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RESEARCH ARTICLE

Bioactivity of *Lantana camara* Flowers: Antilithiatic and Nephroprotective Implications in Male Wistar Rats

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Abstract

Lantana camara is a well-known medicinal plant with various reported therapeutic compounds. This study explores the antilithiatic and nephroprotective effects of *L. camara* flower hydroalcoholic extract. Using animal models and cell culture, we assessed its impact on ethylene glycol-induced nephrolithiasis and gentamicin-induced nephrotoxicity. The ethylene glycol-induced nephrolithiasis model revealed elevated levels of calcium, phosphate, and oxalate, indicating the formation of calculi. Treatment with the hydroalcoholic extract resulted in a significant decrease in uric acid levels, reducing the likelihood of calculi formation. Additionally, the animals treated with the extract showed reduced levels of urea, and creatinine, indicating improved kidney function. The results highlighted the notable difference between the preventive and curative treatments. In the HEK-293 cell culture, the hydroalcoholic extract demonstrated a significant inhibition of abnormal cell morphology induced by gentamicin, a nephrotoxic drug. The extract also exhibited a marked improvement in cell growth compared to the standard treatment. The findings of this study provide substantial evidence supporting the antilithiatic and nephroprotective effects of the hydroalcoholic extract of *L. camara* flowers. The extract effectively reduced the risk of calculi formation, improved renal parameters, and demonstrated potential in mitigating drug-induced nephrotoxicity. These results validate the traditional claims regarding the efficacy of *L. camara* as a therapeutic agent for kidney-related disorders. The hydroalcoholic extract of *L. camara* flowers could serve as a valuable alternative treatment option, offering potential benefits in the management of lithiasis and nephrotoxicity.

Keywords: Folk Medicine, Gentamicin, Nephrolithiasis, Nephroprotection, Verbenaceae

1. Background

Lantana plants are a type of evergreen flowering shrub that belongs to the plant genus *Lantana*. They are renowned for their abundant and attractive flowers, as well as their long-lasting flowering season. *Lantana* is a diverse genus comprising approximately 150 species of shrubs that bloom repeatedly and are classified under the Verbenaceae family. These species of *Lantana* can be found as perennial herbs and upright shrubs, often exhibiting pubescence[1]. While *Lantana* plants are native to hot regions in America and Africa, they have been introduced to various other regions as well. Initially, *Lantana*s gained popularity as garden plants due to their vibrant blossoms, and they also serve as valuable sources of nectar for honey production[2].

Lantana camara, commonly known as red or wild sage, is a widely recognized species of the *Lantana* genus. It is an adaptable ornamental shrub native to the neotropics but has been introduced to other countries where it can be found as both a cultivated plant and a weedy species[3]. This spiky shrub displays an erect growth habit, reaching heights of approximately 2 to 3 meters. Its root system consists of a shallow taproot with

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side roots spreading out to form a mat. The stems and branches are rigid and armed with curved prickles or spines along their edges[4]. The leaves of *Lantana camara* are ovate or ovate oblong in shape, arranged oppositely along the stem. They have rounded toothed edges and may exhibit some hairiness. The inflorescence takes the form of a cylindrical spike or a hemispherical head, typically found in the axils or at the tips of the branches. These inflorescences are composed of numerous small cylindrical flowers. The dense clusters of flowers consist of tiny tubular flowers surrounded by a green involucre. The color of the flowers can vary widely, ranging from white to yellow, pink to rose, and orange to red, with countless combinations. Wild varieties exhibit over 100 different blends of flower colors. The initial flower is often white and tends to change color as it ages. The fruits are small, berry-like drupes, measuring about 3 mm in diameter. They start off green and turn dark purple when fully matured[5,6].

It is worth noting that *L. camara* is considered an invasive weed. It possesses allelopathic properties, releasing chemicals into its surroundings that inhibit the growth of native flora. Despite being categorized as a weed; the plant's hidden potential has been overlooked[7]. *L. camara* has a long history of traditional use in the treatment of various illnesses, including cancer, leprosy, asthma, skin rashes, chickenpox, measles, ulcers, swellings, bilious fevers, rheumatism, malaria, catarrhal infections, and high blood pressure. Additionally, it has been reported to possess insecticidal properties against several insects. In traditional medicine, the infusion of the entire *L. camara* plant was utilized to treat bronchitis, catarrh, and epidemic parotiditis, as well as to reduce fever. A preparation of powdered root in milk was used for alleviating stomach pain[8]. The bark of *Lantana* was employed as a lotion to treat leprous ulcers and other skin injuries due to its astringent activity. Crushed fresh leaves were topically applied to the skin to alleviate conditions such as psoriasis, eczema, rashes, neurodermatitis, tinea, chickenpox, and to stop bleeding from injuries[9]. The leaves were also used in the treatment of cough. Furthermore, a decoction of dried flowers was administered to individuals experiencing haemoptysis and pulmonary tuberculosis[10]. It is worth noting that these traditional uses of *L. camara* have been reported in various studies, indicating its historical significance in traditional medicine.

Phytochemical analysis of various parts of *L. camara* has revealed the presence of several bioactive compounds, including alkaloids, glycosides, steroids, flavonoids, phenolic compounds, carbohydrates, oligosaccharides, essential oils, saponins, tannins, triterpenes, and iridoid glycosides, as well as napthoquinones as major components[11]. The alkaloids derived from *Lantana* have demonstrated beneficial effects on intestinal movements, blood pressure reduction, and improved respiration. Notably, a compound known as verbacoside, isolated from *Lantana* extract, has exhibited antimicrobial, immunosuppressive, and anticancer activities[12]. Furthermore, the processed essential oil derived from *Lantana* has been traditionally used to treat conditions such as leprosy, scabies, and skin irritation[13]. The biomedical application of the extracts of various parts of *L. camara* have been reported in the literature. Fungicidal activity of *L. camara* leaves against different fungi have been reported. Ointment prepared using ethanolic extract of leaves effectively controlled the lesions of bovine dermatophytosis, the disease was not recurred for more than three years[14]. Antibacterial activity of *L. camara* against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis* has been reported[15]. Antidiarrheal activity of the stem extract of *L. camara* was reported. The extract produced inhibitory effect on castor oil induced intestinal secretion and gastrointestinal propulsion[16]. The methanolic extract of *L. camara* leaves has shown significant anti-ulcer and antioxidant activity in gastric and duodenal ulcer models[17]. Notably, the different parts of *L. camara* have also exhibited antilithiatic and nephroprotective activities against various disease-induced models[18-23].

