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PHARMACOLOGICAL EVALUATION, ANTIOXIDANT AND ANTI-UROLITHIC ACTIVITY OF *SINAPIS ARVENSIS* LEAVES EXTRACT IN EXPERIMENTAL RATS

Priyanka Sharma[1]*, Dr. Abhishek Sharma[2], Ms. Payal Saiju[3], Dr. Surendra Jain[4]

Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal Madhya pradesh, India (462021).

***Corresponding Author: Priyanka Sharma**

Lakshmi Narain College of Pharmacy, Kalchuri Nagar, Raisen Road, Bhopal Madhya pradesh, India (462021).

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ABSTRACT

The present study investigates the pharmacological, antioxidant, and anti-urolithic potential of methanolic leaf extract of *Sinapis arvensis* in experimental rats. Methanol extraction yielded 22.45 g from 500 g of dried plant material, with an extraction efficiency of 11.55%. Phytochemical screening revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenolics, proteins, and saponins, which are known to contribute to therapeutic activity. Total phenolic and flavonoid content were measured as 68.3 mg/g and 53.9 mg/g gallic acid and rutin equivalents, respectively. Antioxidant activity assessed by DPPH assay demonstrated moderate radical scavenging activity with an IC_{50} of 48.46 µg/mL, compared to 21.40 µg/mL for ascorbic acid. FTIR analysis confirmed the presence of functional groups related to bioactive constituents. In the ethylene glycol-induced urolithiasis model, the extract, particularly at 400 mg/kg, significantly reduced elevated serum creatinine, urea, calcium, and phosphorus levels, and indicating anti-urolithic effects. These findings support the traditional use of *Sinapis arvensis* and suggest its potential as a natural therapeutic agent for managing urolithiasis and oxidative stress.

KEYWORDS: *Sinapis arvensis*, Methanolic extract, Antioxidant activity, DPPH assay, Urolithiasis, Phytochemicals, FTIR analysis.

1. INTRODUCTION

Medicinal plants have long served as a fundamental source of therapeutic agents, providing effective and affordable remedies for a wide range of diseases **(Shakya, 2016).** In recent years, growing interest in plant-based medicines has been driven by their perceived safety, cost-effectiveness, and rich diversity of bioactive compounds. Many traditional medicinal systems rely heavily on herbal preparations, and scientific validation of these plants has become essential to substantiate their pharmacological potential and identify novel drug candidates **(Ogbuagu *et al.*, 2022).**

Oxidative stress plays a crucial role in the pathogenesis of several chronic and degenerative disorders, including renal diseases. It arises from an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defence mechanisms **(Sachdev *et al.*, 2021).** Excessive ROS can damage cellular components such as lipids, proteins, and DNA, thereby contributing to inflammation, tissue injury, and organ dysfunction. Antioxidants derived from natural sources, particularly plants rich in phenolics and flavonoids, are known to neutralize free radicals and protect against oxidative damage, making them valuable in disease prevention and management **(Akbari *et al.*, 2022).**

Urolithiasis, commonly known as kidney stone disease, is a prevalent urinary disorder characterized by the formation of calculi in the urinary tract. It is associated with high recurrence rates and is influenced by factors such as oxidative stress, altered urinary composition, and impaired renal function **(Malhotra *et al.*, 2022).** Conventional treatments, including surgical procedures and pharmacotherapy, are often costly and may be associated with adverse effects. Consequently, there is

increasing interest in exploring herbal medicines with antioxidant, diuretic, and nephroprotective properties as alternative or complementary approaches for the management of urolithiasis. Pharmacological evaluation of medicinal plants provides scientific evidence for their traditional use and helps identify novel therapeutic agents **(Oswal *et al.*, 2023).** Plants with antioxidant, diuretic, and nephroprotective properties are especially valuable in the management of urolithiasis, as they can reduce oxidative damage, improve renal function, and inhibit stone formation. Experimental animal models, particularly rat models of ethylene glycol–induced urolithiasis, are widely employed to assess the anti-urolithic potential of plant extracts and to study their effects on biochemical and physiological parameters related to kidney health **(Zeng and Jiang 2019).**

