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Afr. J. Biomed. Res. Vol. 27(3s) (November 2024); 5897-5909

Research Article

Evaluation of Anti-Urolithiatic Activity of Selected Medicinal Plants and Its Poly Herbal Formulation in Experimentally Induced Urolithiatic Rats

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Abstract

Introduction: Kidney stones are formed by the crystallization of minerals and salts in the kidneys or urinary tract. Despite significant advances in the knowledge of pathophysiological etiology, therapeutic options remain limited and often expensive. Herbal therapy for the treatment of kidney stones is gaining popularity as an alternative medication.

Methods: The methanolic extracts of *Nelumbo nucifera* (NNE), *Anogeissus latifolia* (ALE), and *Leucas aspera* (LAE) and PHF were administered at a low dose (250mg) and high doses (500mg). Ethylene glycol (0.75%) was used to induce urolithiasis in the experimental rat model. Cystone served as the standard. The vital urine and blood serum parameters were estimated. Kidney homogenates were used for LDH, GSH, and LPO. Further histopathological evaluation changes were presented. The statistical significance of the results was assessed with a threshold set at $p < 0.001$.

Result: Preliminary phytochemical evaluation revealed the presence supportive active phyto-constituents such as of alkaloids, flavonoids, tannins, phenols, saponins, phytosterol, terpenoids and glycosides. Urine analysis indicates that all treated groups had significant antiurolithiasis effects restoring the urine parameters. PHF is more effective than the high- dose extract groups. Blood parameters such as creatinine, uric acid, alkaline phosphatase, BUN, potassium, and alanine amino transferases (ATT) were restored significantly. In kidney tissue homogenates, the amount of LDH, Glutathione (GSH), and lipid peroxidation (LPO) is restored significantly. The efficacy of NNE-treated groups of both low and high dosages was better than ALE and LAE extracts. Polyherbal formulations (PHF) showed higher efficacy than all the groups of high dose-extract.

Conclusion: The selected medicinal plants significantly showed antiurolithiasis activity in EG-induced rats. Histopathological studies demonstrate the better regeneration of glomeruli and very less structural damage in high-dose extracts whereas PHF has shown better results compared to individual high doses. These positive results might be the presence of a supportive phytochemicals group such as saponins, alkaloids, flavonoids and phenols. Hence, it can be concluded that the selected methanolic extracts of *Nelumbo nucifera*, *Anogeissus latifolia*, and *Leucas aspera* have significant antiurolithiasis activity in EG-induced rat model.

Keywords: Anti-urolithiatic, *Nelumbo nucifera*, *Anogeissus latifolia*, and *Leucas aspera*.

1. Introduction

Certain medical conditions, such as urinary tract infections, hyperparathyroidism, and Crohn's disease, can increase the risk of developing kidney stones [1]. Certain medications, such as alpha-blockers and calcium channel blockers, can help relax the muscles in the urinary tract, making it easier for the stones to pass. Extracorporeal Shock Wave Lithotripsy: It is a process that cuts the waves of shock in which the stones can turn in the form of small residues [2].

Ureteroscopy is a better criterion in renal diagnosis. Percutaneous Nephrolithotomy (PCNL): It is suggested for the expulsion of bigger stone complexes [2,3]. According to the reported research, plant metabolites such as saponins, phenols, and flavonoids are supportive phytochemicals against urolithiasis [4]. Further investigation will help to establish the phytochemical screening action that shows the desired pharmacological actions.

Nelumbo nucifera belonging to the Nymphaeaceae family includes treatment in Ayurveda. This plant is used as a diuretic and anti-helminthic and in the treatment of strangury, vomiting, leprosy, skin diseases, and nervous exhaustion. In popular medicine, it is used in the treatment of tissue inflammation, cancer, skin diseases, leprosy, and as a poison antidote [5,6].

Anogeissus latifolia belongs to the family Combretaceae shows treatment of skin injuries, particularly in rats. *Leucas aspera*, a medicinal plant has an antifungal action of extracts containing chloroform and ether of *L. aspera* against *Microsporum gypseum* & *Trichophyton* was discovered in vitro. About 5mg/mL was

determined to be the lowest concentration inhibiting [7,8].

Leucas aspera also known as Thumbai (in Tamil), spread in various regions across the world [9]. Both fungicidal & fungi-static properties were found in *Leucas aspera*. Prostaglandin inhibitory effects Prostaglandin (PG) inhibiting and antioxidant properties were investigated. The alcoholic (ethanol namely) extract was tested for anti-nociceptive, and cytotoxic activity with free radicals, writing inhibition mediated by acetic acid, and brine shrimp lethality bioassay, respectively [10].

2.1 Materials and Methods

2.1.1 Collection of plant material and Preparation of Extract

Nelumbo nucifera, *Anogeissus latifolia*, and *Leucas aspera* were collected from Sri Venkateswara University, Tirupati, 517502, Andhra Pradesh, India. It was then certified by Dr. K. Madhava Chetty, Plant Taxonomist, from the district of Tirupati, AP bearing serial plant numbers 1078, 1080, and 1088 were collected and air-dried for seven days at room temperature and under shady conditions. The Dried plant materials are ground and sieved. The plant's powders are subjected to Soxhlet extraction using methanol as solvent packed into a soxhlet column and extracted with 70% v/v methanol in water at 65–70°C. The obtained methanolic extract of *NNE*, *ALE*, and *LAE* was subjected to evaporation at the temperature scale of 40°C and preceded for further studies. The extract was preserved in air-tight conditions [11-13]. The concentrated extract is used for the screening of possible phytochemicals.

