroprotective agent, offering a novel therapeutic approach for the prevention and treatment of renal dysfunction and nephrotoxicity. The results warrant further investigation into the pharmacological properties of BO extract and its potential applications in clinical practice.
Keywords: *Borago officinalis*, nephroprotection, gentamicin-induced nephrotoxicity, antioxidant, anti-inflammatory, albino rats.

1. INTRODUCTION
Nephrotoxicity can result from the harmful effects of several xenobiotic, including aminoglycosides, cephalosporins, anticancer medications (cisplatin), amphotericin B, and analgesics. One with an estimated lifetime risk of 2-5% in Asia, 8-15% in Europe and the Americas, and roughly 20% in the Middle East, nephrotoxicity is the third most prevalent renal system issue. Certain phytochemical-containing Indian medicinal herbs have been shown in several trials to have positive effects on kidney damage **(Naughton, 2008; Bhattacharjee, 1998).** Acute kidney damage (AKI) affects roughly 1-7% of hospitalized patients and 1-25% of patients in the intensive care unit (ICU). It is a common clinical consequence that is linked to increased morbidity and mortality **(De Mendonca *et al.*, 2000; Nash *et al.*, 2002)** Numerous epidemiological studies have yielded a wide range of estimates on the prevalence of AKI in patients with severe illnesses; these estimates ranged from 15% to 50% **(Case *et al.*, 2013).** Approximately 20% of community and hospital acquired episodes of AKI are caused by drugs, and the percentage among the elderly might reach up to 66%. Drugs are a common cause of AKI **(Kaufman *et al.*, 1991; Kohli *et al.*, 2000).** Among the significant classes of antimicrobial medicines that have been in use for more than 50 years are aminoglycosides **(Swain and Kaplan, 1999).** Aminoglycosides can be used to treat infections that are life-threatening even though new and more potent antibiotics are constantly being found **(Mingeot and Tulkens, 1999).** One of the crucial components of aminoglycosides is gentamicin. Gentamicin may be hazardous to the kidneys. Gentamicin administration causes renal failure, elevated serum creatinine and urea levels, and renal tubular necrosis **(Cuzzocrea *et al.*, 2002; Al-Majed *et al.*, 2002).** The generation of free radicals that may result in renal damage may be the reason of gentamicin's harmful impact. Thus, reducing the production of free radicals could be a strategy to lessen nephrotoxicity. Along, chronic kidney disease (CKD) is a growing global health concern, affecting millions of individuals worldwide. The progression of CKD often results in end-stage renal disease, necessitating renal

replacement therapy such as dialysis or kidney transplantation **(Ruggenenti *et al.*, 2012).** The development of effective nephroprotective agents is of utmost importance in delaying or preventing the progression of CKD and AKI. Traditional medicine has long recognized the therapeutic potential of various plant extracts, including Borago officinalis, commonly known as starflower. *Borago officinalis* has been used for centuries to treat a variety of ailments, including inflammation, pain, and respiratory issues, due to its antioxidant, anti-inflammatory, and antimicrobial properties **(Gruenwald *et al.*, 2007; Asadi *et al.*, 2014).** *Borago officinalis* stem extract (BOSE) has gained particular attention for its potential renal protective effects, as it has been shown to possess anti-inflammatory, antioxidant, and antimicrobial properties that may help prevent the progression of CKD and AKI **(Gracia *et al.*, 2010; Michalak *et al.*, 2023).** The present study aimed to investigate the nephroprotective potential of BOSE in albino rats with experimentally induced nephrotoxicity. In this study, the rats were divided into different groups, with one group receiving BOSE treatment and the others serving as controls. The animals were then subjected to nephrotoxicity induction using a well-established model. The kidneys of the treated group were assessed for histopathological changes, biochemical markers, and antioxidant enzyme activities to determine the efficacy of BOSE in preventing nephrotoxicity. The results of this study will provide valuable insights into the potential of BOSE as a nephroprotective agent and contribute to the development of novel therapeutic strategies for the management of CKD.

2. MATERIAL AND METHOD

2.1 Chemicals

Glacial acetic acid, nitroprusside, sodium hydroxide, and ammonia were procured from Merck, a well-known provider of analytical reagents. Petroleum ether was obtained from Researchlab, while concentrated sulfuric acid was supplied by Fizmerck. Ethanol was sourced from Molychem, and both 95% alcohol and concentrated hydrochloric acid were acquired from Clorofiltind, which also provided chloroform. Magnesium was purchased from Himedia, and the 1% copper sulphate solution was obtained from Rankem.