Sinapis arvensis (family: Brassicaceae), commonly known as wild mustard, is a medicinal plant traditionally used for various therapeutic purposes. Different parts of the plant have been reported to possess anti-inflammatory, antioxidant, and diuretic activities, which are relevant in renal disorders **(Ashwini *et al.*, 2022).** The leaves of *Sinapis arvensis* are particularly rich in phytoconstituents such as flavonoids, phenolic compounds, alkaloids, glycosides, and saponins, all of which are known to contribute to diverse pharmacological effects. Despite its traditional use, scientific evidence supporting the antioxidant and anti-urolithic potential of *Sinapis arvensis* leaves remains limited **(Aboulthana *et al.*, 2025).**

In this context, the present study focuses on the pharmacological evaluation of a medicinal plant extract with emphasis on its antioxidant and anti-urolithic activities. The investigation aims to assess the antioxidant capacity using in vitro assays and to evaluate the anti-urolithic efficacy in experimental rats by analyzing relevant biochemical markers of renal function and stone formation.

2. MATERIAL AND METHODS

2.1 Chemicals

Ethyl acetate and Glacial Acetic Acid were obtained from Vizang Chemicals, a reputable supplier of analytical reagents. Tata Chemicals Limited provided the Sodium Hydroxide. Meitheal Pharmaceuticals provided the Nitroprusside. Conc. H_2SO_4 were obtained from Sun Chemicals. Ethanol were obtained from EID Parry (India) Ltd while Shell chemicals companies provided the Ethylene glycol. Baxter India Pvt Ltd were obtained from Normal saline while Rashtriya Chemicals provided the Ammonia.

2.2 Procurement of plant material

For the present study, *Sinapis arvensis* (500gm) were collected in the month of August 2024 from localized area of Bhopal. The plant was identified and authenticated by botanist. After cleaning, plant part (Leaves) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. It was powdered and stored in air tight bottles.

2.3 Extraction of Plant Material by Soxhlation Process

The Soxhlet extraction has widely been used for extracting valuable bioactive compounds from various natural sources. Generally, a small amount of dry plant material *Sinapis arvensis*is placed in a thimble. The thimble is then placed in distillation flask which contains the methanol and ethyl acetate solvent. When the thimble-holder solution reaches an overflow level, a siphon is used to remove it. Siphon returns the solution to the distillation flask. This solution transports the extracted solutes into the bulk liquid. The solute remains in the distillation flask, while the solvent returns to the plant's solid bed. The process repeats till the extraction is finished. **(Radhika 2017).**

In last calculate the yield of the extracts by following formula:

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

2.4 Quantitative Estimation of Phytoconstituents

After preliminary phytochemical testing confirmed the presence of phenols, alkaloids, flavonoids, saponins, and tannins, the plant material was extracted for quantitative analysis.

2.4.1 Determination of total phenols content by spectrophotometric method

The determination of total phenols content by the spectrophotometric method involves measuring the absorbance of a sample at a specific wavelength after reacting with a phenol-reactive reagent. One commonly used reagent is Folin-Ciocalteu, which reacts with phenolic compounds to produce a blue color. The color intensity is directly related to the sample's total phenol content. To carry out the method, a known volume of the sample is mixed with the Folin-Ciocalteu reagent and a sodium carbonate solution, and then incubated for a certain time. The solution's absorbance is then measured at a wavelength, typically around 765 nm, with a spectrophotometer. A standard curve made from known amounts of phenolic chemicals, such as gallic acid, is used to determine the total phenols in a sample by comparing its absorbance to the curve. This approach is frequently used because it is simple, cost-effective, and reliable for determining the phenolic content of diverse plant-based samples. Gallic acid was used as a standard to prepare calibration curves at concentrations of 20, 40, 60, 80, and 100 µg/mL **(Stratil *et al.*, 2006).**

2.4.2 Determination of total flavonoid content by spectrophotometric method

The total flavonoid content was determined using the aluminum chloride colorimetric method. In brief, 0.2 g of *Sinapis arvensis* extract was dissolved in 1 mL of deionized water. A 0.5 mL aliquot of this solution was

mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of deionized water. The resulting mixture was incubated at room temperature for 40 minutes. After incubation, the absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer, with deionized water serving as the blank. Rutin was used as a standard to prepare calibration curves at concentrations of 20, 40, 60, 80, and 100 µg/mL. These calibration curves were used to determine the total flavonoid content, which was expressed as milligrams of rutin equivalent per gram of dry extract **(Matić *et al.*, 2017).**

