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Pharmacognostical, phytochemical and Antiurolithiatic Activity of ethanolic extract Amoora Cucullata Roxb leaves.

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ABSTRACT

In ayurvedic system of medicine a massive number of medicinal plants are stated to have with antiurolithic activity. The present investigation is concerned with the widely distributed indigenous medicinal plant *Amoora cucullata*, most well known in traditional medicine practice.Urinary lithiasis is generally the results of an imbalance between inhibitors and promoters in the kidneys. **To s**tudy the antiurolithic activity of ethanolic extract of leaves of *Amoora Cucullata Roxb* (ACR) in chemicals induced urolithiasis in Wistar rats.The ethanolic extract of Amoora cucullata subjected to column chromatography for the separation of compounds. The ethanolic extract yielded the compound in the fraction chloroform : acetone (80:20).Treatment with the Ethanolic extracts of *Amoora cucullata* restored the phosphate level, thus reducing the risk of stone formation.

Keywords: *Amoora Cucullata Roxb* (ACR), antiurolithic activity, Ethanolic

INTRODUCTION

Ayurveda, a natural system of medicine, originated in India more than 3,000 years ago. The term Ayurveda is derived from the Sanskrit words ayur (life) and veda (science or knowledge). Thus, Ayurveda translates to knowledge of life. Based on the idea that disease is due to an imbalance or stress in a person's consciousness, Ayurveda encourages certain lifestyle interventions and natural therapies to regain a balance between the body, mind, spirit and environment.[(1)] Nephrolithiasis or renal stone disease remains a significant health problem in the adult population, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate.[(2)]The present-day medical management of nephrolithiasis is either costly or not without side-effects. Invasive procedures for the treatment of nephrolithiasis may cause serious complications and also impose a great load of costs on the healthcare system.

Nephrolithiasis are primarily wedged in the kidneys.[(3)] Human beings are affected by nephrolithiasis since centuries dating back to 4000 B.C. and is the most common disease of the urinary tract by the recent days. The prevention of Nephrolithiasis recurrence remains to be a serious problem in health of the human life.[(4)] The counteraction of stone repeat requires better comprehension of the components associated with stone development.[(5)] Nephrolithiasis have been related with an expanded danger of persistent kidney sicknesses[(6)], end-stage renal disappointment[(7-8)], cardiovascular infections[(8-9)], diabetes, and hypertension[(10)]. It has been proposed that kidney stone might be a fundamental problem connected to the metabolic condition. Nephrolithiasis is liable for 2 to 3% of end-stage renal cases in the event that it is related with nephrocalcinosis[(11)].

MATERIALS AND METHODS

Pharmacognostical studies

The fresh plant material was collected from Karnataka in the month of December 2016. The plant material was taxonomically authentified by Dr. Jayaraman, Botanist, Chennai, Tamil Nadu. About 500gm of the air-dried powdered plant material leaves was extracted successively with solvents of alcohol using Soxhlet extractor.The extract was concentrated in a rotary evaporator at reduced pressure.

The collected plant was taken under care to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin- 5ml + Acetic acid- 5 ml +70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per the schedule. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 – 60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [12,13].

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure[12]. The sections were stained with Toluidine blue[13]. Since toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections were also stained with safranin and Fast – green and IKI for starch.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared[14]. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell components were studied and measured[15].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit[16].

Microscopic Feature of the leaflets

The leaf has thick midrib and smooth and even lamina. The midrib has wide and short conical adaxial part with gradually sloping lateral sides: the abaxial part in broad and semicircular (Fig No. 9 & 10).It is 1.3 mm in vertical plane and 1.4 mm horizontally.

The midrib has prominent cuticle and thin epidermal layer of small narrow cells. The ground tissue consists of small circular compact parenchyma cells.