2.2 Plant collection

300 grams of the therapeutic herb *Borago officinalis* were gathered. Following cleaning, the plant material (stems) was dried for three days at ambient temperature in the shade and for a further three days at 45°C in the oven. To prevent contamination and deterioration, dried plant parts (stems) were kept in airtight glass containers in a dry, cool environment. Verification of the authenticity of a particular traditional plant: A plant taxonomist verified the identity and purity of the medicinal plant *Borago officinalis*.

2.3 Extraction

Plant material was extracted for the current investigation utilizing the Soxhlet apparatus and a continuous hot percolation process. The powdered *Borago officinalis* material was added to the soxhlet apparatus thimble. Soxhlation was carried out at 60°C with a non-polar solvent such as petroleum ether. The plant material that had run out (marc) was dried and then extracted again using methanol. For every solvent, the soxhlation process was continued until no discernible color change was seen in the siphon tube. The extraction process was considered complete when there was no more solvent left behind after it evaporated. The obtained extracts were evaporated at 40°C in a Buchi-type rotating vacuum evaporator. Weighing the dried extract, we calculated the % yield for each extract using the following formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya*et al.*, 2002).**

2.4 Phytochemical investigation

By performing a thorough qualitative phytochemical analysis, the experiment was designed to determine whether or not various phytoconstituents were present. The precipitate formation or color

intensity was utilized to gauge how the body would react to various tests. Standard operating procedures were employed **(Kokate *et al.*, 2000**).

2.5 Quantitative Phytochemical Estimation

2.5.1 TPC

Using the Folin-Ciocalteu Assay, the total phenolic content of the *Borago officinalis* extract was ascertained. Two milliliters of 7.5% sodium carbonate, 2.5 milliliters of Folin-Ciocalteu Reagent, and 0.2 milliliters of *Borago officinalis* extract were combined. Distilled water was added to this combination to dilute it up to 7 mL. After the final solutions were let to remain at room temperature for two hours, the absorbance at 760 nm was determined using spectrophotometry. Gallic Acid Equivalent (GAE) mg/gm standard solutions were used to create calibration curves. Gallic aid was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL The reagent Folin-Ciocalteu is susceptible to reducing agents, such as polyphenols. When they react, they turn blue. This blue hue was determined using spectrophotometry (**Tangco *et al.*, 2015)**.

2.5.2 TFC

The aluminum chloride technique was used to determine the flavonoid concentration. A mixture of 0.5 ml *Borago officinalis* extract solution and 2 ml distilled water was prepared. Following that, 0.15 ml of sodium nitrite (5%) was added and thoroughly stirred. Subsequently, add 0.15 ml of aluminum chloride (10%) and let it stand for an additional 6 minutes. Next, two milliliters of 4% sodium hydroxide were added. The blend was well blended and given a good shake. A UV spectrophotometer was used to assess the mixture's absorbance at 510 nm. Rutin Equivalent (RE) mg/gm standard solutions were used to create calibration curves. Rutin was produced at concentrations of 20, 40, 60, 80, and 100 µg/mL. The calibration curve was used to calculate the total flavonoid concentration, and the results were expressed in milligrams of Rutin equivalent per gram dry extract weight **(Parthasarathy S *et al.*, 2009).**

2.6 DPPH

The flavonoid concentration was established by use of the aluminum chloride procedure. A concoction of 2 ml distilled water and 0.5 ml *Borago officinalis* extract solution was made. Then 0.15 ml of sodium nitrite (5%) was added, and everything was well mixed. Then, pour in 0.15 ml of 10% aluminum chloride and leave it to stand for an extra six minutes. Two milliliters of sodium hydroxide (4%), then that. The mixture was thoroughly mixed and given a brisk shake. Utilizing a UV spectrophotometer, the mixture's absorbance at 510 nm was measured. To construct calibration curves, rutin equivalent (RE) mg/gm standard solutions were utilized. At doses of 20, 40, 60, 80, and 100 µg/mL, rutin was generated. Take 3 milliliters of 0.1 milligram DPPH solution and incubate it for 30 minutes at room temperature in the dark as a control. The control's absorbance against methanol (used as a blank) was measured at 517 nm **(Athavale *et al.*, 2012).**