2.5 Determination of antioxidant activity by the DPPH test

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) test is a popular method for determining antioxidant activity and free radical scavenging capacity in compounds. A DPPH solution (often 0.1 mM) is produced and combined with a sample (extract or chemical). The combination is incubated at room temperature for 30 minutes to an hour, allowing the sample's antioxidants to neutralize the DPPH radicals. This process reduces DPPH's violet color to a pale yellow, which is quantified using a spectrophotometer to record the absorbance at 517 nm. The % inhibition of DPPH radicals is computed using the following formula:

$$\text{Inhibition } (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where A0 represents the absorbance of the DPPH solution in the absence of the sample, and A1 represents the absorbance of the DPPH solution with the sample. The results are compared to a standard antioxidant, such as ascorbic acid or butylated hydroxytoluene (BHT), to determine the sample's antioxidant activity. This approach is commonly used because it is simple, quick, and successful in assessing the antioxidant capabilities of plant extracts and other substances. **(Sirivibulkovit *et al.*, 2018).**

2.6 FTIR analysis

FTIR (Fourier Transform Infrared) spectroscopy is a powerful analytical technique used to identify the molecular composition and functional groups of compounds, such as those present in a *Sinapis arvensis* extract. In this method, the dried methanolic extract is mixed with potassium bromide (KBr) to form a pellet, which is then used to create a clear sample disc suitable for analysis. A 10 mg portion of the extract is combined with KBr to ensure proper dispersion and transparency of the sample. This pellet is then placed in the FTIR instrument, which scans the sample over a wavenumber range from 400 to 4000 cm^{-1}, with a resolution of 4 cm^{-1}. The resulting infrared spectrum provides valuable information about the functional groups and chemical bonds within the extract, enabling researchers to gain insights into its molecular structure and potential bioactive compounds **(Channa *et al.*, 2007).**

2.7 Acute Oral Toxicity Study

Three animals were employed in each phase of the acute toxic investigation, following the OECD 423 guidelines. The current acute oral toxicity research was approved by the Faculty Ethical Committee. Each group consisted of three animals (n=3) who had access to food and running water. All of the rats were randomly assigned to the appropriate group. The treatment groups were orally administered with a single dose of 5, 50, 300, and 2000 mg/kg body weight, respectively, whereas the control group received the respective vehicle, distilled water **(Walum 1998).**

2.8 *In vivo* study of Anti-Urolithic activity in rats

2.8.1 Animals

Animals were chosen at random from the Pinnacle Biomedical Research Institute (PBRI) animal house in Bhopal, India, and then randomly assigned to various treatment groups before being housed in a propylene cage with sterile husk bedding. The animal home had a relative humidity of 30.7%, a temperature of 22±2°C, and a 12:12 light and dark cycle. They were fed standard pellets (Golden Feeds, New Delhi, India) and had access to water as needed. Rats will spend 7 days adjusting to laboratory circumstances before beginning the studies. Each set of studies used a separate group of rats (n=3). The Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI), Bhopal, approved the animal research.

Animals used

- Strain - Rats
- Sex - Male
- Body weight - 200±70g

2.8.2 Ethylene glycol-induced hyperoxaluria model

Ethylene glycol-induced hyperoxaluria model was used to assess the anti-urolithic activity in albino rats. Animals were divided into five groups containing six animals in each.

Experimental protocol

Group I-Acts as a control and is provided with free access to water and regular rat food.

Group II - Lithiatic control: For 28 days, the animals were fed a regular meal along with 0.75% ethylene glycol in their drinking water.

Group III - After being given 0.75% ethylene glycol in their drinking water and being given an oral dose of 200 mg/kg of *Sinapis arvensis* extract, for 28 days.

Group IV - Administered 0.75% ethylene glycol in drinking water, followed by an oral dose of 400 mg/kg of *Sinapis arvensis* extract, for 28 days.