2.1.2 Chemical Agents and Apparatus

Ethylene glycol-AR, (Merck, India), and Cystone (Himalaya Company Limited, India) are selected for the practical studies. If needed selection of the required chemicals and reagents were included with analytical grades. Auto analyzer Semi followed by UV- - spectrophotometer (UV-1700 Pharma-spec, SHIMADZU).

2.1.3 Preliminary phytochemical screening:

The preliminary phytochemical analysis was carried out as per the standard methods [11,12]. The list of presenting groups of phytochemicals is reported in the results.

2.1.4 Animals

Wistar rats (of both sexes) weighing 150-200g were acquired. Animals were subjected to acclimatization for laboratory conditions. Proper, hygienic, environmental conditions like temperature, relative humidity, and *ad libitum* –

standard for feeding were provided according to quarantine conditions. All the work carried out on the animals was by the CPCSEA guidelines and the Research protocols have been approved by the IAEC, JLS, and the Approval. No. was CPCSEA/IAEC/JLS/18/07/22/029 of Jeeva Life Sciences animal house, Medchal, Malkajgiri, Telangana. Experimental research studies were conducted and performed according to CPCSEA & IAEC rules and regulations.

2.15 Acute-toxicity studies

Toxicity studies were conducted according to Organization for Economic Cooperation and Development rules and regulations [14].

2.1.6 Ethylene glycol-induced urolithiasis rat model

To develop urolithiasis in rats, all rats (except the normal group) were fed a commercially pelleted diet with drinking water containing (0.75% v/v) EG for 28 days. The urine, blood, biochemical parameters, and histopathological studies were carried out. [15,16].

Table 2.1.6.1. Treatment schedule

Group No.	Group Name	Treatment
Group 1	Normal control	Vehicle for 28 days
Group 2	Disease control	Water with 0.75 percent Ethylene Glycol (0.75% v/v) was given to the individual rats for 28 days.
Group 3	Standard group	Cystone (500 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 4	Test group 1	NNE (250 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 5	Test group 2	NNE (500 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 6	Test group 3	ALE (250 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 7	Test group 4	ALE (500 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 8	Test group 5	LAE (250 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 9	Test group 6	LAE (500 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 10	Test group 7	PHF (250 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.
Group 11	Test group 8	PHF (500 mg/kg, orally) is given from the 14 th day to the 28 th day with 0.75 percent ethylene glycol in drinking water.

NNE, ALE, LAE, and PHF: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

2.17 Kidney's Histological Studies

Histopathological evaluation was carried out using a microtome. The kidneys were isolated, fixed in formalin (10%), and embedded in paraffin for 5mm thick sections. Sections measuring 5mm thick were stained with hematoxylin and eosin. The specimen was examined under a microscope [17].

2.1.8 Statistical Analysis

All the results were scrutinized by analysis of

variance (Dunnett's comparison test) using Prism (v8.2) and articulated as mean and SD. The significance ($p < 0.05$) was considered.

3. Results:

3.1 Preliminary phytochemical screening:

Preliminary phytochemical evaluation revealed the presence of supportive active phyto-constituents such as alkaloids, flavonoids, tannins, phenols, saponins, phytosterol, terpenoids, and glycosides.

Table 3.1. Preliminary phytochemical result for selected plant extracts

S. No.	Phytochemicals	NNE	Results	
			ALE	LAE
1.	Alkaloids	-	+	+
2.	Flavonoids	+	+	+
3.	Tannins	+	+	+
4.	Phenols	+	+	+
5.	Carbohydrates	+	+	+
6.	Saponins	+	+	+
7.	Phytosterol	+	+	-
8.	Terpenoids	+	+	+
9.	Glycosides	+	+	+
10.	Anthraquinones	-	-	-

(+) Present; (-) Absent

NNE, ALE, LAE, and PHF: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

3.2 Acute oral toxicity:

In acute oral toxicity evaluated on selected rats (both sexes) for 14 days, no signs and symptoms of toxicity were noticed at the limit-test dose of 2000mg/kg administered as per the OECD guidelines.

3.3 Anti-urolithiatic activity on ethylene glycol induced rat model:

The selected methanolic plant extracts of NNE, ALE, LAE, and PHF were studied for their Anti-urolithiatic activity on ethylene glycol (0.75% v/v) induced rats. All the results of urine parameters analysis are presented in **Table 3.3.1.1** and **Figure 3.3.1.1**. The blood parameters are presented in **Table 3.3.2** and **Figure 3.3.2**.