2.7 FT-IR

FT-IR spectroscopy was carried out using a Perkin Spectrum BX spectrophotometer to determine whether the functional groups were present in the separated fraction (A) of the PI methanolic extract. Thermo Nicolet model 6700 spectrum spectrometer was used to evaluate the material after it had been dried and crushed using KBr pellets. A mixture of 2% finely dried sample was used to make a disk containing 100 mg of KBr, which was subsequently analyzed using an IR spectrometer. Spectra of infrared light were obtained between 400 and 4,000 cm-1 **(Lucien*et al.*, 2008).**

2.8 Acute Toxicity Study

Three animals of the same sex are used in each phase of the step-by-step process for the acute toxic class technique outlined in the guidelines. An assessment of the test substance's acute toxicity may need, on average, two to four phases, and contingent on the animals' mortality and/or moribund state. One of the specified doses of the material is given orally to a group of experimental animals. Three animals of the same sex are used in each phase of the methodical testing process for the chemical. The next stage will be determined by whether compound-related mortality of the animals dosed at one step is present or absent, meaning that if more testing is not required, three additional animals will be dosed at the same dose and three other animals will be dosed at the next higher or lower dose level. Every phase involves the use of three animals. One of four fixed dose levels—5, 50, 300, or

2000 mg/kg body weight—is chosen to constitute the initial dosage. **(Guideline Document on 1996**).

2.9 Experimental work

2.9.1 Animals required

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). The animals used of either sex were of approx 200-250g body weight in range. Before the experiment for the acclimatization, the animals were kept in groups of six in individual cages with temperature controls set at 22 ± 2°C. After that every animal received a consistent supply of water and a standard meal (golden feed, New Delhi).

2.9.2 Experimental protocol

The 14-day experimental protocol was planned. Five groups, each consisting of six rats, were created from rats of both sexes. Oral preparations with a feeding tube were administered to the rats in each group (**Rad *et al.*, 2017**).

Group-I: served as a normal control and received a daily dose of 1 mL/kg of normal saline.

Group II: 40 mg/kg of gentamycin was administered intraperitoneally (i.p.) at the same time for 14 days, serving as a nephrotoxic control.

Group III: 200 mg/kg of silymarin orally as the typical nephroprotective medication

Group IV: 100 mg/kg of *Boragoofficinalis*extract combined with Gentamycin (p.o.)

Group V: 200 mg/kg of *Boragoofficinalis*extract combined with Gentamycin (p.o.)

2.9.3 Blood collection techniques used in the present study

Animals were slaughtered under a light ether anesthesia on the fifteenth day, which marked the conclusion of the experimental period. Using a fine capillary, a retro-orbital vein puncture was used to collect the blood, which was then placed in an anticoagulant tube and left to stand for 30 minutes at 37°C before the serum was separated and the biochemical markers were assessed by centrifugation.

2.9.4 Preparation of kidney homogenate

After the kidney was swiftly removed, ice-cold saline (0.9% NaCl) was immediately perfused. Using a homogenizer, a piece of the kidney was homogenized in cold Tris-HCl buffer (0.025 M, pH 7.4). After centrifuging the homogenate for ten minutes at 5,000 rpm, the supernatant was collected and utilized in a variety of biochemical tests.

2.10 Analysis of general parameters

2.10.1 Estimation of urine volume

For a full day, the animals are housed in different metabolic cages. After a day, the volume of pee from each rat is measured. Urine is treated to remove dietary wastes and feces. Additionally, a measuring cylinder is used to determine the urine's volume.

2.10.2 Estimation of Body weight

Upon completion of the trial, every group of animals was maintained separately within their cages. Take out the food and water, weigh each animal separately, and record the results.

2.10.3 Analysis of serum biochemical parameters

- **Estimation of Serum Creatinine (Slot C *et al.*, 1965)**

A, B, C, D, and E were the labels on five test tubes. Where E is considered blank, C and D are considered test, and A and B are considered standard. 2 ml of distilled water were pipetted into E (blank), 0.5 ml of serum and 1.5 ml of water into C&D (test), and 1.5 ml of water and 0.5 ml of creatinine standard (3 mg/dl) into A&B (standard). In each of the five test tubes, 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added.