The vascular system is large and solitary. It consists of abaxial wide bowl-shaped outline with laterally expanded wings and adaxial flat, lid-like vascular plate. Both the abaxial bowl and adaxial plate are collateral having the phloem on the outer part of the xylem. Both the adaxial plate and the abaxial are of xylem have short, parallel lines of narrow circular thin walled vessels and thick walled dense fibres.

Phloem consists of dense, narrow straight lines of phloem elements. Along the outer zones of the adaxial and abaxial vascular segments occur thick bands of sclcrenchyma cells. The cells arc narrow thick walled and lignified. The adaxial vascular segment is 200µm thick and 950µm wide. The abaxial are is 150µm wide.

Secretory cavities occupy the major portion of the central zone the midrib. The cavities are angular in outline measuring upto 90µm in diameter. The cavities have elliptical epithelial cells all along the circumference.

Crystals

Calcium oxalate crystals are abundant in the midrib. Prismatic types of crystals are seen around the secretory cavities in the central core of the ground tissue. Smaller prismatic as well as druse types of crystals are densely distributed in the phloem tissue.

Lamina is smooth surfaced with less distinct differentiation of the mesophyll tissues. It is 180µm thick. The epidermal cells are square shaped and have thick, smooth cuticle. The mesophyll consists of vertically elongated dense palisade cells both on the adaxial and abaxial sides.The adaxial zone of palisade tissue consists of three layers of cells and the zone is 60µm in height. The abaxial palisade is two layered 30µm in height. In the median portion of the lamina occur vertical filaments comprising of five or six spherical cells. The vertical partition filaments form wide air chambers in between Dilated circular cells with prismatic crystals and wide secretory cavities are frequent in the mesophyll tissue.

Leaf Margin

The marginal pat of the leaf is bunt and semi circular. It has been more prominent cuticle and radially oblong rectangular epidermal cells. The mesophyll has compact parenchyma cells. Circular, Small vascular bundle seen in the marginal part.

Powder Microscopy

The powder of the leaf, when examined under the microscope shows the following elements

1. Peltate Scales: Thin plate of several, elongated narrow cells united laterally forming star like scales are very frequent in the powder. The scale is attached to the epidermis of the leaf by means of a short a stalk cell. The cells of the peltate scale are dead cells; their walls are thick and lignified walls. The scale is 190 pm in diameter.
2. Long, narrow worm like fibres are seen in the powder (Fig.18). The fibers have thick lignified walls and narrow lumen. The fibres are 900µm to 1 mm long and 10µm thick.
3. Square shaped sclerenchyma cells are very common in the powder. The cells have thick lignified walls wide, empty lumen. The cells are 40 x 40 µm in size..
4. Prismatic calcium oxalate crystals are abundant in the leaf fragments. They are of various shape and size. Some of them are cuboidal, some are rhomboidal and others are double pyramids. The size of the crystals ranges from 10-12pm.

Phytochemical analysis

The extract was screened for various constituents (alkaloids, saponins, tannins, anthraquinones, sterol, flavonoids,

terpenoids, glycosides, simple sugars) using standard procedure.

Animal Used for the Study

The Adult Male wistrar rats weighing between (180-200 g) were used to calculate LD50. They were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). The animals were fed daily with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions.

Acute toxicity study

Experimental groups of Ratswas treated orally (p. o.) with aqueous extract of *AmooraCucullata*leaves at doses of (2000 mg/kg), control group of animals received normal saline by the same routes. Diazepam (2 mg/kg) was administered orally. All drugs were freshly prepared before each experiment. The doses of extracts were calculated to administer 1 ml of the suspension of extracts to the Rats of 100 g. The procedure was followed as per OECD 423 guidelines (OECD/OCDE. 2002). The extract was administered orally at a dose of 2000 mg/kg body weight. Rats were kept under observed for 14 days to register possible mortality.

Ethylene Glycol (EG) Induced Urolithiasis Model Antiurolithiatic Activity Study

Chemicals

All the chemicals and reagents were purchased from Merck, Mumbai, India. Solvents and all the reagents used were of analytical grade. The creatinine kit and uric acid diagnostic kits were used to estimate serum creatinine and uric acid level.

