

Antiuro lithiatic Potential of *Eleusine indica* Linn. (GOOSE GRASS) Root Extract on Ethylene Glycol Induced Nephrolithiasis in *Rattus norvegicus* (ALBINO RATS)

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Abstract: The study was mainly aimed to determine the antiuro lithiatic potential of different concentrations of *E. indica* root extract on ethylene glycol induced nephrolithiasis in *Rattus norvegicus* (albino rats). It specifically targeted the effects the plant extract will pose on serum parameters (creatinine, BUN and uric acid) nitrituria, proteinuria, calcium oxalate excretion, and the histopathology of kidney tubules and glomeruli of albino rats. In the study, root of *E. indica* was collected, dried and extracted using distilled water of 80°C-100°C. The actual experimental period lasted for 4 weeks. The study involved 5 test groups; negative control group (T₋), positive control group or lithogenic group (T₊), two prophylactic groups (T₁ and T₂) receiving different concentrations of *E. indica* root extract (T₁- 500mg/kg and T₂- 800mg/kg) and one curative group receiving 800mg/kg of *E. indica* root extract after being induced with kidney stones during the first 2 weeks of the experimental period. All the test groups received 0.75% ethylene glycol (stone inducing chemical) in water for 4 week ad libitum with the exception of the negative control. At the end of the study, there was elevated levels of serum creatinine, BUN and uric acid among the lithogenic group which was normalized in all the three treatment groups. There was no significant difference between the serum parameters of the *E. indica* treatment groups and that of negative control ($P < 0.05$). There was the presence of nitrituria, proteinuria, high pH and high levels of calcium oxalate excretion in the lithogenic group. But again all these parameters were normalized in the three *E. indica* treatment groups. *E. indica* root extract also maintained the normal structure of kidney glomeruli and tubules among T₁ and T₂ (prophylactic groups) and repaired kidney defects in T₃ (curative group). *E. indica* root extract thus possess antiuro lithiatic potentials and can be used to prevent and cure nephrolithiasis in albino rats.

Keywords: Antiuro lithiatic, Ethylene Glycol, Nitrituria, Proteinuria, Nephrolithiasis

1. Introduction

Urinary calculi disease or kidney stones is a pathological condition (-iasis) in which stones (lith-) are formed in the kidneys (Nephro-); hence the name Nephrolithiasis. Many people around the globe suffer from this disease and there is an increasing prevalence of it through the twentieth century until today [1]. In the United States alone approximately 1 in every 11 individuals is affected with Nephrolithiasis [2]. Kidney problems is reported to be Philippines 7th leading cause of death and some studies show that about 2.3% of the total Filipino population has kidney stones [3]. This ancient disease has claimed the lives of many individuals especially

those in the Middle East and had a mortality rate of about 19,000 deaths per year between 1990 and 2010 [4]. About 85% of kidney stones are caused by accumulation of calcium oxalate or uric acid crystals in the kidney tubules [5].

The widespread of diseases such as Nephrolithiasis in our world today has gotten many researchers to probe into the good old traditional medicinal plants to find solutions since most of these fatal modern diseases were not dominant during the ages where medicinal plants were widely used [1]. More so the emergence of drug resistant organisms and increasing prices of medicines calls for the discovery of new

and less expensive plant-based medicines. The geological background and global position of the Philippines has made it one of the most bio-diversified countries in the world and as such an excellent center to pursue medicinal plant-based researches. This country is rich in many plants species including the *Eleusine indica* commonly known as goose or wire grass.

E. indica is a herb found in the family poaceae (grass family). It is an annual grass which bears 3-8 narrow spikes in its inflorescence with firm fibrous roots [6]. This small xerophyte is distributed mostly throughout the warmer areas of the world and it is found in all the habitable continents present in about 150 countries across the globe [7]. It is considered as one of the most invasive weeds and it is very difficult to control. However, in spite of all the odds about this grass, it is traditionally used as a medicinal plant to treat many diseases [8]. A review paper published by the institute of the Philippine medicinal plant in 2015 on *E. indica* shows that the entire plant especially the root is traditionally used to enhance diuresis [9]. In spite of the fact that this plant is traditionally known to have some potentials to enhance the discharge of urine, no vivid scientific study has been conducted on the potential it has on the aversion of kidney stones [10]; [11]. This boosted the interest of researchers to pursue this study. Since *E. indica* is easily accessible and has a wide distribution, a study of this sort will be advantageous to people from all around the globe. More so an affirmative results from the study will economically reduce the millions of money which is spent in controlling this weed since the plant will become an important asset to the world's health sector.

Ethylene Glycol is an antifreeze organic substance which has the potential to induce calcium oxalate stones when ingested in small amounts and therefore highly useful in kidney stones based researches among rats and other vertebrates [12].

This study establishes the antiurolithic potential of different concentrations of *E. indica* root extract on ethylene glycol induced nephrolithiasis in *Rattus norvegicus*.