- **Estimation of Serum Blood urea nitrogen (BUN) (Fawcett, J.K *et al.*,1960)**

The Berthelot method (Fawcett and Scott, 1960) was used to estimate the blood urea using a commercial kit from Kamini Life Sciences Pvt. Ltd. in Hyderabad, India. To create the test, standard, and blank, 1000 µl of working reagent-I, which contained urease reagent, and a combination of salicylate, hypochlorite, and nitroprusside were added to 10 µl of serum, 10 µl of standard urea (40 mg/dl), and 10 µl of filtered water. Following thorough mixing, each test tube was incubated for five minutes at 37 °C. After that, 1000 µl of reagent-II, which contained alkaline buffer, was applied to

each test tube. The test tubes were then incubated for five minutes at 37 °C. The transformation of urea into ammonia and carbon dioxide is catalyzed by urease. Indo phenol, a blue-green chemical, is produced when a combination of salicylate, hypochlorite, and nitroprusside combines with the ammonia that is so released. Spectrophotometrically measured at 578 nm, the intensity of the color produced is directly proportional to the concentration of urea in the sample. The following formula was used to determine the blood urea:

$$\text{Blood urea (mg/dl)} = \frac{\text{Absorbance of test} \times 40}{\text{Absorbance of std}}$$

3. RESULTS

3.1. Percentage Yield

Table 1: Percentage Yield of crude extracts of *Borago officinalis* extract

S.No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Boragoofficinalis*	Pet ether	285	1.64	0.57%
2		Methanol	299	6.16	2.06%

3.2 Preliminary Phytochemical study

Table 2: Phytochemical testing of extract

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Absent	Present
Glycoside		
Borntrager test	Present	Present
Legal's test	Present	Present
Killer-Killiani test	Present	Present
Carbohydrates		
Molish's test	Absent	Absent
Fehling's test	Absent	Absent
Benedict's test	Absent	Absent
Barfoed's test	Absent	Absent
Proteins and Amino Acids		
Biuret test	Absent	Absent
Flavonoids		
Alkaline reagent test	Absent	Present
Lead Acetate test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Saponin		
Foam test	Present	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Absent	Absent
Libbermann-Burchard's test	Absent	Absent

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3.3 Quantitative Analysis

3.3.1 Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

Table 3 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.142
2.	40	0.175
3.	60	0.190
4.	80	0.237
5.	100	0.272

Table 4 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.173
2.	40	0.203
3.	60	0.277
4.	80	0.316
5.	100	0.331

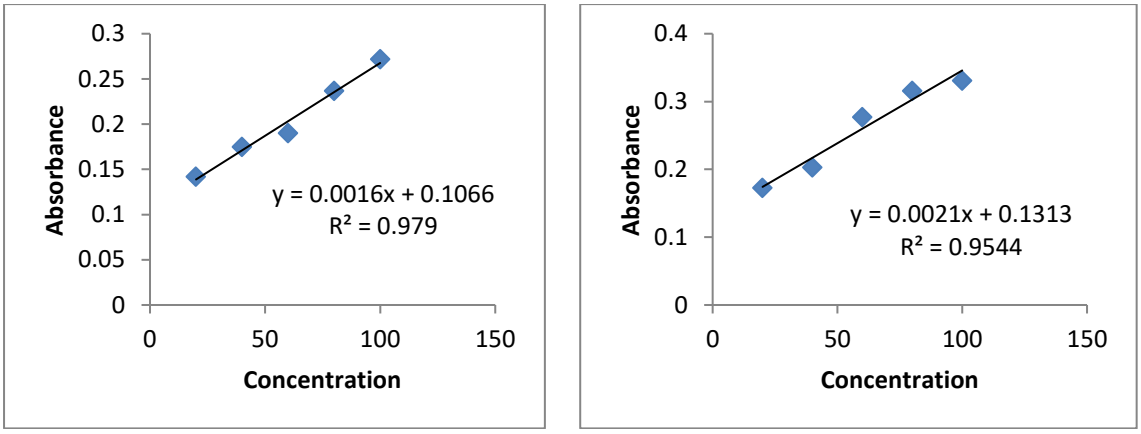

Figure 1: Represent standard curve of Gallic acid and Rutin

3.3.1.1 Total Phenolic Content and Total Flavonoid Content in extract

Table 4: Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.136	61.33mg/gm
2	0.177	
3	0.189	