Group V-Received standard anti-urolithiatic drug, cystone 750mg/kg

2.8.3 Analysis for Anti-Urolithic activity

- **Serum analysis**

One hour after the final dose of treatment, blood was collected from the retro-orbital sinus, and serum was separated by centrifugation at 10,000 ×g for 10 minutes. The serum was then analyzed spectrophotometrically for key renal biomarkers, including creatinine, uric acid, and blood urea nitrogen (BUN), using diagnostic kits. These markers are crucial in assessing kidney function, as elevated levels can indicate impaired renal function or urolithiasis. The spectrophotometric analysis provided accurate quantification by measuring the color change resulting from the reaction between the analytes and specific reagents, offering insight into the effectiveness of the treatment in supporting renal health **(Lu *et al.*, 2018).**

- **Urine analysis**

During blood collection, all animals were placed in individual metabolic cages to collect pee samples for 5 hours. The volume of urine was calculated and the samples were tested for total protein and creatinine. These parameters were tested with diagnostic tools that allow precise quantification. Total protein levels assist measure kidney function and potential injury, whereas creatinine levels indicate renal filtration efficiency. The examination of these markers aids in determining the treatment's impact on kidney health and its ability to prevent or treat urolithiasis **(Gounden *et al.*, `2024).**

- **Kidney homogenate analysis**

Following the euthanasia of each animal, the abdomen was carefully opened to remove both kidneys. Excess tissue was trimmed off, and the kidneys were preserved in 10% neutral formalin for further analysis. To prepare the kidneys for homogenization, they were dried at 80ºC in a hot air oven for 30 minutes. After drying, 100 milligrams of the kidney tissue was boiled in 10 ml of 1N hydrochloric acid for 30 minutes to facilitate the breakdown of cellular components. The boiled tissue was then homogenized to create a consistent kidney homogenate, which was used for biochemical analysis to assess various markers of kidney function and damage **(Golla *et al.*, 2023).**

3. RESULTS AND DISCUSSION

3.1 Procurement of plant material

Table 1: Percentage Yield of plant material.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Sinapis arvensis	Methanol	500	22.45	11.55

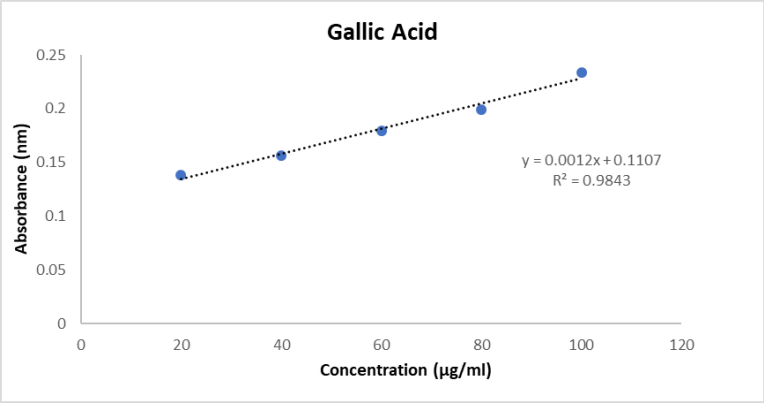
3.2 Phytochemical Test

Table 2: Phytochemical test of Leaves extract of methanol.

Experiment	Presence or absence of phytochemical test
	Methanol extract
Alkaloids	
Dragendroff's test	Positive (+ ve)
Mayer's reagent test	Positive (+ ve)
Wagner's reagent test	Positive (+ ve)
Hager's reagent test	Positive (+ ve)
Glycoside	
Borntrager test	Positive (+ ve)
Legal's test	Positive (+ ve)
Killer-Killiani test	Positive (+ ve)
Carbohydrates	
Molish's test	Positive (+ ve)
Fehling's test	Positive (+ ve)
Benedict's test	Positive (+ ve)
Barfoed's test	Positive (+ ve)
Proteins and Amino Acids	
Biuret test	Positive (+ ve)
Ninhydrin test	Positive (+ ve)
Flavonoids	
Alkaline reagent test	Positive (+ ve)
Lead Acetate test	Positive (+ ve)
Tannin and Phenolic Compounds	
Ferric Chloride test	Positive (+ ve)
Saponin	
Foam test	Positive (+ ve)
Test for Triterpenoids and Steroids	
Salkowski's test	Negative (- ve)
Libbermann-Burchard's test	Negative (- ve)