2. Methods

2.1. Plant Acquisition Preparation and Extraction

Eleusine indica plants were collected from Indang-Cavite, Philippines within the months of November and December, 2016. The plant was identified and authenticated by a Botanist at De La Salle University-Dasmariñas. In collection, the plants were uprooted and the whole roots up till an inch of the stem were harvested on the spot. They were then washed thoroughly with water to remove all the soil particles and air dried at room temperature of 25°C (± 2) [13].

This dried roots were boiled with low heat for about 15 minutes after which the solvent was filtered out and the residue discarded [14]. The filtrate containing the desired extracts was then cooled, poured into a clean bottle and stored in a refrigerator. 1 mL of the cooled liquid sample was placed on a pre-weighed petri dish. The water in the sample

was evaporated and weighed again to determine the exact weight of extract per mL.

2.2. Acquisition of Albino Rats

Thirty (30) healthy male albino rats of *Rattus norvegicus* species which were 5-6 weeks old weighing between 200-250 grams and fed with standard dry pellets and water ad libitum prior to study was bought from the Food and Drugs Administration (FDA), Alabang - Muntinlupa City Philippines [14]. They were then grouped into five (5) test groups (T_+ , T_+ , T_1 , T_2 and T_3) with each group containing six (6) albino rats. As advised by veterinarian two albino rats were be put in a single highly ventilated standard rat cage having dimensions of 35.5 cm long, 25.4 cm wide and 12.7 cm high. The cages was then tagged with their test group labels (T_+ , T_+ , T_1 , T_2 , T_3) and arranged in a random blocking set up to ensure equitable distribution of other environmental factors [14]. The rats were then kept under standard condition (25°C \pm 2°C) in 12 hours dark and light cycles [15]. They had access to standard dry pellets and water ad libitum. They were kept in the farmhouse for 2 weeks before the start of the experimental period to ensure that they are well acclimatized to their new environment. An approval of a study protocol was sort from the Institutional Animal Ethics Committee according to the regulation of Committee for the Purpose of Control and Supervision of Experiments on Animals before the inception of all the experimental period. The experiment was conducted in accordance with accepted standard guidelines for the care and use of animals in scientific research [14].

2.3. Ethylene Glycol Induction and Treatments with Plant Extract

From the commencement of the experimental period, each of the groups were administered different supplies which were maintained throughout the entire experimental period. Albino rats in T_- (negative or vehicle control) was maintained on standard dry pellets and drinking water. All the other groups; T_+ , T_1 , T_2 and T_3 received calculi inducing treatment of 0.75% v/v ethylene glycol in drinking water ad libitum within the entire experimental period [14]. T_+ which served as the lithogenic group was not given any other treatment apart from the 0.75% v/v ethylene glycol in water. T_1 , T_2 and T_3 served as the treatment groups who received different concentration of *E. indica* roots extract. 500mg/kg and 800mg/kg of root extract were administered to albino rats in T_1 and T_2 respectively from 1st day to the 28th day of ethylene glycol induction and served as the prophylactic groups [16]. T_3 on the other hand served as the curative group and was be administered 800mg/kg of *E. indica* root extract at the beginning of week 3 (day 15) of the experimental period. The concentration of *E. indica* given to each of the groups did not exceed 3.45g/kg body weight, the LD₅₀ of *E. indica* in albino rats [8]. The entire treatment period lasted for 4 weeks.

2.4. Data Gathering and Statistical Analysis

At the end of the experimental period, all animals were kept

in individual metabolic cages, and a urine and blood samples were collected from some randomly selected rats in each group [16]. The blood and urine were then taken to the diagnostic laboratory for Serum analysis and urinalysis. The urine volume, pH, general urinalysis parameters, calcium oxalate crystals and nitrites levels were determined from the urine samples. Serum creatinine, Blood Urea Nitrogen (BUN) and uric acid levels were determined in serum analysis [14]. Blood was collected from the jugular vein near the heart of rat under light ether anesthesia. The serum in the blood was then be separated by centrifugation at $10,000 \times g$ for 10 min and analyzed for the creatinine, uric acid, urea, and blood urea nitrogen (BUN) at the diagnostic laboratory (Hi-precision diagnostic laboratory, Dasmariñas-Cavite, Philippines).

In addition to the collection of the urine and blood, one (1) randomly selected rat from each group was killed through the gas chamber. An incision was made through the abdominal cavity and the kidneys were then removed. The kidneys were stored in 37% formaldehyde and taken to the diagnostic laboratory (Hi-precision diagnostic laboratory, Dasmariñas-Cavite) for histopathological analysis of the kidney. A longitudinal section of the kidneys were obtained, stained

and observed under the microscope. The degree of damage to the glomerulus and other kidney tubules were used as the baseline for establishing the effect of *E. indica* extract on kidney stones.

All data was expressed as mean \pm SD. Differences and was statistically analyzed using one way analysis of variance (ANOVA) *t* to find out the statistical significant difference of the result. [17].

3. Results

Serum Analysis

Table 1. Average Serum Creatinine Levels for each Group Expressed mg/dL, Their Standard Deviation from the Mean, and Significant Differences.