Table 5: Total Flavonoid Content

S.No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.147	17.83mg/gm
2	0.161	
3	0.192	

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3.4 *In vitro* Antioxidant Assays

3.4.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 6: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.483	51.261
40	0.434	56.205
60	0.343	65.388
80	0.284	71.342
100	0.144	85.469
Control	0.991	
IC50	21.98	

Table 7: DPPH radical scavenging activity of methanol extract of *Boragoofficinalis*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.519	43.831
40	0.465	49.675
60	0.454	50.865
80	0.413	55.303
100	0.367	60.281
Control	0.924	
IC50	49.84	

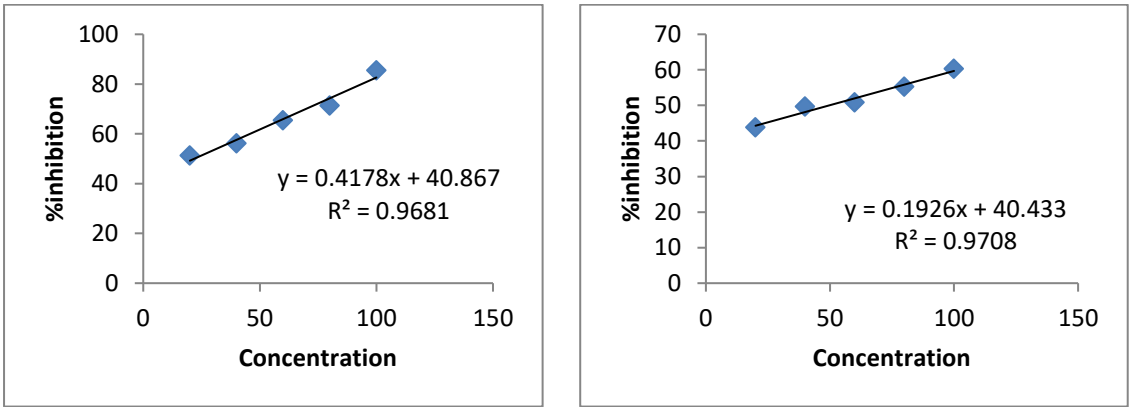

Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid and extract of *Borago officinalis*

3.5 Functional group identified by FTIR Study

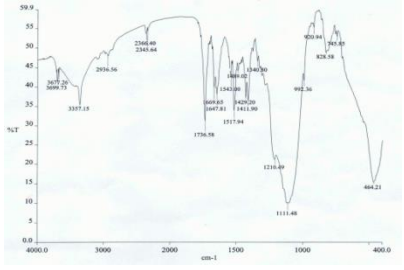

Figure 3: FTIR of *Boragoofficinalis*

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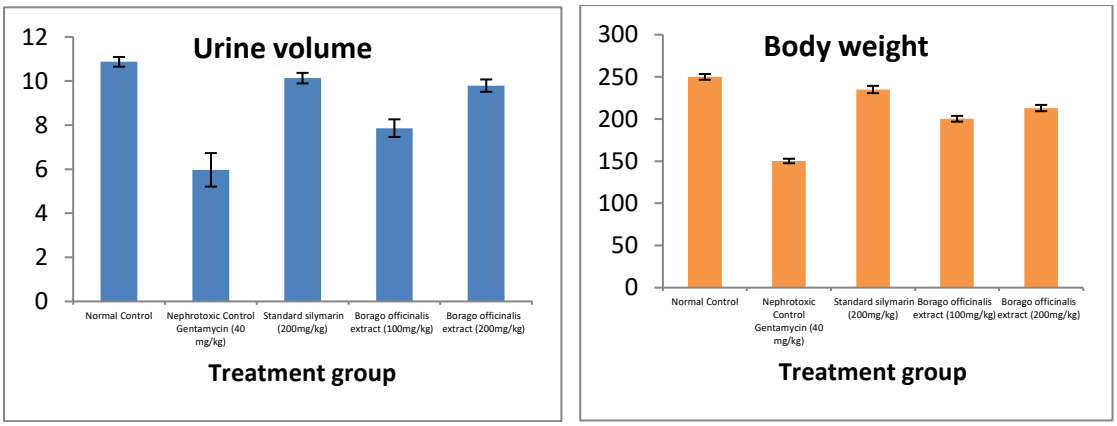
3.6 Analysis of general parameters