3.3 Qualitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation



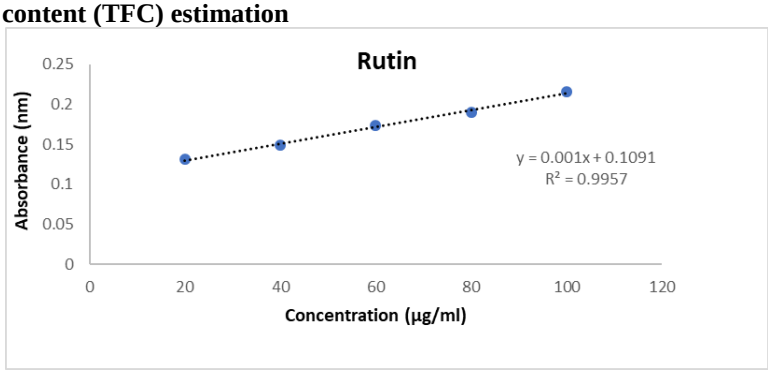
Graph 1: Represent standard curve of Gallic acid.

3.3.1.1 Total Phenolic Content in *Sinapis arvensis* extract

Table 3: Total Phenolic Content.

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.138	68.3 mg/gm
0.189	
0.210	

3.3.2 Total Flavonoids content (TFC) estimation



Graph 2: Represent standard curve of Rutin.

3.3.2.1 Total Flavonoid Content in *Sinapis arvensis* extract

Table 4: Total Flavonoid Content.

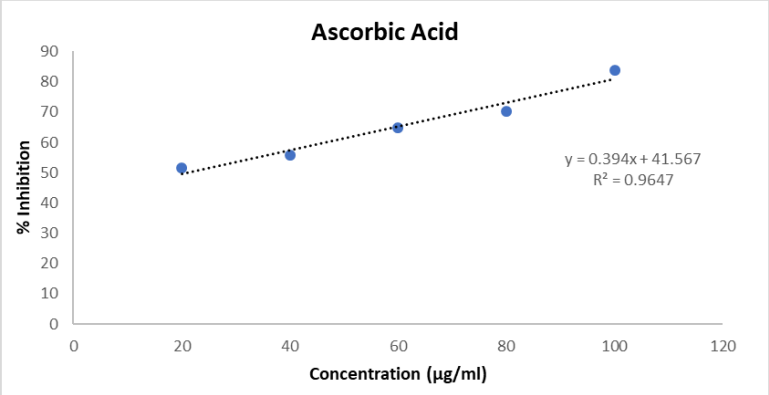
Absorbance	*TFC in mg/gm equivalent of Rutin*
0.130	53.9 mg/gm
0.162	
0.199	

3.4 Anti-Oxidant Activity

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

Table 5: DPPH radical scavenging activity of Std. Ascorbic acid.

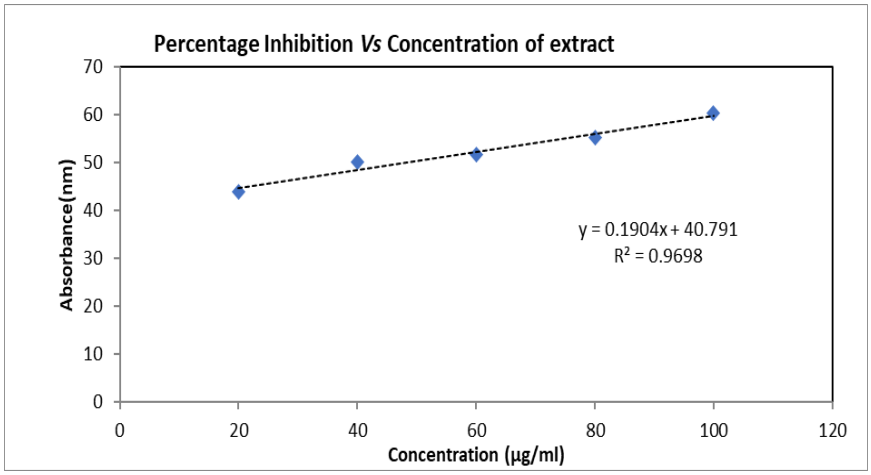
Concentration (µg/ml)	Absorbance	% Inhibition
20	0.482	51.557
40	0.440	55.778
60	0.351	64.723
80	0.296	70.251
100	0.162	83.718
Control	0.995	
IC 50	21.40	



Graph 3: DPPH radical scavenging activity of Std. Ascorbic acid.