Test Groups	Average (mg/dL)	\pm SD
T ₋	0.26	$\pm 0.001^x$
T ₊	0.37	$\pm 0.013^y$
T ₁	0.22	$\pm 0.008^x$
T ₂	0.25	$\pm 0.014^x$
T ₃	0.25	$\pm 0.018^x$

Legend: Different superscript denotes significant difference. Same superscript denotes no significant difference

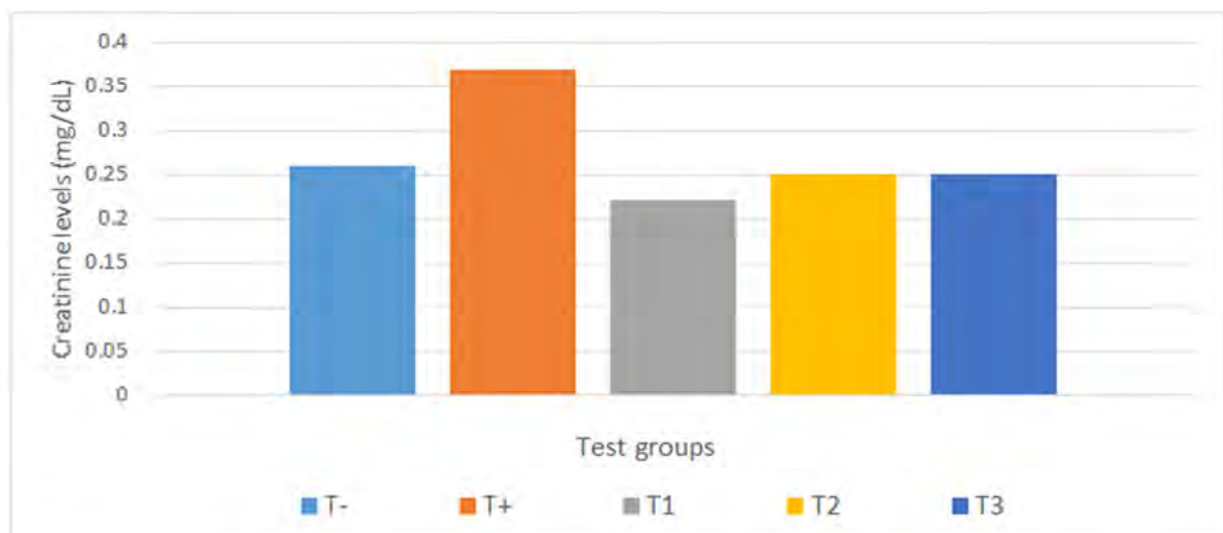


Figure 1. Effects of *E. Indica* on the Serum Creatinine Levels of Albino Rats.

Figure 1 shows a bar chart of the serum creatinine levels of the various test groups used in the study. From the figure it can be observed that, there is a higher level of serum creatinine among the T₊ (Positive control or lithogenic group). However these creatinine levels are normalized among the various *E. indica* treatment groups. The serum creatinine of the various treatment groups as seen from the figure is almost at the same level like that of the negative control group.

Table 2. Average Blood Urea Nitrogen (BUN) Levels of Various Test Groups.

Test Groups	Average (mg/dL)	\pm SD
T ₋	12.32	$\pm 0.65^x$
T ₊	23.44	$\pm 0.49^y$
T ₁	11.14	$\pm 0.15^x$
T ₂	16.13	$\pm 0.19^x$
T ₃	14.34	$\pm 0.19^x$

Legend: Different superscript denotes significant difference
Same superscript denotes no significant difference

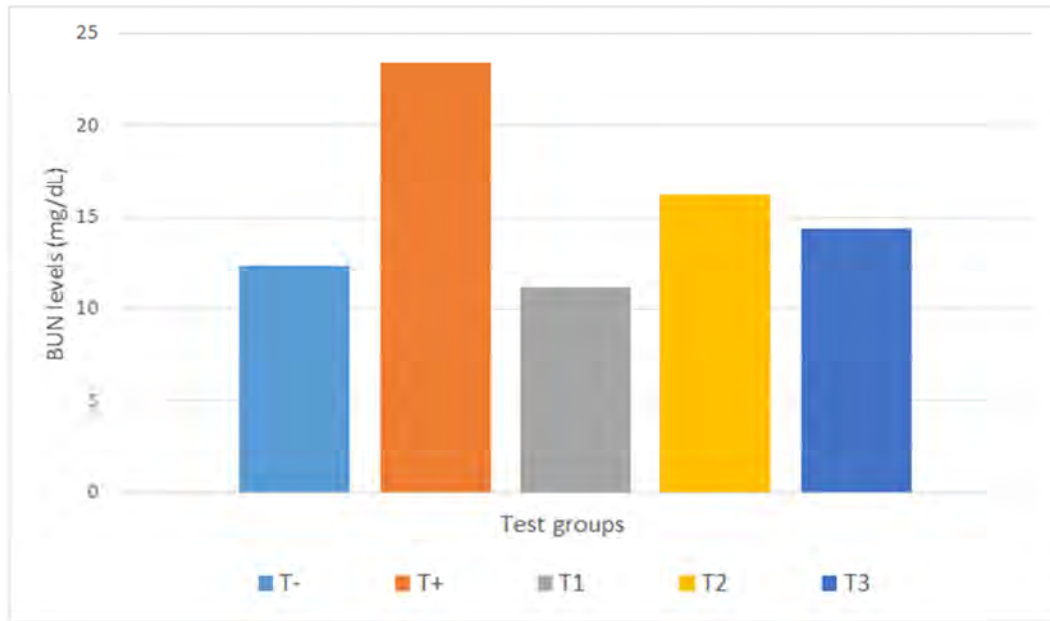


Figure 2. Effects of *E. indica* Root Extract on Blood Urea Nitrogen Levels of Albino Rats.