3.3.1 In urine analysis (Table 3.3.1.1 and

Figure 3.3.1.1), we examine calcium, magnesium, phosphate, and oxalates. The results suggest that all treated groups have shown significant antiurolithiasis activity in ethylene glycol-induced rats. Calcium, magnesium, and phosphate levels were increased in the disease control group treated with ethylene glycol whereas all the extracts treated groups significantly reduced the levels of Calcium, magnesium, and phosphate levels. However, polyherbal formulations (PHF) have shown better efficacy compared to high-dose extracts treated groups. In the case of oxalates, the levels were decreased in the disease control group and significantly increased in all the tested groups whereas the PHF have shown better results.

Table 3.3.1.1. The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene Glycol induced rats on urine parameters.

Groups	Treatment (kg/p.o)	Test Description			
		Calcium (mg/dl)	Magnesium (mg/dl)	Phosphate (mg/dl)	Oxalates (mg/dl)
Normal	Vehicle	0.45±0.03	1.83±0.02	3.83±0.05	5.79±0.07
Control	EG (0.75%v/v)	2.40±0.06	2.88±0.04	6.88±0.05	4.25±0.08
Standard	Cystone (500mg)	1.50±0.02	2.16±0.18	4.56±0.04	5.20±0.05
Test Group - 1	<i>NNE</i> (250mg)	1.90±0.06***	2.70±0.04*	6.64±0.13*	4.10±0.04
Test Group - 2	<i>NNE</i> (500mg)	1.90±0.03***	2.61±0.05***	5.74±0.17***	4.53±0.10***
Test Group - 3	<i>ALE</i> (250mg)	2.10±0.01***	2.83±0.08	6.63±0.06**	4.23±0.15
Test Group - 4	<i>ALE</i> (500mg)	2.00±0.05***	2.69±0.08*	6.15±0.16***	4.21±0.06
Test Group - 5	<i>LAE</i> (250mg)	2.00±0.03***	2.70±0.18*	6.11±0.14***	4.07±0.04*
Test Group - 6	<i>LAE</i> (500mg)	1.90±0.02***	2.64±0.11**	6.07±0.10***	4.18±0.14
Test Group - 7	<i>PHF</i> (250mg)	1.50±0.02***	2.49±0.06***	5.20±0.04***	5.03±0.10***
Test Group - 8	<i>PHF</i> (500mg)	1.20±0.05***	2.11±0.07***	4.17±0.14***	5.41±0.17***

*P < 0.05, **P < 0.01, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test. ANOVA: Analysis of variance, *NNE*, *ALE*, *LAE*, and *PHF*: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