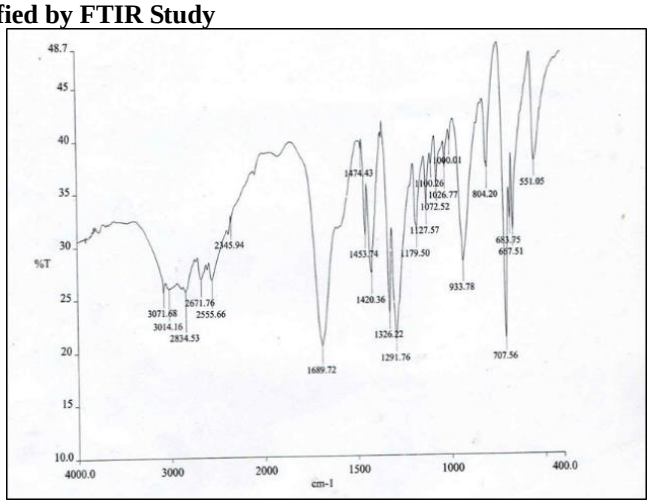
***Table 6: DPPH radical scavenging activity of methanol extract of* Sinapis arvensis.**

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.525	43.850
40	0.467	50.053
60	0.452	51.657
80	0.419	55.187
100	0.371	60.320
Control	0.935	
IC50	48.46	



Graph 4: Represents the Percentage Inhibition* Vs *Concentration of extract.

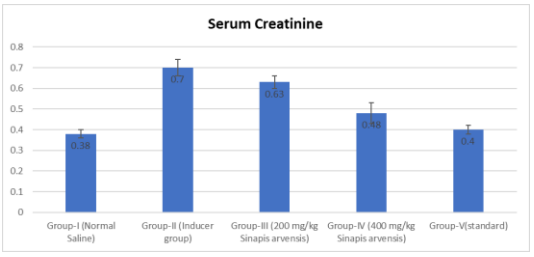
3.5 Functional group identified by FTIR Study



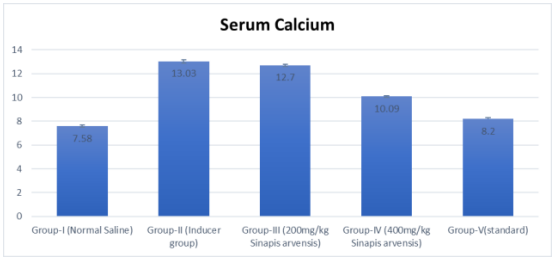
Graph 5: FTIR's Analysis of *Sinapis arvensis* extract.

3.6 The activity of ethylene glycol-induced antiurolithiasis

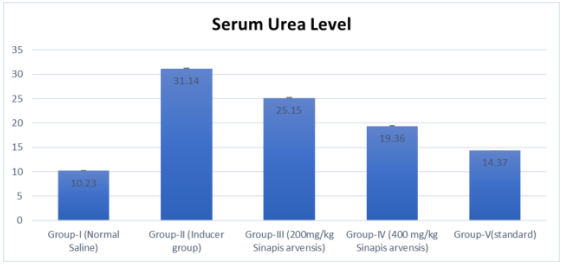
Study Groups	Serum Creatinine Level (mg/dL)	Serum Calcium Level (mg/dL)	Serum Urea Level (mg/dL)	Serum Phosphorus Level (mg/dL)
Group-I (Normal Saline)	0.38±0.02	7.58±0.09	10.23±0.05	7.98±0.05
Group-II (Inducer group)	0.70±0.04	13.03±0.13	31.14±0.12	10.14±0.17
Group-III (200 mg/kg *Sinapis arvensis)*	0.63±0.03	12.70±0.08	25.15±0.09	9.56±0.07
Group-IV (400 mg/kg *Sinapis arvensis)*	0.48±0.05	10.09±0.07	19.36±0.07	8.99±0.10
Group-V(standard)	0.40±0.02	8.20±0.06	14.37 ±0.02	7.80±0.08



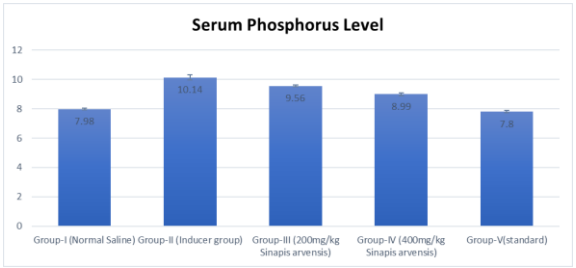
Graph 6: The serum creatinine level is displayed as a bar graph.