Figure 2 is a bar chart showing the levels of Blood Urea Nitrogen (BUN) among the various test group. From the chart is can be clearly seen that the BUN levels of T₊ (lithogenic group) is highly elevated as compared to the other groups. The BUN levels of the various *E. indica* treatment groups (T₁, T₂, T₃) is almost at par with the BUN levels of the negative control group.

Table 3. Uric Acid Levels of Various test Groups.

Test Groups	Average (mg/dL)	± SD
T ₋	0.99	± 0.011 ^x
T ₊	2.83	± 0.014 ^y
T ₁	0.82	± 0.010 ^x
T ₂	0.83	+ 0.010 ^x
T ₃	0.99	+ 0.019 ^x

Legend: Different superscript denotes significant difference.
Same superscript denotes no significant difference.

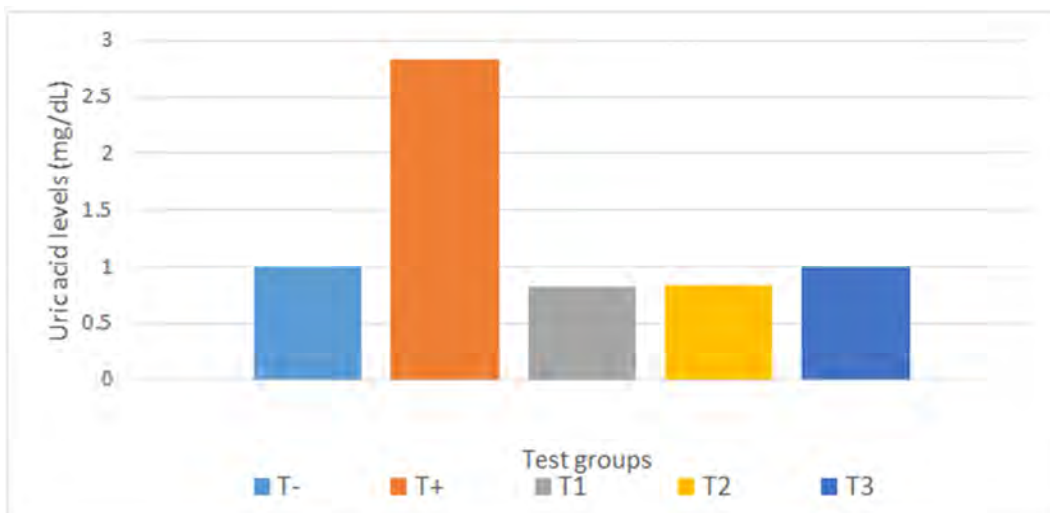


Figure 3. Effects of *E. Indica* Root Extract on Serum Uric acid Levels of Albino Rats.

Figure 3 is a bar chart showing the serum uric acid levels among various test groups. From the figure, it can be seen that the uric acid levels in the lithogenic group is highly elevated. The uric acid levels of the various *E. indica*

treatment groups and that of the negative control group is at par. The levels of uric acid is lowered among the *E. indica* treatment groups (T₁, T₂, T₃).

Table 4. Urine pH, Proteins, Nitrites and Calcium Oxalate Crystals Observed in the Urine of Rats.

Test Groups	pH	Protein Excretion	Nitrate Excretion	CaOX crystals
T ₋	8.0	+	Negative	Few
T ₊	9.0	++++	Positive	Many
T ₁	8.0	+	Negative	Few
T ₂	8.0	+	Negative	Few
T ₃	8.0	+	Negative	Few

Reference values: pH (normal; 6.00-8.00), proteins(normal; +,++), nitrite (normal; negative) CaOX (normal; few).

Urinalysis

From Table 4, it can be seen that all the test groups with

the exception of T₊ has a pH of 8.0. T₊ on the other hand has a higher urine pH of 9.0. urine proteins found in all test groups were found to be minimal represented by (+) while that of the lithogenic group, T₊ was found to be very high represented by (++++). All test groups tested negative for excretion of nitrate with the exception of T₊ which tested negative. More so many calcium oxalate (CaOX) crystals were found in the urine of the lithogenic group (T₊) while all other groups had just few CaOX crystals.

Histopathology

The pictures of H&E stained kidney section of the test groups are shown below.