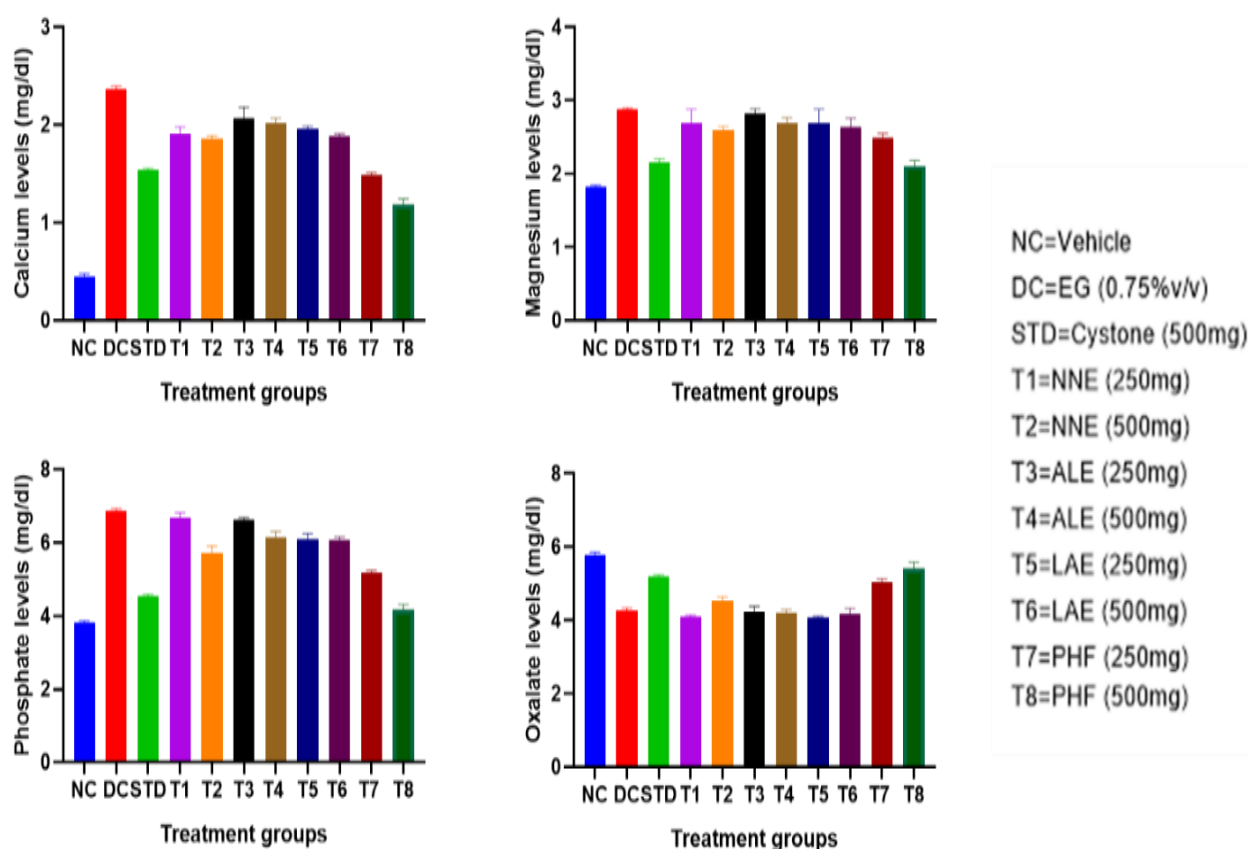


Figure 3.3.1.1. The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on urine parameters.

*P < 0.05, **P < 0.01, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test. ANOVA: Analysis of variance, NNE, ALE, LAE, and PHF: Nelumbo nucifera, Anogeissus latifolia, Leucas aspera, and polyherbal formulation.

3.3.2 In blood parameters analysis (Table 3.3.2 and Figure 3.3.2), we examine creatinine, uric acid, alkaline phosphatase, BUN, potassium, and alanine amino Transferases (ATT) levels. The results suggest that all treated groups have shown antiurolithiasis activity in ethylene glycol-induced

rats. Creatinine, uric acid, alkaline phosphate, BUN, potassium, and AAT levels were increased in the disease control group treated with ethylene glycol whereas all the extracts treated groups significantly reduced. However, polyherbal formulations (*PHF*) have shown better efficacy (p< 0.001) compared to high-dose extracts treated groups. However, *ALE*-treated groups showed (p<0.01) efficacy in creatinine, uric acid, BUN, and AAT levels. Whereas *NNE*-treated groups with low and high doses have shown superlative efficacy (p< 0.001) compared to *ALE* and *LAE* extract treated groups.

Table 3.3.2.1: The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on blood parameters.

Groups	Treatment (mg/kg/p.o)	Test Description					
		Creatinine (mg/dl)	Uric (mg/dl)	acidAlkaline Phosphatase (IU/L)	BUN (mg/dl)	Potassium (mEq/L)	AAT (mEq/L)
Normal	Vehicle	0.65±0.007	1.77±0.03	74.60±1.36	19.40±0.14	3.54±0.06	16.66±0.24
Control	EG (0.75%v/v)	1.1±0.07	3.75±0.03	94.78±1.68	29.68±0.46	5.37±0.17	49.34±0.63
Standard	Cystone(500mg)	0.75±0.02**	2.16±0.04**	76.36±2.80**	22.05±0.91**	3.45±0.12**	20.99±0.57**
		*	*	*	*	*	*
Test Group 1	-NNE (250mg)	0.91±0.03**	2.22±0.10**	78.34±2.31**	25.38±0.33**	3.46±0.37**	31.32±1.27**
		*	*		*	*	*
Test Group 2	-NNE (500mg)	0.84±0.04**	2.12±0.02**	73.62±2.15**	23.98±0.91**	3.20±0.06**	25.20±0.97**
		*	*	*	*	*	*
Test Group 3	-ALE (250mg)	1.00±0.04**	2.86±0.06**	82.55±1.27*	27.69±0.28**	3.95±0.07**	34.36±1.33*
		*	*			*	
Test Group 4	-ALE (500mg)	0.93±0.03**	2.76±0.02*	75.74±1.17**	24.62±1.28**	4.39±0.14**	28.86±0.83**
					*	*	
Test Group 5	-LAE (250mg)	0.92±0.05**	2.80±0.12**	79.74±1.13**	25.14±1.79**	3.65±0.12**	33.80±0.76**
		*		*	*	*	*
Test Group 6	-LAE (500mg)	0.87±0.08**	2.29±0.15**	73.20±2.67**	23.38±0.92**	3.25±0.09**	25.99±1.15**
		*	*	*	*	*	*
Test Group 7	-PHF (250mg)	0.78±0.03**	2.39±0.09**	71.18±1.3***	23.17±1.32**	3.13±0.07**	23.31±0.60**
		*	*		*	*	*
Test Group 8	-PHF (500mg)	0.71±0.04**	2.10±0.06**	64.84±1.61**	20.29±0.43**	2.91±0.10**	20.46±1.06**
		*	*	*	*	*	*

*P < 0.05, **P < 0.01, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test.