Graph 7: A bar graph shows the amount of calcium in the serum.



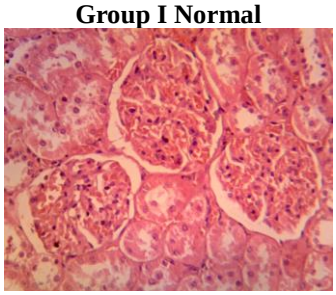
Graph 8: A bar graph illustrates the level of serum urea.



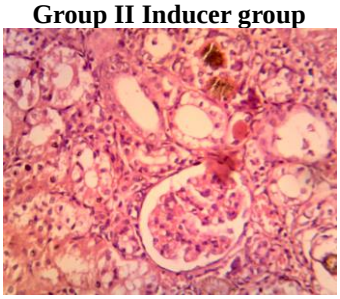
Graph 9: The serum phosphorus level is shown as a bar graph.

3.7 Hostopathological examination

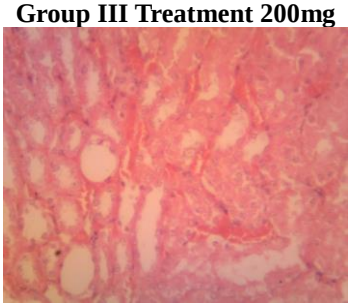
Group I Normal



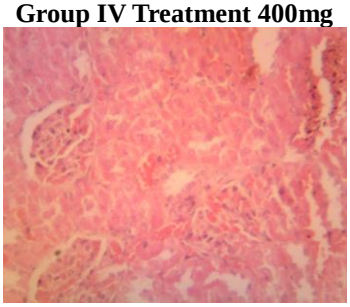
Group II Inducer group



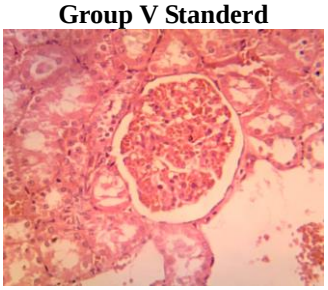
Group III Treatment 200mg



Group IV Treatment 400mg



Group V Standerd



The methanolic extraction of *Sinapis arvensis* produced a yield of 11.55% (22.45 g from 500 g of dried plant material), indicating the presence of a considerable amount of methanol-soluble phytoconstituents. Methanol effectively extracted bioactive compounds such as alkaloids, flavonoids, phenolics, glycosides,

carbohydrates, proteins, and saponins, while triterpenoids and steroids were absent. This phytochemical profile supports the plant's traditional medicinal use and its potential for further pharmacological studies, particularly in urolithiasis management.

The extract showed high Total Phenolic Content (56.91 mg/g gallic acid equivalents) and Total Flavonoid Content (53.09 mg/g rutin equivalents), suggesting strong antioxidant potential. In the DPPH assay, the extract demonstrated notable radical scavenging activity, though weaker than ascorbic acid, confirming its role as a natural antioxidant source. In vivo studies revealed that *Sinapis arvensis* extract significantly reduced ethylene glycol–induced elevations in serum creatinine, calcium, urea, and phosphorus levels, especially at 400 mg/kg. These improvements, comparable to standard treatment, indicate pronounced antiurolithic activity, likely due to the plant's antioxidant and diuretic properties.

4. CONCLUSION

The study found that *Sinapis arvensis* has considerable antioxidant and anti-urolithic capabilities, which supports its traditional use in the treatment of oxidative stress and kidney stones. Methanol was discovered to be the most effective solvent for extracting a variety of bioactive chemicals, including alkaloids, flavonoids, and glycosides. The extract's high phenolic and flavonoid concentration adds to its significant antioxidant properties, which may help reduce oxidative damage. The findings suggest that *Sinapis arvensis* could serve as a valuable natural remedy for the prevention and treatment of kidney stones and oxidative stress-related conditions. However, further clinical research is needed to confirm its safety and efficacy in humans.

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