The reports mentioned provide a scientific background by identifying the bioactive compounds present in *L. camara*, highlighting their potential therapeutic activities, and indicating the traditional uses and reported effects of different parts of the plant. The mention of antilithiatic and nephroprotective activities of different parts of *L. camara* against various disease-induced models in the reports connects directly to the proposed work. Although no definitive evidence has been provided, previous studies have suggested the potential benefits of *L. camara* flowers in these contexts. This study explores the largely overlooked potential of

L. camara flowers, aiming to validate their antilithiatic and nephroprotective effects.

Kidney stones, or nephrolithiasis, are solid mineral and acid salts deposits that form within the kidneys. They can vary in size and composition, with common types including calcium oxalate, calcium phosphate, and uric acid stones. The formation of kidney stones is influenced by factors such as dehydration, dietary habits, and genetic predisposition. These stones can cause intense pain when they obstruct the urinary tract, leading to symptoms like flank pain, hematuria, and urinary urgency. Nephrotoxicity, on the other hand, refers to kidney damage caused by exposure to toxic substances, including certain medications, heavy metals, and environmental toxins. It can result in impaired kidney function, characterized by elevated levels of serum creatinine and blood urea nitrogen. Understanding the basics of kidney stones and nephrotoxicity is crucial for identifying preventive measures and developing interventions to mitigate their impact on renal health.

2. Materials and Methods

2.1 Plant Materials, Animals, Reagents and Chemicals

Lantana camara flowers were collected from rural areas of Medchal district, Hyderabad, Telangana in the month of June. The authentication of the flowers was performed by a taxonomist in Titupathi. Male Wistar albino rats weighing between 150-250 grams were procured from Vab Bioscience, Musheerabad, Hyderabad. The rats were acclimated in standard laboratory conditions for one week prior to the experiments. Throughout the study, ethical standards for animal handling were strictly adhered to. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) under the registration number 1447/Po/Re/S/11/CPSCEA-51/A. Gentamicin was received as a gift sample from Celon labs, Hyderabad. Human embryonic kidney (HEK-293) cells were obtained from CCMB (Centre for Cellular and Molecular Biology), Hyderabad. Quercetin was purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore. All other chemicals, solvents, and reagents used in the study were procured from SD Fine Chem Ltd., Hyderabad.

2.2 Extraction Method

The freshly collected flowers were carefully washed with water to eliminate any unwanted dirt particles. Subsequently, the flowers were air-dried in the shade until they became brittle. The dried flowers were then powdered using a blender. The powdered flowers were packed into a Soxhlet apparatus and subjected to defatting using petroleum ether. Afterward, the contents were removed from the Soxhlet and further dried to eliminate all remaining moisture. The dried powder was once again placed into the Soxhlet apparatus and extracted with 50 ml ethanol and 50 ml water for a duration of 72 hours. The resulting extract was concentrated using a rotary evaporator under reduced pressure at a temperature of 40°C. The percentage of extract yield was calculated, and the remaining sample was stored in a dark environment for future use. The obtained powder was stored at 4 °C and suspended in distilled water prior to its use[24].

2.3 Acute Toxicity Study

The acute toxicity assessment of the hydroalcoholic extract obtained from *Lantana camara* flowers was conducted following the guidelines outlined by the OECD (Organization for Economic Co-operation and Development) guideline 423. A total of six rats were randomly selected for the assay and subjected to overnight fasting. Initially, an oral dose of 50 mg/kg of body weight was administered, and the animals were observed for a period of 14 days. If four out of the six animals exhibited mortality, the administered dose would be considered as the lethal dose. However, if only two animals died, the same dose would be repeated using fresh animals to confirm the lethal dose. In the absence of any mortality, the procedure would be repeated with incremental doses of 100, 200, 400, 800, 1000, and 2000 mg/kg. The therapeutic dose for subsequent experiments would be determined based on the results obtained from the toxicity study.

2.4 Antilithiatic Activity

2.4.1 Experimental Design for Ethylene Glycol Induced Nephrolithiasis

This experiment was designed to evaluate the antilithiatic effect of the extract derived from *L. camara* flowers on renal issues in a nephrolithiasis-induced model using male Wistar albino rats. A total of 42 animals were

utilized for this study, divided into seven groups, with six rats in each group. Throughout the experiment, all animals were provided unrestricted access to food. The study was conducted in two approaches: curative and preventive studies. Group A served as the plain control group and received a normal diet along with ad libitum access to water. The remaining groups were administered ethylene glycol (0.75%) in their drinking water to induce the formation of renal calculi from day 1 to day 28. Group B was designated as the disease control group. Animals in Group C were treated with the reference drug Cystone (750 mg/kg) from day 14 to day 28. Animals in Groups D to G received different concentrations of the test extract, as indicated below.