ANOVA: Analysis of variance, *NNE*, *ALE*, *LAE*, and *PHF*: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

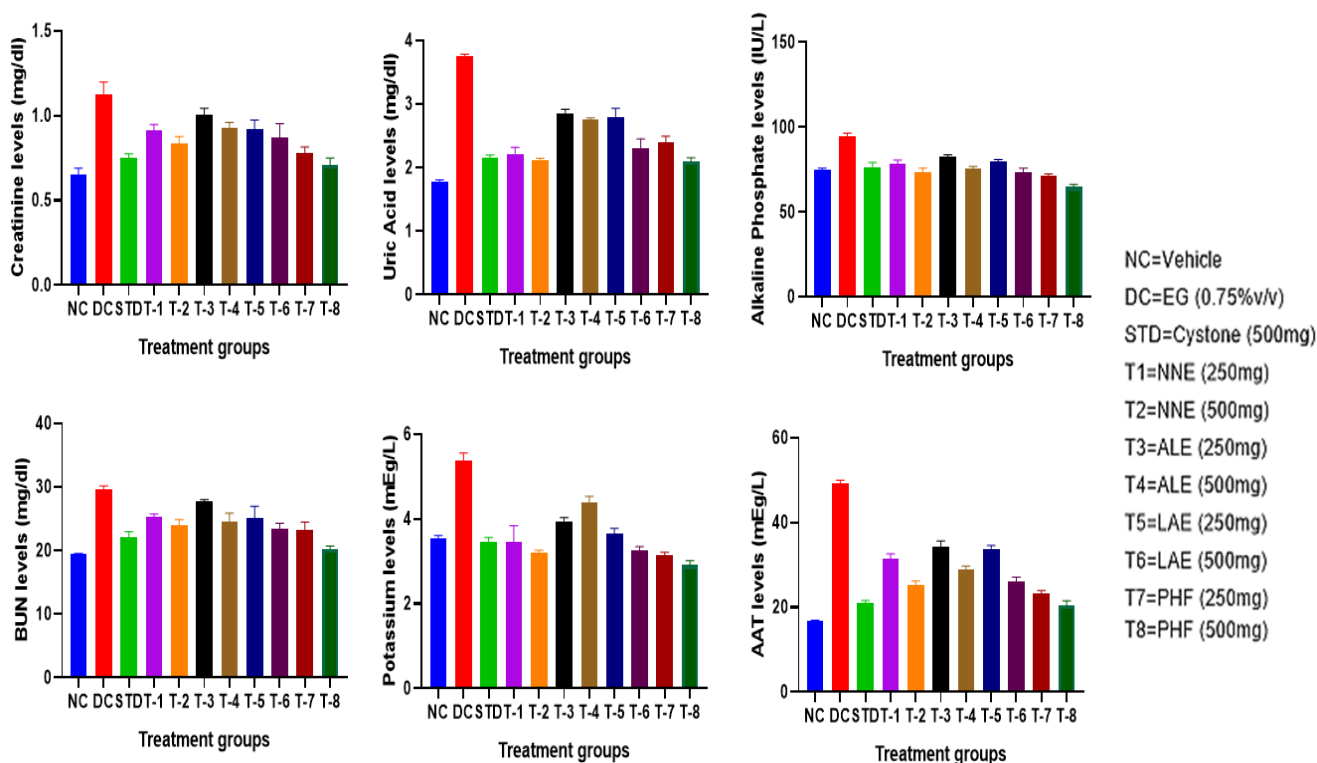


Figure 3.3.2.2. The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on blood parameters.

*P < 0.05, **P < 0.01, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test. ANOVA: Analysis of variance, *NNE*, *ALE*, *LAE*, and *PHF*: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation

3.3.3. In kidney tissue homogenate analysis (Table 3.3.3 and Figure 3.3.3), we estimated the levels of lactate dehydrogenase (LDH), Glutathione (GHS), and lipid peroxidation (LPO) levels. The results suggest that all treated groups

have shown antiurolithiasis activity in ethylene glycol-induced rats. The levels of LDH and lipid peroxidation were increased, and GSH levels decreased in the disease control group treated with ethylene glycol. whereas all the extracts treated by the groups significantly restored these levels. However, *NNE*-treated groups with low and high doses have shown better efficacy (p< 0.001) compared to *ALE* and, *LAE* extracts treated groups. However, polyherbal formulations (*PHF*) have shown superlative efficacy (p< 0.001) compared to high-dose extracts treated groups.

Table 3.3.3.1: The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on tissue homogenates.

Groups	Treatment (mg/kg/p.o)	Test Description		
		LDH (nmol/min/mL)	GHS (nmol/min/mL)	LPO (mEg/L)
Normal	Vehicle	349.97±4.71	3.69±0.22	1.45±0.13
Control	EG (0.75%v/v)	744.45±8.49	1.51±0.08	2.69±0.18
Standard	Cystone (500mg)	445.83±13.41***	2.21±0.06***	2.09±0.05***
Test Group - 1	<i>NNE</i> (250mg)	562.10±17.80**	1.96±0.06**	2.24±0.07***
Test Group - 2	<i>NNE</i> (500mg)	482.63±9.10***	2.16±0.14***	2.15±0.06***
Test Group - 3	<i>ALE</i> (250mg)	582.60±17.48**	1.45±0.15***	2.03±0.09**
Test Group - 4	<i>ALE</i> (500mg)	494.96±7.14**	1.76±0.14*	2.25±0.07**
Test Group - 5	<i>LAE</i> (250mg)	551.32±15.17*	1.80±0.17**	2.22±0.10**
Test Group - 6	<i>LAE</i> (500mg)	465.13±8.17**	1.81±0.14***	2.08±0.09**
Test Group - 7	<i>PHF</i> (250mg)	429.37±11.94***	2.06±0.08***	1.87±0.04***
Test Group - 8	<i>PHF</i> (500mg)	403.76±7.32***	2.42±0.15***	1.51±0.13***