Ethylene glycol induced urolithiasis model

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

Treatment protocol

The grouped animals received the treatment as follows
Group I – Received normal diet and served as controls.
Group II - *Lithiatic control:* The animals were given normal diet and 1% Ethylene glycol in drinking water, for 28 days.
Group III - Received 1% ethylene glycol in drinking water and then treated with standard drug cyston 750 mg/kg orally, for 28 days.
Group IV - Received 1% Ethylene glycol in drinking water and then treated with Aqueous extracts of SM(*Stellaria media*) at a dose of 250mg/kg orally, for 28 days.
Group III - Received 1% ethylene glycol in drinking water and then treated with Ethanolic extract of SM at a dose of 500mg/kg orally, for 28 days.

Collection and Analysis of Urine

All animals were kept in metallic cage separately, and urine samples of 24 h were collected on the 28th day, and a drop of concentrated hydrochloric acid was added to the urine sample before being stored at 4°C. Animals had free admittance to drinking water during the urine collection period([17-19]). The collected urine sample was analyzed for urine volume, calcium, oxalate, phosphate, total protein, blood urea nitrogen (BUN), uric acid, and creatinine content using commercially available kit

Histopathological Studies

At the end of the experiment, few rats were sacrificed by euthanasia and remove the kidney then fixed in 10% formalin and tissue were then embedded in paraffin blocks for preparing

STATSTICAL ANALYSIS

All values were expressed as mean ± SEM, and data was analyzed by one way analysis of variance (ANOVA) followed by Newman-Keuls multiple range tests using Graph Pad In Stat and $p < 0.05$ was considered noteworthy.

Acute toxicity

Plant a dose of 5000 mg/kg had no adverse effect on the behavioral responses of the tested Rats up to 14 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane, behavior patterns, tremors, salivation and diarrhea of the Rats. There was no mortality observed and recorded weight loss is normal. Based on the above observation fix the doses 250 and 500 mg/kg for anxiolytic activity

Urinary excretion of calcium and phosphorous

In the present study, chronic induction of EG (0.75% v/v) to male Wistar rats resulted in significant ($P<0.001$) increase in urinary excretion of calcium and phosphorous. Whereas the cystone-treated group III animals were shown significant reduction in calcium ($P<0.0001$) and phosphate ($P<0.001$) levels. Similarly treatment with plant extract significantly lowered the elevated levels of calcium ($P<0.0001$) and phosphate ($P<0.0001$) in 250 mg/ kg and 500 mg/kg as compared to EG induced group II animals (Table 1& Fig 1). The plant extract 250 mg/ kg treated group of animals calcium and phosphorous levels were constantly less when compared to 400mg/ kg treated group of animals.

Table No.1. Effect ethylene glycol induced urolithiasis on the changes between calcium and phosphorous

S. No	Treatment	Dose (mg/kg)	Calcium level in urine (mg/dl)	Phosphorous level in urine (mg/dl)
1	Normal control	5ml/kg	9.15±0.16	2.02±0.02

2	Control	5ml/kg	21.5±1.77	6.4±0.08
3	Positive control	750	12.6±0.18	3.4±0.01
4	Plant extract	250	19.5±0.16	5.7±0.07
5	Plant extract	500	12.6±0.173	3.45±0.01