Group A: Control

Group B: Disease (lithiasis) induced group

Group C: Treated with standard drug – Cystone (750 mg/kg) from day 14 to 28

Group D: Treated with test sample (200 mg/kg) from day 1 to 28 (preventive)

Group E: Treated with test sample (400 mg/kg) from day 1 to 28 (preventive)

Group F: Treated with test sample (200 mg/kg) from day 14 to 28 (curative)

Group G: Treated with test sample (400 mg/kg) from day 14 to 28 (curative)

2.4.2 Evaluation of Serum and Urinary Parameters

On the 29th day of the study, serum and urine analyses were conducted. Under anesthetic conditions, blood samples were collected from all animals via the retro-orbital plexus and centrifuged at 15000 rpm for 10 minutes to obtain serum samples. The levels of uric acid, urea nitrogen, and creatinine in the serum were determined using an automated clinical chemistry analyzer (Roche Cobas C501). Urine samples were collected from the rats on different days, specifically the 14th and 28th days of the study. The collected urine samples were analyzed for calcium, phosphate, and oxalate content using an automated system. Microscopic examination of the urine samples was performed at a magnification of 100x using a light microscope (Olympus DX 45, Japan). The left kidneys of the animals subjected to ethylene glycol-induced nephrolithiasis were excised, weighed, fractionated, and fixed in 10% Neutral Buffered Formalin (NBF) for histopathological examination. The tissue specimens were dehydrated using ethanol, cleared using xylene, and then embedded in paraffin. Sections of 4 µm thickness were cut from the embedded specimens and stained with hematoxylin-eosin. Microscopic examination was conducted to identify the deposition of calcium oxalate and assess any associated histological changes.

2.5 Nephroprotective Activity

2.5.1 In Vitro Cell Line Studies

Thawing and revival of HEK-293 cell lines preserved in liquid nitrogen were carried out following standard protocols. The cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) supplemented with inactivated fetal bovine serum. The cell cultures were incubated in a CO_2 incubator at 37°C. Upon reaching confluence, the cells were treated with trypsin to detach them from the culture surface. The trypsinized cells were then combined with an adequate number of media to deactivate the trypsin. Subsequently, the cells were centrifuged at 1500 rpm for 4 minutes, the pellet was resuspended and diluted in media. Further dilutions were made to achieve the desired number of cells for counting using a hemocytometer and the trypan blue exclusion method. The final seeding density was set at 10,000 cells per well for cultivation. After 24 hours of seeding, the cells were treated with test samples at various concentrations (10, 50, 100, 150, 250, and 300 µg/ml). Following 24 hours of treatment, cell viability assays and morphological examinations were performed.

2.5.2 In Vivo Nephroprotective Activity

This study followed a previously reported method with slight modifications[25]. A total of 36 Wistar male rats were randomly divided into six experimental groups, each receiving different treatments. The animals were kept in metallic cages for 24 hours prior to the experiment to acclimate to the laboratory conditions. Group I animals were administered normal saline (1 ml/kg) for 23 days and served as the plain control. Group II animals received subcutaneous injections of gentamicin (40 mg/kg) for 13 days, followed by oral administration of normal saline (1.0 mL) from days 14 to 23. Animals in group III were treated with gentamicin (40 mg/kg) for 13 days, followed by oral administration of the test extract at a dose of 200 mg/kg from days 14

to 23. Animals in group IV received gentamicin (40 mg/kg) for 13 days and were treated with the test extract at a dose of 300 mg/kg from days 14 to 23. Group V animals received gentamicin (40 mg/kg) for 13 days and were treated with the test extract at a dose of 400 mg/kg from days 14 to 23. Animals in group VI received gentamicin (40 mg/kg) for 13 days and were treated with quercetin (50 mg/kg) from days 14 to 23. On the 24th day, blood samples were collected from the tail vein to assess renal function. Serum levels of uric acid, urea, and creatinine were measured biochemically. Histological examination was performed to evaluate any changes in kidney tissues among the different treatment groups.

2.6 Statistical Analysis

The experimental data were presented as mean ± SD (n = 6). Statistical analysis was conducted using one-way ANOVA. Results with a p-value less than 0.005 were considered statistically significant. The histopathological findings were evaluated through semi-qualitative analysis.

3. Results

The hydroalcoholic extract of dried flowers was obtained through the soxhlet extraction process, resulting in a practical yield of less than 30 percent. A preliminary phytochemical analysis confirmed the presence of polyphenols, flavonoids, tannins, and coumarins in the extract. To determine the safety and therapeutic dosage of the extract in experimental animals, acute toxicity studies were conducted. These studies aimed to identify the LD_{50} values, which represent the dose at which the extract can cause mortality or harmful effects when administered in repeated doses. The toxicity studies also helped in assessing any potential side effects induced by the test sample. It was found that the hydroalcoholic extract, even at a concentration of up to 2000 mg/kg body weight, did not cause any mortality or adverse effects. The LD_{50} value of the extract was determined to be greater than 2000 mg/kg. Therefore, a starting therapeutic dose of 1/10th of the LD_{50} dose (200 mg) was chosen. Based on these findings, the range of 200 to 400 mg/kg body weight was selected for further experiments.

3.1 Antilithiatic Activity

3.1.1 Serum Biochemical Analysis

A statistically significant increase ($p<0.001$) in the levels of creatinine, urea nitrogen, and uric acid was observed in group B (diseased group) compared to group I (plain control). These elevated levels indicate nephritic damage and reduced kidney function. However, animals treated with the reference drug and different concentrations of test samples exhibited a significant decrease in the levels of these parameters compared to the disease control group. Furthermore, a significant ($p<0.01$) difference was observed between the curative and preventive groups, as shown in Table 1.

3.1.2 Urine Volume

A significant decrease ($p<0.001$) in urine output was observed in the diseased group compared to the plain control group. Conversely, animals treated with the standard treatment (Group C) showed a significant increase ($p<0.001$) in urine output compared to the disease control group. Animals treated with the test phytochemical exhibited a remarkable increase ($p<0.001$) in diuresis compared to the disease control group. Both preventive and curative treatment with the test samples resulted in an increase in urine output compared to the disease control group. The effect was dose-dependent, as shown in Table 2. Treatment with the standard and test samples significantly reduced polyuria associated with lithogenic treatment. This led to the dilution of urinary electrolytes, resulting in the elimination of calcium and phosphorus from the urine and reducing the chances of saturation and precipitation. Consequently, the formation of calculi was eliminated. Additionally, a significant increase in the excretion of urinary and serum calcium was observed in the treatment groups.

3.1.3 Urine Analysis

Urine samples were collected from various groups on the 28th day and analyzed for pH, specific gravity, glucose, erythrocytes, leukocytes, bilirubin, urobilinogen, ketones, protein, nitrite, calcium, phosphate, and oxalate. The results are presented in Table 3.