*P < 0.05, **P < 0.001, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test. ANOVA:

Analysis of variance, *NNE*, *ALE*, *LAE*, and *PHF*: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

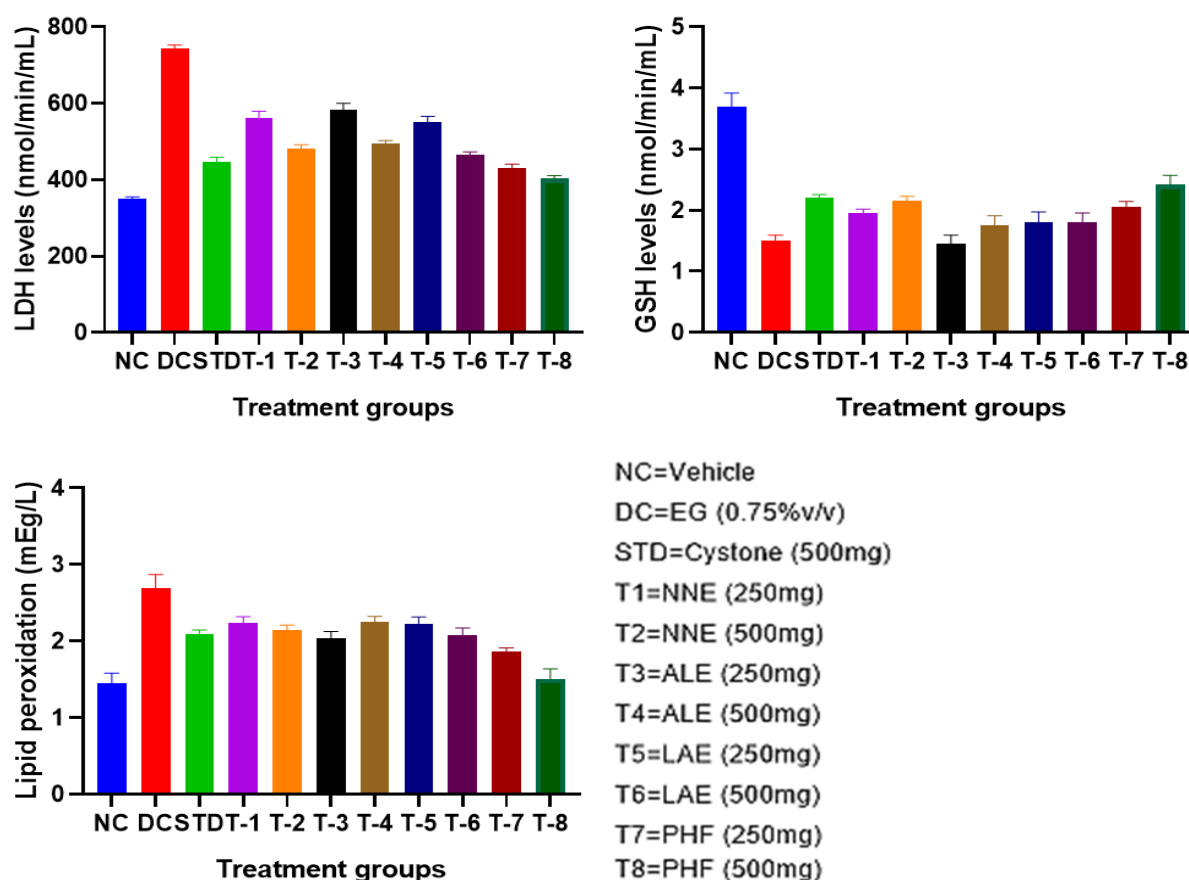


Figure 3.3.3.2: The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on tissue homogenates.

*P < 0.05, **P < 0.01, ***P < 0.001. Values are mean±SD, n = 6, using one-way ANOVA followed by Dunnet's multiple comparison test. ANOVA: Analysis of variance, *NNE*, *ALE*, *LAE*, and *PHF*: *Nelumbo nucifera*, *Anogeissus latifolia*, *Leucas aspera*, and polyherbal formulation.

3.3.4 Histopathological evaluation:

The cytoplasm of columnar epithelial cell stains in the coloration of pink in the tubules of Bowman's capsule at which glomerulus seems to be normal in vehicle-treated groups. Bowman's capsule surrounds glomerular degeneration with loss of capillaries [18], Degeneration and loss of tubular architecture are visible in the tubules, as is the build-up in the tubules' centers in cystone treated group [19]. Bowman's capsule surrounds a glomerulus that has suffered a loss of capillaries. Bowman's capsule surrounded by a tuft of capillaries shows that the glomerulus has recovered its usual structure in all the extract treatment groups. A few tubules, however, seemed to have modest degeneration, as seen by the build-

up of fluid in the core in extract- treated groups [20]. The tubules had a normal structure and were recovering normally. Most tubules have returned to their usual structure and function and few tubules, on the other hand, had modest degeneration, as shown by accumulation in the tubules' centers in all extract treatment groups. **(NC)** Normal Control with normal glomeruli with renal tubular morphology. **(DC)** Urolithiatic rats with crystal contents with damages **(T1- T6)** urolithic rats treated with extracts low and high doses (250mg/kg.b.wt and 500mg/kg.b.wt) displayed low crystal contents, lesser degradation of glomeruli and tubules **(T7 and T8)** urolithic rats treated with low and high doses (250mg/kg.b.wt and 500mg/kg b.wt) of *PHF*'s displayed low crystal contents, lesser degradation of glomeruli and tubules compared to standard and all the doses of individual extracts. Hence, this is considered that the polyherbal formulations have better efficacy compared to individual high doses of extracts. The histology of the kidney sections was presented in **Figure 3.3.4.1**.