3.6.1 Estimation of urine volume and Body weight

Table 8: Urine volume and Body weight

Groups	Urine volume	Body weight
Normal Control	10.87±0.22	250±3.404
Nephrotoxic Control Gentamycin (40 mg/kg)	5.97±0.76	150.22±2.657
Standardsilymarin(200mg/kg)	10.13±0.24	235±4.354
*Boragoofficinalis*extract (100mg/kg)	7.86±0.40	200.32±3.42
*Boragoofficinalis*extract (200mg/kg)	9.79±0.28	213±3.741



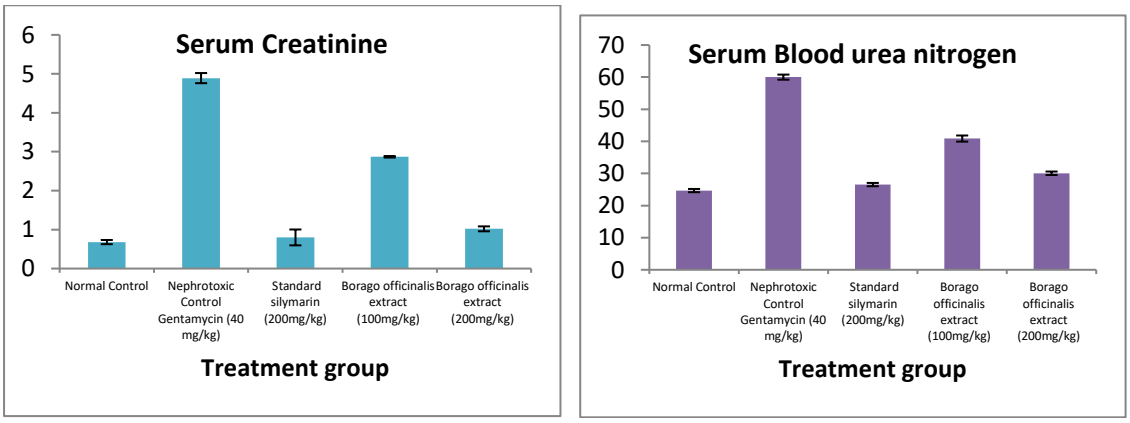
Graph 1: Urine volume and Body weight

3.7 Analysis of serum biochemical parameters

3.7.1 Estimation of Serum Creatinine and Serum Blood urea nitrogen(BUN)

Table 9: Serum Creatinine and Serum Blood urea nitrogen

Groups	Serum Creatinine	Serum Blood urea nitrogen
Normal Control	0.68±0.054	24.67±0.504
Nephrotoxic Control Gentamycin (40 mg/kg)	4.89±0.130	60±0.791
Standardsilymarin(200mg/kg)	0.80±0.204	26.54±0.51
*Boragoofficinalis*extract (100mg/kg)	2.87±0.019	40.87±0.95
*Boragoofficinalis*extract (200mg/kg)	1.02±0.063	30.06±0.54



Graph 2: Serum Creatinine and Serum Blood urea nitrogen

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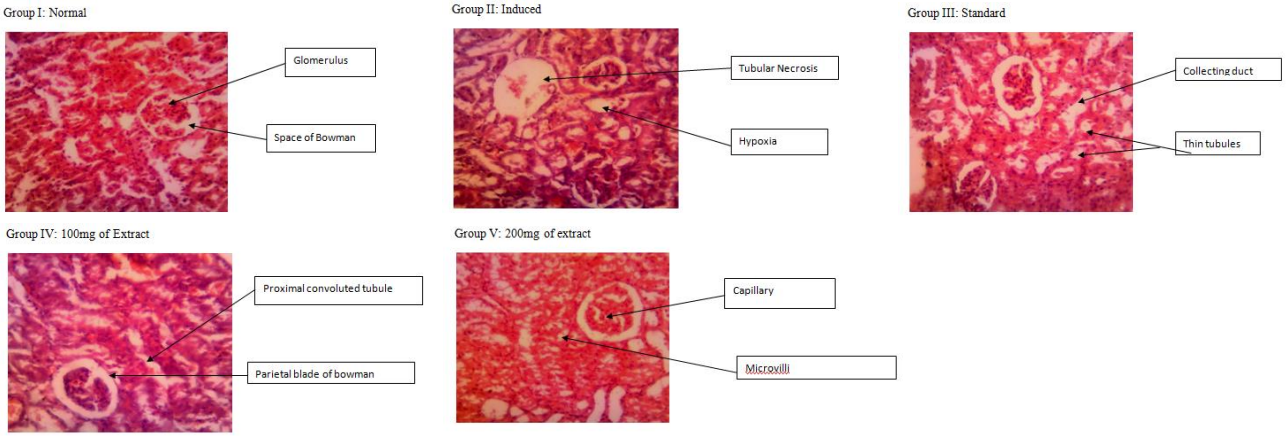
3.8 Histology