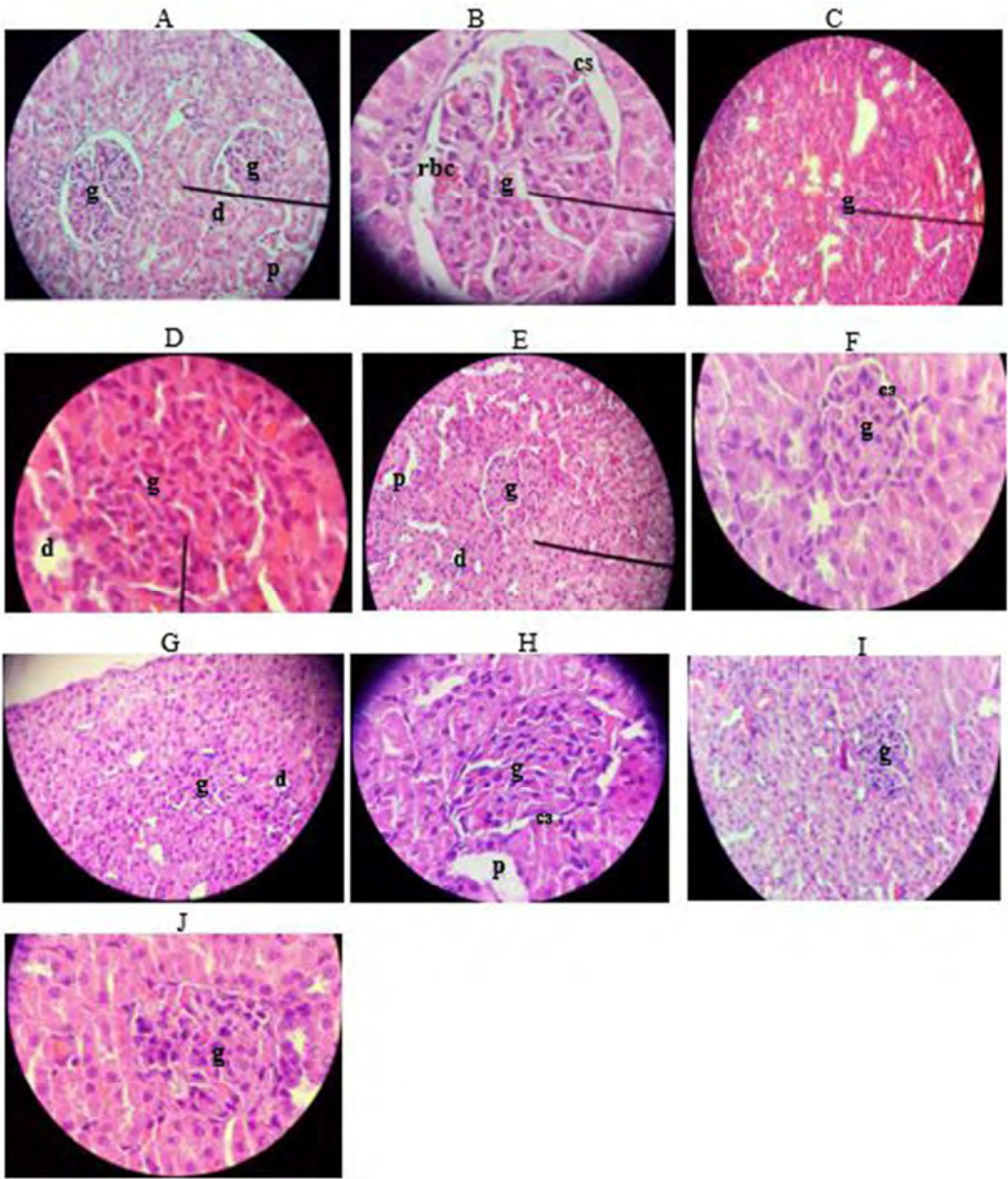


Figure 4. Histopathology of H&E Stained Kidney Sections of the Various Test Groups.

Labels A-B (negative control or T₋), C-D (positive control or T₋), E-F (prophylactic group 1 or T₁), G-H (prophylactic group 2 or T₂), I-J (curative group or T₃)
Legend: “g” represents glomerulus, “p” represents proximal convoluted tubules, “d” represents distal convoluted tubules, “cs” represents capsular space, “rbc” represents red blood cells.

4. Discussion

Nephrolithiasis is associated with the formation of stones in the kidneys. Ethylene Glycol used in the study leads to the creation of calcium oxalate stones in kidney tubules. These stones obstruct the kidney tubules and destroy the glomerulus of the nephrons. Glomerular filtration rate decreases due to the obstruction to the flow of urine. As a result, waste products from the blood which should be filtered out in the kidneys remain and accumulate in the blood. Prominent among these waste products that are retained in the blood include nitrogenous wastes such as creatinine, BUN, and uric acid [18].