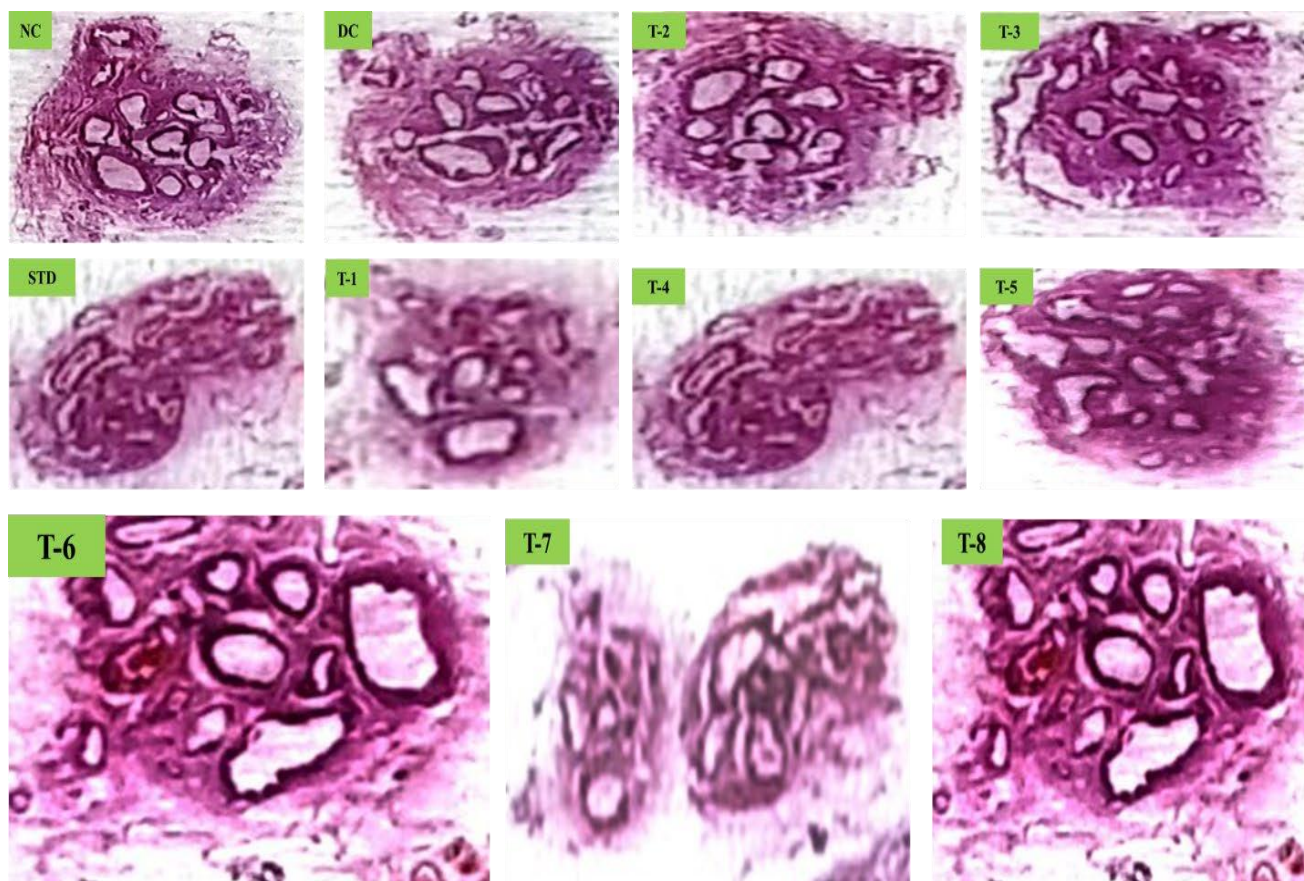


Figure 3.3.4.1: The effect of methanolic extracts of *NNE*, *ALE*, *LAE*, and *PHF* on Ethylene glycol-induced rats on kidney histopathology.