4. DISCUSSION

The methanolic extract of *Boragoofficinalis*underwent phytochemical examination, which revealed the presence of alkaloids, phenolics, flavonoids, saponins, glycosides, tannins, and phenolics. Total flavonoid content (TFC) and total phenolic content (TPC) were determined for a quantitative phytochemical experiment. The TPC was computed using gallic acid as the standard, while the TFC was computed using rutin as the standard. Outcomes displayed in Tables 4 and 5. The *Borago officinalis* extract's DPPH radical scavenging activity demonstrated a percent inhibition of 60.28%, with an IC 50 value of 49.84µg/ml. As a reference drug, ascorbic revealed an IC 50 value of 21.98µg/ml and a percent inhibition of 85.46%. Up to a dosage of 2000 mg/kg body weight, no symptoms of toxicity were observed in the acute toxicity investigation. As a result, the study will use the 1/10th and 1/5th doses, or 100 mg/kg and 200 mg/kg, respectively. Using *Borago officinalis* at doses of 100 and 200 mg/kg body weight for 14 days significantly lowered the serum level of creatinine with a significant weight gain and increased urine output when compared with the nephrotoxic control group. In the current study, rats treated with a single dose of Gentamycin showed a marked reduction of body weight as compared to the normal group. This was also accompanied by a marked reduction of glomourular filtration rate. When gentamycin was administered to control rats, a characteristic pattern of nephrotoxicity was observed, characterized by a significant rise in serum blood urea nitrogen (BUN). Rats given a dose of *Borago officinalis* while on gentamycin showed a decrease in their plasma levels of blood urea nitrogen (BUN).

5. CONCLUSION

The present study was undertaken to scientifically evaluate the nephroprotective activity of the methanolic extract of *Borago officinalis*. The phytochemical investigation revealed the presence of alkaloids, flavonoids, glycosides, tannins and phenols in *Borago officinali.* The administration of Gentamycin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Therefore it is an effective and an ideal model for nephrotoxicity research. The evaluation of renal parameters on nephrotoxic rats with *Borago officinalis* showed significantly elevate the attenuated body weight, urine volume and significant reduce in elevated serum creatinine level, which supports its Nephroprotective activity. Histopathological studies on isolated kidney revealed that the *Borago officinalis*, reversed the kidney damage and also restored normal kidney architecture. In summary, the *Borago officinalis* methanolic extract showed statistically significant nephroprotective activity. The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties.

6. REFERENCES

- Al-Majed, A. A., Mostafa, A. M., Al-Rikabi, A. C., & Al-Shabanah, O. A. (2002). Protective effects of oral arabic gum administration on gentamicin-induced nephrotoxicity in rats. Pharmacological Research, 46(5), 445-451.