Creatinine is a major metabolic waste produced from muscle metabolism. The kidneys maintain the blood creatinine in a normal range by filtering it out of the body. As such when there are kidney dysfunctions, especially with ultra-filtration, creatinine levels will be elevated in the blood. Creatinine has therefore been found to be a fairly reliable indicator of kidney function. A rise in serum creatinine level is a late marker, observed only with marked damage to functioning nephrons and as such signifies impaired kidney function or kidney disease [19]. The higher levels of creatinine seen among the T_+ shows that the kidney function had been highly compromised. This problem was corrected by *E. indica* root extract in T_1 , T_2 , and T_3 . The lowered levels of serum creatinine indicated that normal kidney function has been restored. It can be deduced from Table 1 that there was no significant difference between the creatinine levels of the treatment groups and that of the negative control group. That is to say that *E. indica* was able to maintain the normal levels of serum creatinine among the treatment groups. It prevented the ethylene glycol from inducing its kidney destructive effects on the kidneys of rats by preventing the formation of stones in the kidney tubules. The normal levels of serum creatinine as seen in all the treatment groups is a clear indicator of the antiurolithiatic effect of the plant extract.

Blood Urea Nitrogen is also another indicator for determining kidney function among mammals. Blood urea nitrogen (BUN) test measures the amount of nitrogen in the blood that comes from the waste product urea. One of the main functions of the kidney is to filter out nitrogenous waste which comes from proteins. Since proteins cannot be stored in the body, excess proteins undergo deamination in the liver to be converted into urea which is then excreted out of the body [20]. When there is a kidney disease like nephrolithiasis which lead to kidney injury, this process is impeded and these nitrogenous wastes are retained in the blood in high amounts. As such high BUN values signifies kidney dysfunction and is one ways to diagnose the nephrolithiasis [21]. The normal values of BUN in 6 - 10 week old albino rats is 10-20 mg/dL [22]. In the study it can be seen that the negative control and all the treatment groups had their BUN levels within this range showing normal kidney function. Thus there was no significant difference between the BUN levels of the negative control and the treatment groups ($P <$

0.05). The lithogenic group on the other hand had a really high levels of BUN. *E. indica* root extract was able to reduce and maintain the BUN levels among the treatment groups (T_1 , T_2 , and T_3) suggesting preventive function and curative function of *E. indica* root extract. Other studies which looks at the phytochemicals of *E. indica* suggest that the plant contains enormous amount of tannins [23]. Tannins have been found decrease blood urea nitrogen content [24]. The normalized BUN levels seen among the treatment groups may therefore be due to the presence of tannins in the plant.

Uric acid is another parameter that is used to detect kidney function. Uric acid is naturally produced in the body out of breakdown of purine containing dietary substances. But since uric acid is a metabolic waste it is required to be excreted out of the body. This work of excretion is done by the kidneys. When ethylene glycol induced in albino rats forms calcium oxalate stones in the kidneys, glomerular filtration is highly affected and as such uric acid excretion is compromised. This leads to high levels of uric acid in serum as seen in the positive control (T_+). Serum uric acid is commonly elevated in subjects with chronic kidney disease (CKD). Uric acid also have the tendency to form crystals in kidneys leading to nephrolithiasis. Raising the uric acid level in rats was found to induce glomerular hypertension and renal disease as noted by the development glomerular injury [25]. In line with this, the lowered serum uric acid levels in *E. indica* root extract treatment groups (T_1 , T_2 and T_3) suggests normal kidney function and aversion of nephrolithiasis. Thus there was no significant difference between the uric acid levels of the treatment groups and the negative control (T_-).

Urinalysis parameters can also be used to determine the presence of kidney stones. Urine pH is an important parameter in diagnosing the presence or absence of kidney stones. The formation of various types of kidney stones is strongly influenced by urinary pH. A highly alkaline pH favours the crystallization of calcium and phosphate containing stones, whereas and acidic urine pH promotes uric acid or cystine stones. [26] [27]. The high alkaline Urine pH of the positive control group suggests the possible presence of calcium oxalate stones which is induced by ethylene glycol. The alkaline levels are reduced among the treatment groups as that in the control suggesting the possible prevention or reduction stones.

Proteinuria is one indicator of kidney damages among kidney stone victims. It refers to the condition where there are enormous amount of proteins in the urine. The healthy kidney do not allow the proteins to pass out; thus prevents protein filtration. However when kidney function is compromised due to the presence of kidney diseases like nephrolithiasis, the glomerular filters allow proteins like albumin to leak out from the blood to the urine. Proteinuria may accelerate kidney disease progression to end-stage renal failure [28]. The results of this study showed a high levels of proteins present in the urine of the positive control group (lithogenic group) which is not present in the treatment groups. The aversion of proteinuria in the treatment groups

serves as major indicator that *E. indica* root extract does have preventive and curative properties against nephrolithiasis.

Under normal circumstances, urine is a sterile substance, so the presence of any anomalous components is a potential sign of infection. A positive test for nitrites in the urine is called nitrituria. The purpose of nitrite test in urine is to detect whether bacteria are present, as they convert nitrate to nitrite in urine [29]. Nephrolithiasis has the possibility of increasing the developing of urinary tract infection due to the destruction of the mucosal walls of the urinary organs making it easy for other bacteria present to proliferate and cause urinary tract infection. It can be seen from the table that both the negative control group and the treatment groups tested negative for nitrite. The lithogenic group which tested positive for nitrite is a clear indicator of a possible urinary tract infection. This problem is not seen in the *E. indica* treatment groups which suggest the absence of urinary tract infection [30] [31].