3.1.4 Microscopic Analysis

The normal control group exhibited the absence of crystal formation in urine samples. However, in

Table 1. Levels of serum biochemical parameters

Biochemical Parameter	Group						
	A	B	C	D	E	F	G
	Plain Control	Disease control	Standard treatment	preventive (200 mg/kg)	preventive (400 mg/kg)	curative (200 mg/kg)	curative (400 mg/kg)
Blood urea nitrogen	24.17 ± 0.31	40.17 ± 0.40*	25.32 ± 0.40#	28.83 ± 0.70#	24.84 ± 0.74#	30.5 ± 0.72#$	28.67 ± 0.57#$
Creatinine	0.56 ± 0.02	0.90 ± 0.03*	0.58 ± 0.04#	0.67 ± 0.01#	0.58 ± 0.03#	0.71 ± 0.03#$	0.63 ± 0.02#$
Uric acid	1.78 ± 0.06	5.96 ± 0.08*	1.85 ± 0.05#	2.08 ± 0.04#	1.87 ± 0.09#	2.26 ± 0.07#$	2.13 ± 0.06#$

*Comparison with Plain control, #Comparison with disease control, @Comparison with standard treatment, $Comparison with preventive group

Table 2. Urine volume of different group of animals

Group	Urine volume in ml	
	14th day	28th day
A – Plain control	10.5 ± 0.71	11.33 ± 0.21
B- Disease control	3.83 ± 0.31*	3.16 ± 0.30*
C – Standard treatment	6.83 ± 0.30#	9.17 ± 0.31#
D - Preventive (200 mg/kg)	9.16 ± 0.31#@	10.52 ± 0.71#@
E - Preventive (400 mg/kg)	9.87 ±0.31#@	10.66 ± 0.33#@
F - Curative (200 mg/kg)	6.66 ± 0.33#$	8.89 ± 0.31#$
G - Curative (400 mg/kg)	8.33 ± 0.33#$	9.17 ± 0.45#$

*Comparison with Plain control, #Comparison with disease control, @Comparison with standard treatment, $Comparison with preventive group

the disease-induced group, urine samples displayed crystals of calcium oxalate with distinct rectangular shapes. Treatment with the standard drug cystone resulted in smaller crystals or almost no crystals. The groups treated with the test sample showed a significant reduction in crystal formation. Remarkably, the groups treated with HLC (hydroalcoholic extract of *L. camara*) exhibited the highest level of prevention in crystal growth compared to the disease group (Figure 1).

3.1.5 Histopathology

The histopathology studies demonstrated the absence of renal calculi and associated deformities in the plain control group (Group A). In the disease control

Table 3. Results of urine analysis

Parameter	Group						
	A	B	C	D	E	F	G
	Control	Disease control	Standard treatment	preventive (200 mg/kg)	preventive (400 mg/kg)	curative (200 mg/kg)	curative (400 mg/kg)
pH	6.08 ± 0.23	8.66 ± 0.21*	6.54 ± 0.18#	6.96 ± 0.22	6.58 ± 0.23	7.12 ± 0.40	6.83 ± 0.10
Specific gravity	1.02 ± 0.001	1.04 ± 0.008	1.02 ± 0.001	1.03 ± .004	1.01 ± 0.008	1.07 ± .004	1.05 ± 0.003
Glucose	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Erythrocytes	Ab	150 ± 44.7*	5 ± 2.24#	6.6 ± 2.1	Ab	8.9 ± 1.61$	7.8 ± 1.32$
Leucocytes	Ab	41.67 ± 10.54*	8.33 ± 5.27#	15.17 ± 5.2	12.5 ± 5.2@	20.32 ± 4.5$	18.68 ± 2.34$
Bilirubin	Ab	0.83 ± 0.32*	0.17 ± 0.12#	0.25 ± 0.18	0.17 ± 0.10	0.42 ± 0.20$	0.33 ± 0.16$
Urobilinogen	0.25 ± 0.15	1.0 ± 0.12*	0.4 ± 0.1#	0.55 ± 0.20	0.4 ± 0.18	0.7 ± 0.19	0.85 ± 0.15
Ketone	1.66 ± 1.05	22.5 ± 8.7*	1.66 ± 1.32#	2.0 ± 5.8	1.7 ± 1.1	3.33 ± 2.1$	4.16 ± 2.0$
Protein	Ab	20 ± 4.47*	3.33 ± 2.1#	3.5 ± 2.23	Ab@	8.3 ± 4.7$	6.66 ± 4.94$
Nitrite	Ab	0.91 ± 0.08*	0.75 ± 0.17#	0.41 ± 0.20	0.25 ± 0.11	0.66 ± 0.16	0.42 ± 0.20
Calcium	24.17 ± 0.31	40.17 ± 0.40*	25.32 ± 0.40#	28.83 ± 0.70	24.84 ± 0.74	30.5 ± 0.72	28.67 ± 0.57
Phosphate	0.56 ± 0.02	0.90 ± 0.03*	0.58 ± 0.04#	0.67 ± 0.01	0.58 ± 0.03	0.71 ± 0.03	0.63 ± 0.02
Oxalate	1.78 ± 0.06	5.96 ± 0.08*	1.85 ± 0.05#	2.08 ± 0.04	1.87 ± 0.09	2.26 ± 0.07	2.13 ± 0.06

*Comparison with Plain control, #Comparison with disease

group (Group B), deposits of calcium oxalate crystals were observed in renal cells, with the majority of crystal deposition occurring in the tubules. Epithelial desquamation, cellular inflammation, and blood vessel congestion were also observed. Significant improvement in these abnormalities, including a reduction in calcium oxalate depositions, was observed in the cystone and test sample treated groups (Figure 2). The administration of the standard drug and testing samples to animals with lithiasis prevented the supersaturation of calcium oxalate, thereby reducing the deposition in renal tubules. These findings further confirm the antilithiatic effect of the extract in the ethylene glycol-induced urolithiatic model.