Discussion:

Kidney failure is indicated by an elevated creatinine level. Hypomagnesemia, hypercalciuria, and hyper-phosphaturia were the disorders caused by the induction of ethylene glycol. The creatinine level in the blood will increase if the kidneys are unable to eliminate creatinine from the blood. In most cases, calcium and phosphorus act as a buffer. By depositing phosphorus on the bones and other tissues, excessive phosphorus levels may cause low serum calcium as renal disease progresses. Uric acid crystals may cling to the surface of the renal epithelial cells, causing an immediate inflammatory reaction. In addition, kidney stones and a decreased glomerular filtration rate are also possible side effects (GFR) [18]. Serum ALP concentrations are often used to identify hyperparathyroidism and renal bone disease. When a CKD patient's blood ALP levels are elevated, it frequently means that their bones are resorbing quickly. The amount of urea nitrogen that is produced in the body because of the breakdown of protein is the quantity that is measured by BUN [19]. It is (ultrahigh dosage) (high dose). Eliminated from the body through the urinary system, namely the urine. An increase in BUN may be the consequence of a decline in renal function brought on by disease or damage to the kidneys. This may be the case in certain cases. For patients with renal failure, a build-up of potassium is a common complication that occurs when the kidneys cannot eliminate excess. Hyperkalemia, or high potassium levels in the blood, is a possible consequence of severe renal disease [20, 21]. The current research study notices that the calculi insurgents into the cells and tissues of kidneys in toxic control-treated rats. Formation of renal

calculi and crystal saturation may tend to show variations in weight in either of the kidneys by the release of interstitial fluids and alterations in histological changes. So, the current research study has the inhibition activity of stone formation in all aspects. The genus of selected plants (*Nelumbo nucifera*, *Anogeissus latifolia*, and *Leucas aspera*) extracts have free radical scavenging action, with inhibition of lipid peroxidation [8,9]. These effects are due to constituents like Flavonoids, Tannins, Saponins, Phytosterol, Phenols, Terpenoids, etc., which tend to have probably anti-urolithic and nephron-protective actions. Tissue injury is indicated by an increase in LDH. As an example: Many tissues, including the blood and heart as well as the kidneys and brain, might be affected. As a result of any damage to these tissues, LDH and other body fluids are discharged into the bloodstream. Glutathione Stimulating Hormone (GSH) is critical for the kidneys to function normally [22]. Oxidative stress may lead to tissue damage and an increased risk of illness if it persists for an extended period. Bowman's capsule tends to have a loss of capillaries along with the degeneration of the glomerulus, according to histological studies [23]. Tubular degeneration, tubular morphological cells, and tissue damage loss occur and are minimized by a conventional medication by cystone, respectively, are seen. Both the low doses of both drugs demonstrate normal architecture of the glomerulus and have normal recovery of cells and tissues in the tubules. All the doses of extracts show recovery and normal architecture of the glomerulus the tubes showed normal architecture and recovery. Methanolic forms of *Nelumbo nucifera*, *Anogeissus latifolia*, and *Leucas aspera*

tend to have an anti-urolithic action that can be exerted by inhibiting the stone formation followed by damage to the internal surfaces of the renal tubules of Bowman's capsule and glomerulus in the kidneys. Thus, it can be enhanced that the recovery and growth of the cells and tissues can be seen with the replacement of degenerative and necrotic areas in the renal system. The increase in the volume of urine, magnesium in urine followed by the improvement of biomarkers of renal functions by decreasing LPO and relinquishment of antioxidants. So, all the factors mentioned above show that *PHF* reduces the genesis of calcification along with crystalline stones thereby inhibiting the aggregation and supersaturation of stone initiation and progress in the urinary bladder by promoting increased urinary volume. So, this research study escalates that the inclusion of all the constituents may contribute to the nephrolithiasis action.

Conclusion:

The phytochemical analysis revealed the presence of various supportive constituents for antiurolithiasis activity in rats. Urine analysis indicates that all treated groups had significant antiurolithiasis effects restoring the urine parameters. *PHF* is more effective than the high-dose extract groups. Restore levels of creatinine, uric acid, alkaline phosphatase, BUN, potassium, and alanine amino transferases (ATT) were noticed. Notably, the polyherbal formulations *PHF* showed higher efficacy when compared to high-dose extracts. In kidney tissue homogenates, the amount of LDH, Glutathione (GSH), and lipid peroxidation (LPO) is restored significantly. All treated groups exhibited significant antiurolithiasis activity compared with the ethylene glycol-induced rats. The efficacy of *NNE*-treated groups of both low and high dosages was better than *ALE* and *LAE* extracts. Polyherbal formulations (*PHF*) showed higher efficacy than all the groups of high dose-extract. Hence, it can be concluded that the

selected methanolic extracts have significant antiurolithiasis activity in EG- induced rat model. However, further extension studies are required to prove this activity in various models.

Additional Information: NA

Author Contributions: All authors contributed equally to the manuscript.

Conflict of Interest: No conflict of Interest.

Research Fellowship and Scholarship Approval: None

Acknowledgments

I, sincerely, convey my sincere gratitude and regards to the Director, Dr. Subhranshu Panda, Sir, Jaipur National University, Jaipur, Dr. G. Tharun Kumar, Sir, Professor, for their sincere support, and to the Principal, Dr. Vijaya kuchana Madam, Teegala Krishna Reddy College of Pharmacy, Medbowli, Hyderabad, for library studies and prominent guidelines assistance etc., Jeeva Life Sciences Labs, Medchal, Malkajgiri, Telangana state for contributing adequate laboratory requirements and assistance for conducting the research project by me in a safety and hygienic environment.

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