The presence of Calcium Oxalate crystals in urine is another trademark indicator to determine the presence of nephrolithiasis. Calcium oxalate crystals in the urine are the most common constituent of human kidney stones. Kidney stones form when the urine contains more crystal-forming substance such as calcium and oxalate than the fluid in your urine can dilute [32]. Naturally, urine contains calcium oxalate crystals but the presence of numerous amount of these crystals suggest the possible presence of stones in the kidneys. The ability of *E. indica* root extract to reduce the number of calcium oxalate crystals among treatment groups shows the antiurolithiatic property of this plant.

Histopathology is one of the ideal ways to detect the presence of nephrolithiasis and its effects on the kidney. In histopathology, what actually happens in the kidney tubules is clearly observed and understood. The glomerulus is a tuft of small blood vessels called capillaries located within Bowman's capsule within the kidney. This tuft of capillaries is supported by a matrix of collagen and other components that are called mesangium. In addition, the tuft is surrounded by a layer of flat cells that forms something like a tapestry that envelope it; the visceral epithelial cells or podocytes. The network of capillaries, the mesangium, and the podocytes form the glomerular tuft. Glomerular capsule is made up of outer basement membrane and inner epithelium. Between glomerular capsule and visceral epithelial cell layer is capsular space. Abnormalities can be in basement membrane, epithelium, and capsular space [32]. A normal kidney histopathology will have a well-defined capsular space, basement membrane and the red blood cells will be mostly observed in the glomerulus, with few spread out at some points among the tubules. More so, Haematoxylin and Eosin (H & E) staining for normal kidney yields the cytoplasm of the cuboidal cells of distal and proximal convoluted tubules as pale pink while the nucleus is stained purple with distinct and visible nucleolus. The nuclei of the various cells are not stuck together and each cell is seen as separated from the other cell.

The kidney tubule cells of the lithogenic group (T₊) in this

study was found darkly stained as compared to the negative control and treatment groups. The entire nucleus of the lithogenic group appeared darkly stained purple and the nucleolus was not clearly distinct and visible (figure 4). In nephrolithiasis, the formation of CaOX stones reacts with certain stone forming proteins and creates a complex that reacts with the kidney tubules [34]. These complexes (crystals) attaches themselves unto apical microvilli of surrounding renal cells leading to inflammations. Some cells of the tubules transport these complexes through endocytosis in order to digest them. If they are unable to dissolve these crystals, cells start to undergo changes in some important parts such as cytoskeleton and microfilament. The inflammations created by crystal interaction with cells and proliferation of these microfilaments in the cells make the cytoplasm appear darkly stained. Inflammations also creates adhesions which when leads to membranous of renal proximal tubular cells and ultimately cell apoptosis [34]. Apoptotic cells undergo cell shrinkage, condensation and deep eosinophilia of the cytoplasm and pyknosis; irreversible condensation of chromatin in the nucleus [35] [36]. This is what made the cells in the lithogenic group appear with darkly stained cytoplasm and deep purplish nucleus. All these symptoms are not seen in all the three *E. indica* root extract treatment groups (T₁, T₂, T₃). The cytoplasm of the kidney tubules of the groups were pale pink, the nucleolus were distinct and visible as that of the negative control. All these evidences clearly shows the antiurolithic property of the plant.

From the histopathology sections (refer to figure 4), it could be clearly seen that the glomerular capsule of the lithogenic group which were affected with nephrolithiasis were deformed. The bowman's space (urinary space) was not clearly defined. The squamous epithelial and basement membrane of the Bowman's capsule were also deformed. The nucleus of the podocytes was overlapping and there was a lot of red blood cells seen outside the glomerulus. In the normal kidney, Red blood cells are too large to pass through the small holes in the glomerular capillaries. Thus the kidney filtrate do not contain red blood cells [37]. If they are found to proliferate outside the glomerulus, it suggest that the capillaries has been enlarged and the ultrafiltration process has been compromised. Crystals formed in nephrolithiasis cause expansions of the capillaries and lesions in the glomerulus which leads to glomerulonephritis. This condition in turn leads to the seeping out of RBC in to the capsular space and kidney tubules which leads to haematuria [38]. This is greatly observed in the H&E sections of the lithogenic group (refer to 'C' and 'D' from figure 4). However, the situation is averted in the *E. indica* root extract treatment groups. All the three treatment groups showed normal structure of the glomerulus. The capsular space, single squamous epithelial cells and basement membranes of the bowman's capsule could be observed as intact in the treatment groups like that of the negative control group. More so, no red blood cells were found within the tubules and capsular space of T₁ and T₂ (the prophylactic groups)

which proves that *E. indica* root extract is an excellent preventive treatment for nephrolithiasis and as such a good nephron-protective plant. Few red blood cells were found in the capsular space T₃ (curative group) which suggested that the kidneys were still in the process of healing (refer to 'I' and 'J' from figure 4).