3.2 Nephroprotective Activity

3.2.1 Cell Line Studies

The effectiveness of the hydro alcoholic extract derived from *L. camara* flowers was evaluated in a drug-induced cytotoxicity model using HEK-293 cells. The cytoprotective potential of the extract against gentamicin-induced damage was determined by assessing cell viability. HEK-293 cells were exposed to various concentrations of the extract (10, 50, 100,

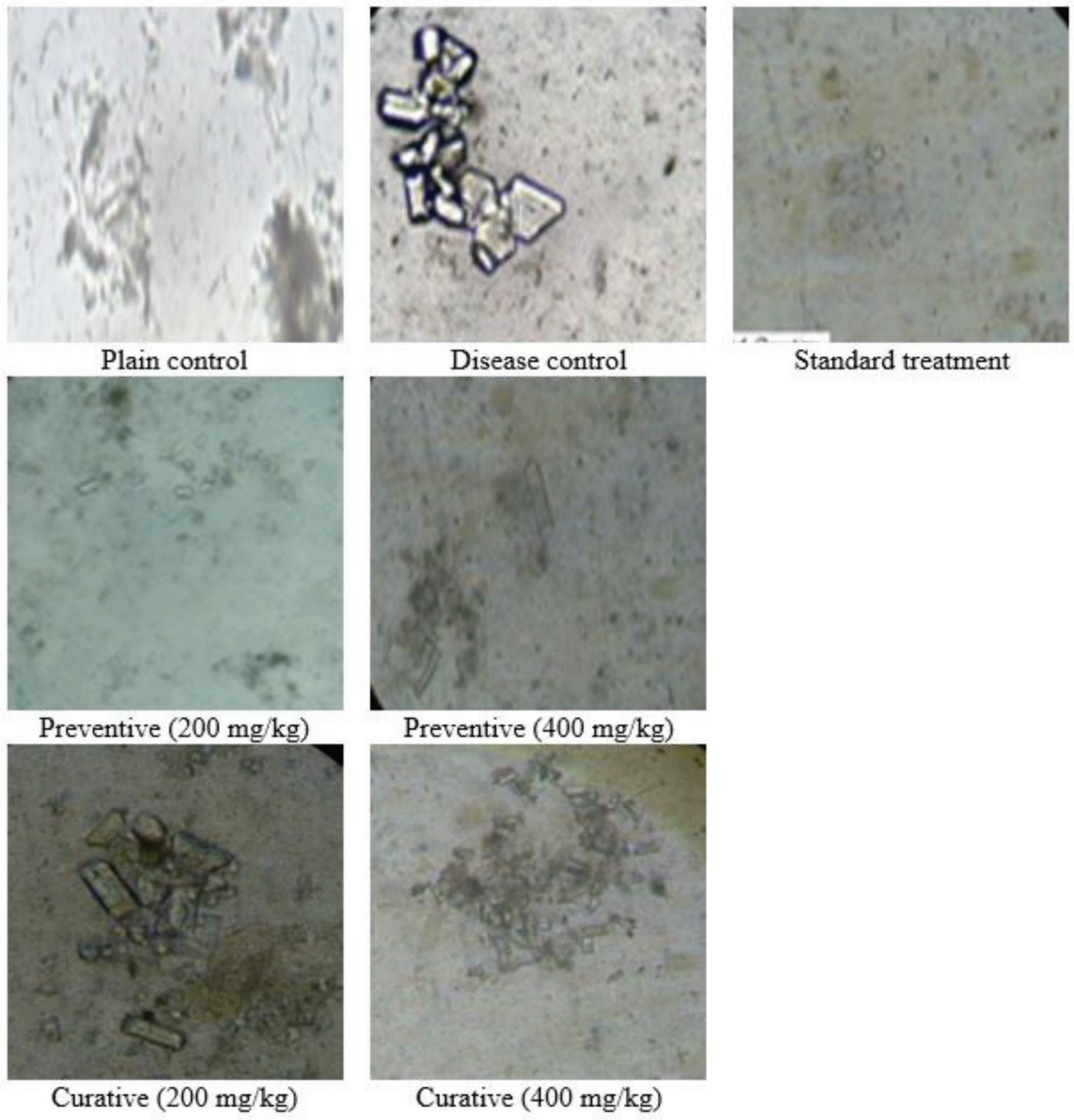

Figure 1. Microscopic images of urine samples.

150, 250, and 300 µg/ml). Treatment with gentamicin significantly ($p<0.001$) decreased cell viability, resulting in cellular shrinkage and vacuolation compared to the normal control group. However, when the cells were treated with the test extract, a significant improvement in cell viability was observed. The cell viability increased by 9-22% compared to the gentamicin control group. The EC50 value of the extract was determined to be 6.872 µg/ml.

3.2.2 *In-Vivo Study*

The treatment groups were able to prevent the gentamicin-induced increase in creatinine levels, demonstrating a significant protective effect against drug-induced toxicity in rats. Gentamicin (GM) administration resulted in evident signs of nephrotoxicity and marked renal dysfunction compared to the control groups, as confirmed by elevated serum creatinine levels (Table 4).

Histopathological examination of kidney tissues from the plain control group, diseased group, standard treatment group, and sample-treated groups is presented in Figure 3. The plain control group exhibited normal renal cell structure. In contrast, the animals treated with gentamicin (disease control) showed smaller glomeruli, interstitial edema in tubular cells, and mild hyaline casts in their lumens. Animals treated with the standard drug exhibited congested glomeruli along with significant degeneration of the renal tubules. The presence of luminal hyaline casts and mild stromal interstitial mononuclear cellular infiltration was observed in the standard treatment