- Asadi-Samani, M., Bahmani, M., & Rafieian-Kopaei, M. (2014). The chemical composition, botanical characteristic and biological activities of Borago officinalis: a review. Asian Pacific journal of tropical medicine, 7, S22-S28.
- Athavale, A., Jirankalgikar, N., Nariya, P., & Des, S. (2012). Evaluation of In-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. Int J Pharm Sci Res, 3, 2543-9.
- Baidya, B., Gupta, S. K., & Mukherjee, T. (2002). An extraction-based verification methodology for MEMS. Journal of Microelectromechanical Systems, 11(1), 2-11.
- Bhattacharjee SK. Hand Book of Medicinal Plants. (1998).2nd edition. Pointer Publishers Jaipur.2:228.
- Case, J., Khan, S., Khalid, R., & Khan, A. (2013). Epidemiology of acute kidney injury in the intensive care unit. Critical care research and practice, 2013.
- Cuzzocrea, S., Mazzon, E., Dugo, L., Serraino, I., Di Paola, R., Britti, D., ... & Salvemini, D. (2002). A role for superoxide in gentamicin-mediated nephropathy in rats. European journal of pharmacology, 450(1), 67-76.
- De Mendonça, A., Vincent, J. L., Suter, P. M., Moreno, R., Dearden, N. M., Antonelli, M., ... & Cantraine, F. (2000). Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. Intensive care medicine, 26, 915-921.
- Fawcett,J.K, and J.E. Scott. (1960). determination of serum blood ureanitrogen.
- Garcia-Herreros, C., Garcia-Iñiguez-de-Ciriano, M., Astiasarán, I., & Ansorena-Artieda, D. (2010). Antioxidant activity and phenolic content of water extracts of *Borago officinalis* L: Influence of plant part and cooking procedures.
- Gruenwald, J., Brendler, T., & Jaenicke, C. (2007). PDR for herbal medicines. Thomson, Reuters.
- Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs
- Kaufman, J., Dhakal, M., Patel, B., & Hamburger, R. (1991). Community-acquired acute renal failure. American journal of kidney diseases, 17(2), 191-198.
- Kohli, H. S., Bhaskaran, M. C., Muthukumar, T., Thennarasu, K., Sud, K., Jha, V., ... & Sakhuja, V. (2000). Treatment-related acute renal failure in the elderly: a hospital-based prospective study. Nephrology dialysis transplantation, 15(2), 212-217.
- Kokate CK, Purohit AP and Gokhale SB.(2000). Textbook of Pharmacognosy, NiraliPrakashan.; 1–4.
- Luciene Gonçalves Palmeira Moraes , Renata Sanches Ferreira Rocha , LíviaMalufMenegazzo, Eudes Borges De Araújo , KeizoYukimitu, João Carlos Silos Moraes, (2008). Infrared Spectroscopy: A Tool For Determination Of The Degree Of Conversion In Dental Composites, Journal Applied Oral Science.16(2):145-9
- Michalak, M., Zagórska-Dziok, M., Klimek-Szczykutowicz, M., & Szopa, A. (2023). Phenolic profile and comparison of the antioxidant, anti-ageing, anti-inflammatory, and protective activities of *Borago officinalis* extracts on skin cells. Molecules, 28(2), 868.
- Mingeot-Leclercq, M. P., & Tulkens, P. M. (1999). Aminoglycosides: nephrotoxicity. Antimicrobial agents and chemotherapy, 43(5), 1003-1012.
- Nash, K., Hafeez, A., & Hou, S. (2002). Hospital-acquired renal insufficiency. American Journal of Kidney Diseases, 39(5), 930-936.
- Naughton, C. A. (2008). Drug-induced nephrotoxicity. American family physician, 78(6), 743-750.
- Parthasarathy S, Bin Azizi J, Ramanathan S, Ismail S, Sasidharan S, Said MI, *et al.*, (2009) Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from Mitragynaspeciosa (Rubiaceae Family) leaves. Molecules 14: 3964-3974

- Rad, A. K., Mohebbati, R., & Hosseinian, S. (2017). Drug-induced nephrotoxicity and medicinal plants. Iranian Journal of Kidney Diseases, 11(3), 169.
- Ruggenenti, P., Cravedi, P., & Remuzzi, G. (2012). Mechanisms and treatment of CKD. Journal of the American Society of Nephrology, 23(12), 1917-1928.
- Slot, C. (1965). Plasma creatinine determination a new and specific Jaffe reaction method. Scandinavian journal of clinical and laboratory investigation, 17(4), 381-387.
- Swain, R. A., & Kaplan-Machlis, B. (1999). Therapeutic uses of vitamin E in prevention of atherosclerosis. Alternative Medicine Review, 4(6), 414-423.
- Tangco J.V.V., Angustia D.A., Jelynne P.T. (2015). Nutrional Analysis, Phytochemical Screening & Total Phenolic Content of Basellaalbaleaves from Philippines. International Journal of Pharmacognosy& Phytochemical research, Philippines, 7(5);1031-1033