Studies which looked into the phytochemicals present in *E. indica* shows that the plant is rich in flavonoids [23]. Flavonoids has also been found to prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation) which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones [34]. More so, the presence of high levels of flavonoids in a plant makes it possible for it to have preventive properties on the development of nephrolithiasis and the prevention of renal tubular inflammations caused by this pathologic condition. [39] [40]. Therefore, flavonoids present in *E. indica* may therefore be a possible reason for its ability to prevent inflammations in the kidney tubules and to prevent nephrolithiasis among albino rats.

From all the results explained above, it is clearly seen that *E. indica* root extract exhibit a great effect on Serum creatinine, Blood Urea Nitrogen (BUN) and uric acid levels of albino rats. The plant extract reduces these nitrogenous waste substances in the blood to normal levels in all the three *E. indica* treatment groups (T₁, T₂, T₃) as that observed in the negative control group. The presence of normal levels of these parameters suggests the preventive and curative properties of the plant extract against nephrolithiasis. The root extract also exhibit effects on the excretion of urinary nitrite, proteins and calcium oxalate crystals of albino rats. It prevents the formation of urinary nitrites, a major indicator of urinary infections among all treatment groups. The plant extract also prevents proteinuria (the presence of high levels of proteins in urine) among all *E. indica* treatment groups which suggest normal glomerular filtration. More so the levels of calcium oxalate crystals seen in the urine of treatment groups are just few as compared to the lithogenic group. All these suggest the absence of nephrolithiasis among treatment groups supporting the fact that *E. indica* root extract indeed has antiurolithic properties and as such can be used to prevent and cure nephrolithiasis. *E. indica* root extract also exhibit effects on the structure of the glomerulus and kidney tubules of albino rats. The plant extract prevents the disruption of normal structure of the glomerulus and tubules of rat's kidney among the prophylactic groups (T₁, T₂). It also shows restoration of already disrupted glomeruli and kidney tubules back to the normal structure among curative treatment group (T₃). The plant extract maintains the normal structure of glomerular capillaries, podocytes, mesangium, basement membrane, capsular space, and the entire Bowman's capsule among all the treatment groups. Presence of red blood cells in capsular space and kidney tubules which can lead to hematuria are totally prevented among the prophylactic groups (T₁, T₂) and highly reduced in curative group (T₃). Cell lesions, membranous and

apoptosis which are highly present among lithogenic group was prevented and corrected in all *E. indica* treatment groups. All these are clear indications of the antiurolithic property of *E. indica* root extract.

In spite of the fact that, all concentration of *E. indica* root extract used were able to maintain these parameters within the normal range, the concentration used for T₁ (500mg/ml) showed the best results. This concentration reduced serum creatinine, BUN and uric acid drastically to the best levels. It also prevented the excretion of urinary nitrite, proteins calcium oxalate crystal. More so, it fully maintained the normal structure of the kidney tubules and glomerulus like that observed in the negative control group.

5. Conclusion

E. indica root extract exhibit a great effect on Serum creatinine, Blood Urea Nitrogen (BUN) and uric acid levels of albino rats as there was no significant difference between negative control and treatment groups (P value < 0.05). *E. indica* root extract also exhibit effects on the excretion of urinary nitrite, proteins and calcium oxalate crystals of albino rats. It prevents nitrituria, proteinuria and oxaluria among treatment groups. The formation of urinary nitrites, a major indicator of urinary infections among all treatment groups. *E. indica* root extract also exhibit effects on the structure of the glomerulus and kidney tubules of albino rats. The plant extract prevents the disruption of normal structure of the glomerulus and tubules of rat's kidney among the prophylactic groups (T₁, T₂). It also shows restoration of already disrupted glomeruli and kidney tubules back to the normal structure among curative treatment group (T₃). There is a slight difference in the effects that different concentration of *E. indica* root extract posed on serum, urine and histopathological parameters utilized in the study. In spite of the fact that, all concentration of *E. indica* root extract used were able to maintain these parameters within the normal range, the concentration used for T₁ (500mg/ml) showed the best results as it reduced the concentration serum creatinine, BUN and uric acid drastically to the best levels and prevented nitrituria, proteinuria and oxaluria among treatment groups.

All the above, clearly proves that *E. indica* root extract has antiurolithic effects against ethylene glycol induced nephrolithiasis in *Rattus norvegicus* (albino rat).

Recommendations

To further improve the study the researchers recommend;

1. To replicate the same study should make use of many curative study groups and should extend the time of administration of treatment to the curative group.
2. To conduct further study to determine the exact phytochemicals responsible for the antiurolithic property of *E. indica* root extract.
3. To replicate the study among higher mammals like guinea pigs and rabbits to determine if the plant extract will

pose the same antiurolithic effects on nephrolithiasis induced in these mammals.

4. To look into the effects different part *E. indica* have on the aversion of nephrolithiasis.

5. To conduct further on the same topic making use of a different extraction solvent like ethanol or methanol.

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