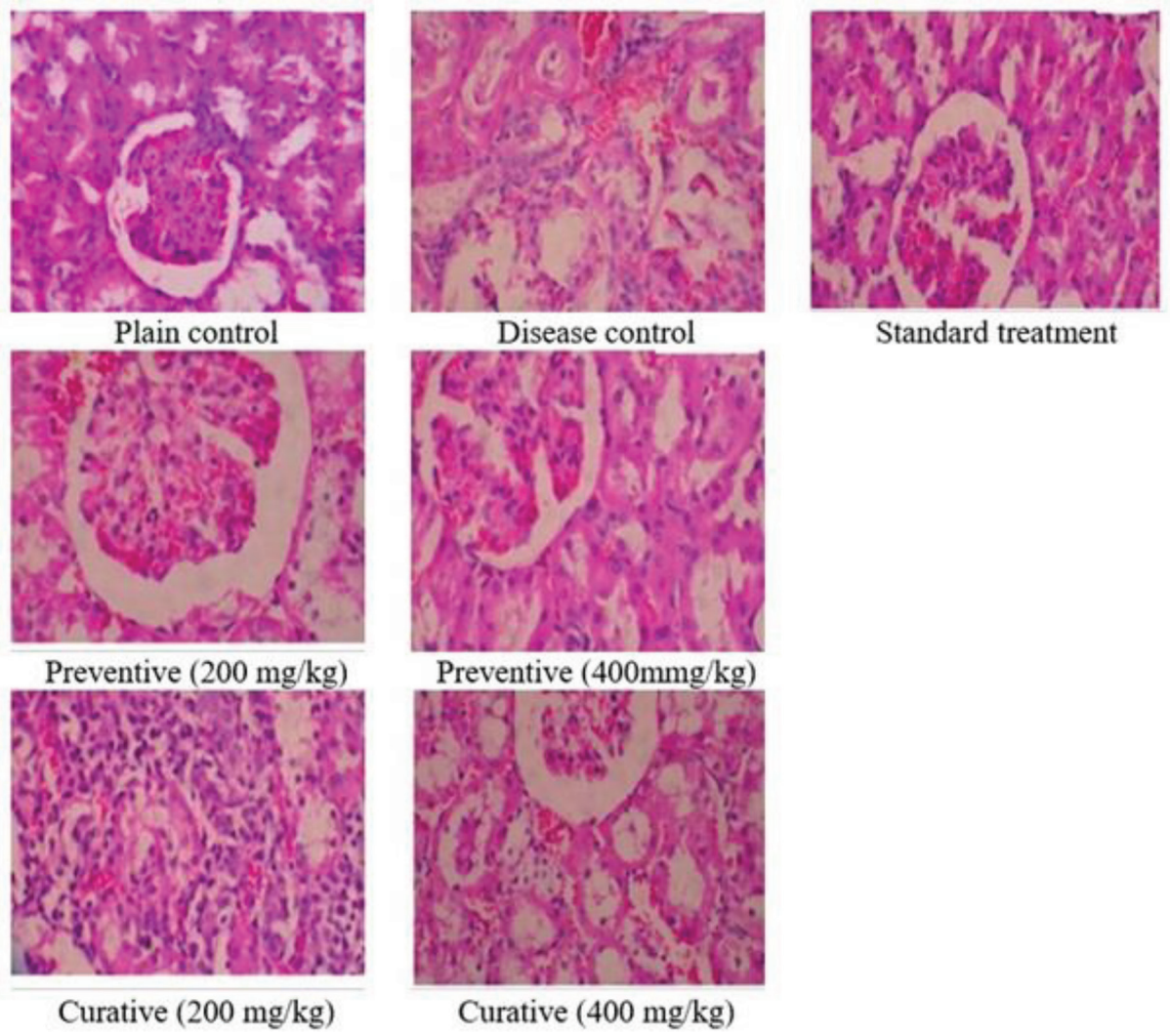

Figure 2. Histopathological findings of various groups- ethylene glycol-induced urolithiatic model.

Table 4. Serum biochemical parameters

Group	Urea ($mgdl^{-1}$)	Uric acid ($mgdl^{-1}$)	Creatinine ($mgdl^{-1}$)
Normal control (group I)	23.23 ± 1.45	2.08 ± 0.07	0.33 ± 0.05
Toxicant-gentamicin (group II)	97.46 ± 1.57**	3.84 ± 0.09**	4.46 ± 0.06**
Test sample 200mg/kg (group III)	42.78 ± 1.61 ns	2.49 ± 0.05 ns	1.35 ± 0.09*
Test sample 300mg/kg (group IV)	46.80 ± 1.98**	2.72 ± 0.08**	1.38 ± 0.05**
Test sample 400mg/kg (group V)	27.94 ± 1.78**	2.35 ± 0.07**	0.53 ± 0.06**
Standard treatment – Quercetin (group VI)	26.99 ± 1.25**	2.80 ± 0.05**	0.46 ± 0.03**

group. Animals treated with the test samples (at doses of 200 mg/kg and 300 mg/kg of body weight) showed moderate-sized glomeruli with few luminal casts, mild congestion, and mild tubular degeneration compared to the disease control group. Animals treated with the higher dose (400 mg/kg) of the test sample displayed reduced glomerular congestion and very mild tubular degeneration. These findings indicate the significant nephroprotective activity of the hydroalcoholic extract of *L. camara* flowers against degenerative kidney injury caused by gentamicin. The results of the histopathological analysis are consistent with the findings of the biochemical parameters.

4. Discussion

L. camara, a widely utilized traditional medicinal plant, holds great therapeutic potential. It has been traditionally used in various regions for treating numerous health conditions. The significance *of L. camara* as a valuable medicinal plant has been emphasized through both ethnomedical knowledge and scientific research. Although several ethno pharmacological studies have suggested the potential antilithiatic and nephroprotective effects of *L. camara* flowers, there is still a lack of authenticated scientific evidence for these traditional uses. Therefore, this study aims to investigate the antilithiatic and nephroprotective activity of the hydroalcoholic extract derived from *L. camara* flowers.

The kidneys play a vital role in various important functions, including the elimination of waste products and toxins from the bloodstream, as well as maintaining electrolyte balance. Currently, nephrolithiasis and nephrotoxicity are significant global concerns. Nephrolithiasis refers to the formation of hard, non-metallic mineral deposits in the kidney, bladder, or urethra[25]. The development of these stones is often linked to factors such as fluid intake, diet, metabolic disorders, and obesity. Calcium oxalate formation is a primary cause of lithiasis, which has a high prevalence worldwide. Nephrotoxicity, on the other hand, is the second most common kidney problem and a leading cause of morbidity and mortality. Exposure to various drugs and chemicals is a major contributing factor to nephrotoxicity[26]. The therapeutic management of kidney diseases is costly and often requires hospitalization for dialysis and surgery. As a result, extensive research is being conducted to explore safe, effective, and natural approaches for the treatment and prevention of kidney diseases. In countries like India and Brazil, there is increasing focus on utilizing traditional medicine to reduce the burden on healthcare and promote accessible healthcare options[27].

The antilithiatic effect of the hydroalcoholic extract of *L. camara* flowers was investigated in a nephrolithiasis-induced model using male Wister albino rats. The study was conducted in two ways: curative and preventive approaches. To evaluate the antilithiatic activity, biochemical analysis of serum and urine samples was performed. Male Wister albino rats have been extensively utilized in the study of urolithiasis due to the similarities in the lithiasis process between rats and humans. Urolithiasis can be induced using various methods, with two predominant approaches. The first method involves acute induction through a single large dose, while the second method involves chronic induction using small doses of lithogens over an extended period. Different lithogens can be employed

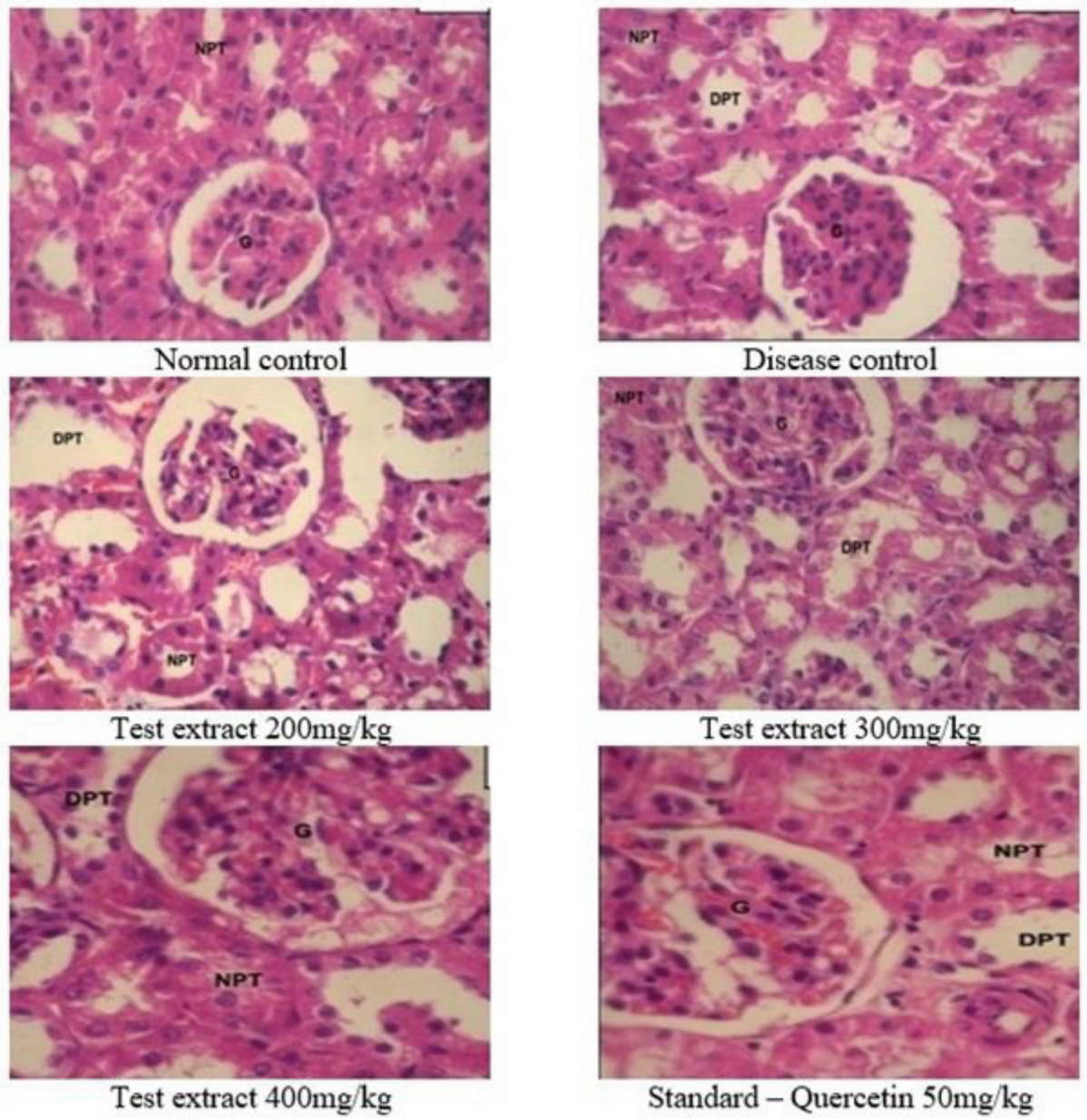

Figure 3. Histopathological findings of various groups- gentamicin-induced nephrotoxicity.

to induce lithiasis[28]. In this study, renal calculi were induced using 0.75% ethylene glycol in drinking water.

In the antilithiatic activity analysis, the extract exhibited significant improvements in serum biochemical parameters related to kidney function, such as creatinine, urea nitrogen, and uric acid levels. These improvements indicate a reduction in nephritic damage and improved kidney function. Additionally, the extract increased urine output and showed favorable changes in urine composition, including pH, protein levels, and the presence of calculi-forming components like calcium, phosphate, and oxalate. Microscopic analysis and histopathology studies further confirmed the preventive and curative effects of the extract in reducing the formation and deposition of calcium oxalate crystals, a common type of urinary stone.

Group B (diseased group) exhibited an increase in urinary pH towards the alkaline range (pH 8.66) compared to the plain control group. Animals treated with cystone and the test treatment showed a progressive decrease in urine pH. Urine glucose was absent in all groups, and the presence of ketone

bodies, nitrites, and urobilinogen was not statistically significant. However, the presence of protein indicated damage in the lithiasis-induced animals (diseased group). Animals fed with cystone and the test samples showed remarkable recovery, while the diseased group exhibited proteinuria, indicating severe nephritic damage. The groups treated with the test sample demonstrated favorable improvement in a dose-dependent manner. Elevated levels of calcium, phosphate, and oxalate were observed in the calculi-induced group, which are considered components involved in calculi formation. Animals treated with cystone and the test sample showed reduced concentrations of these calculi-forming components. However, the effect of the test sample decreased with higher doses. The preventive groups exhibited the most significant effect, with decreased amounts of calcium, phosphates, and oxalates, which in turn prevented the formation of calculi.

The induction of lithiasis significantly increased the levels of calcium in both serum and urine in the disease control group. This higher concentration of urinary calcium may form complexes with negatively charged inhibitors. Ethylene glycol-induced lithiasis resulted in higher concentrations of oxalate in both serum and urine. Treatment with the standard and test samples effectively controlled lithogen-induced hypercalciuria and hyperoxaluria. Elevated uric acid levels were observed in urolithic rats, which can alter the solubility of calcium oxalate and decrease the inhibitory effect of drugs used to treat lithiasis. However, all the treatments decreased the uric acid levels, reducing the chances of calculi formation. In diseased animals, the glomerular filtration rate significantly declined due to the obstruction of urine outflow by calculi in the system. As a result, the levels of waste products such as urea, uric acid, and creatinine in the blood decreased. The different groups of animals treated with the standard and test samples showed reduced levels of urea, uric acid, and creatinine. These results demonstrated a significant difference between the preventive and curative treatments.

Prolonged usage of gentamicin is known to cause both ototoxicity and kidney problems. The nephrotoxicity of gentamicin is primarily attributed to its selective accumulation in the proximal convoluted tubules through absorptive endocytosis. This accumulation triggers the development of lysosomal phospholipidosis, leading to tubular necrosis, which is a key pathological mechanism responsible for the reduced renal function. Nephrotoxicity induced by gentamicin is associated with increased levels of endothelin-1, upregulation of transforming growth factor-β, significant infiltration of monocytes/macrophages into the renal cortex and medulla, induction of apoptosis, and oxidative stress[29].

Regarding nephroprotective activity, cell line studies using HEK-293 cells demonstrated the potential of the extract to protect kidney cells from damage. Although the specific mechanisms of action were not mentioned in the summary, the observed reduction in cellular inflammation, epithelial desquamation, and blood vessel congestion in the histopathology analysis indicate the protective effect of the extract on renal cells. The results suggest that the hydroalcoholic extract derived from *L. camara* possesses antilithiatic and nephroprotective properties, making it a potential therapeutic agent for the prevention and treatment of urolithiasis and associated kidney damage. Further research is needed to elucidate the underlying mechanisms of action and to assess the safety and efficacy of the extract in human subjects.

The observed antilithiatic and nephroprotective effects of *L. camara* flower hydroalcoholic extract may be attributed to its rich composition of bioactive compounds. The reduction in uric acid levels suggests a potential modulation of purine metabolism, hindering calculi formation. Improved kidney function markers indicate a possible enhancement of renal filtration and excretion processes. The extract's inhibitory effect on abnormal cell morphology in gentamicin-induced nephrotoxicity may involve antioxidant and anti-inflammatory properties, shielding cells from oxidative stress. The traditional uses of *L. camara* in treating various ailments align with reported bioactive compounds, supporting its multifaceted therapeutic potential. Further mechanistic studies are warranted to elucidate the specific pathways and molecular interactions responsible for these observed effects.

Based on the current findings, future research could focus on isolating and characterizing the specific bioactive compounds responsible for the antilithiatic and nephroprotective effects observed in *L. camara* flowers. Mechanistic studies should delve into the molecular pathways and cellular interactions

underlying these effects. Optimization of extraction methods, formulation development, and exploration of combination therapies could enhance the efficacy and applicability of *L. camara* flower extracts. Progressing from preclinical trials to well-designed clinical trials will further validate their safety and therapeutic potential in human subjects. Additionally, research avenues may include examining the impact on specific kidney disorders, exploring various dosage forms, conducting long-term safety studies, and utilizing bioinformatics analysis to elucidate systems-level effects. These endeavors aim to contribute to the comprehensive understanding and effective utilization of *L. camara* flowers in addressing kidney-related disorders. Moreover, the stability of phytosomes is a crucial consideration in pharmaceutical and herbal formulations. Phytosomes are complex structures formed by the interaction of natural phospholipids with phytoconstituents, enhancing their bioavailability. To ensure the efficacy and shelf-life of phytosome-based products, it is essential to have proper storage conditions (temperature, humidity, and light exposure).

5. Conclusion

This study evaluated the potential benefits of the hydroalcoholic extract of *L. camara* flowers in the context of kidney-related conditions, such as nephrolithiasis and nephrotoxicity. The extract demonstrated significant antilithiatic activity, as evidenced by the prevention and reduction of calcium oxalate crystal formation in the kidneys. It also showed nephroprotective effects by improving renal function, reducing nephritic damage, and preventing the accumulation of calculi forming components. Biochemical analysis revealed that the extract effectively reduced the levels of creatinine, urea nitrogen, uric acid, and other parameters associated with kidney dysfunction. The observed increase in urine output and improvement in urinary electrolyte balance further supported its therapeutic potential in the management of kidney diseases. Furthermore, histopathological examination confirmed the protective effects of the extract against renal damage induced by lithiasis and drug toxicity. The extract mitigated cellular abnormalities, inflammation, and congestion in the renal tissues, promoting the restoration of normal kidney structure. Overall, these findings highlight the promising antilithiatic and nephroprotective properties of the hydroalcoholic extract of *L. camara* flowers. Further research and clinical studies are warranted to explore its full therapeutic potential and determine its optimal dosage and administration regimen for the treatment and prevention of kidney diseases.

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