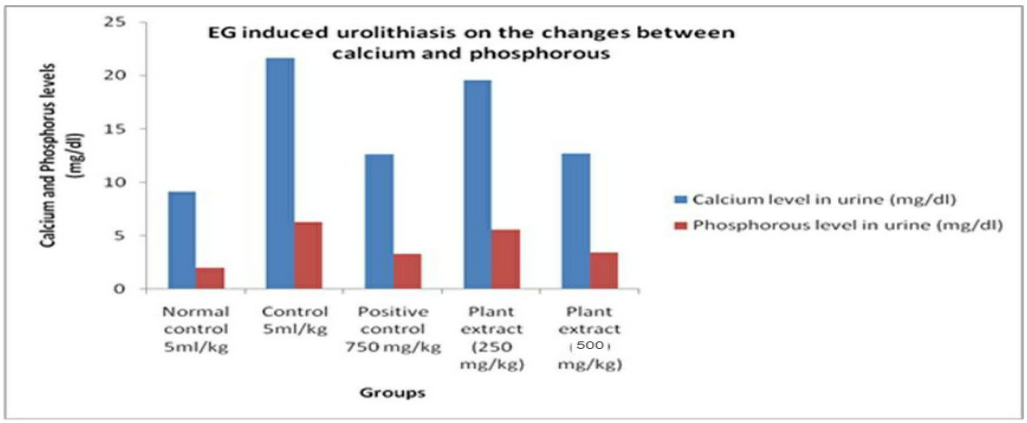

Figure No.1. EG induced urolithiasis on the changes between calcium and phosphorous

Blood Urea Nitrogen

The blood urea nitrogen (BUN), levels was significantly (P<0.0001) increased in EG-induced group II animals. While the BUN levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the BUN levels was significantly (P<0.0001) decreased in both 250mg/ kg and 500 mg/kg groups. The plant extract 200 mg/ kg treated group of animals BUN level was constantly less when compared to 400mg/ kg treated group of animal.

Table No.2. Effect ethylene glycol induced urolithiasis on blood urea nitrogen

S. No	Treatment	Dose (mg/kg)	Blood urea nitrogen (mg/dl)
1	Normal control	5ml/kg	18.7±0.16
2	Control	5ml/kg	42.6±3.6
3	Positive control	750	22.64±3.6
4	Plant extract	250	38.66±4.0
5	Plant extract	500	23.79±0.26

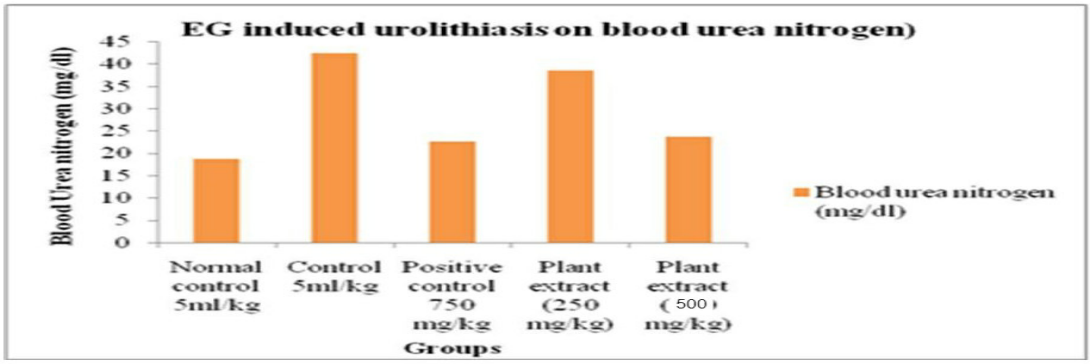

Figure No.2. EG induced urolithiasis on Blood Urea Nitrogen

Serum creatinine

On collected and isolated Serum creatinine, levels were significantly (P<0.0001) increased in EG-induced group II animals. While the serum creatinine levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the serum creatinine levels was significantly (P<0.0001) decreased in both 250mg/ kg and 500 mg/kg groups. The *Strellaria media* extract 200 mg/ kg treated group of animals serum creatinine level was constantly less when compared to 400mg/ kg treated group of animals.

Table No.3. Effect ethylene glycol induced urolithiasis on serum creatinine

S. No	Treatment	Dose (mg/kg)	Serum creatinine (mg/dl)
1	Normal control	5ml/kg	2.3±0.06

2	Control	5ml/kg	6.6±0.09
3	Positive control	750	3.2±004
4	Plant extract	250	4.6±0.05
5	Plant extract	500	2.2±0.03

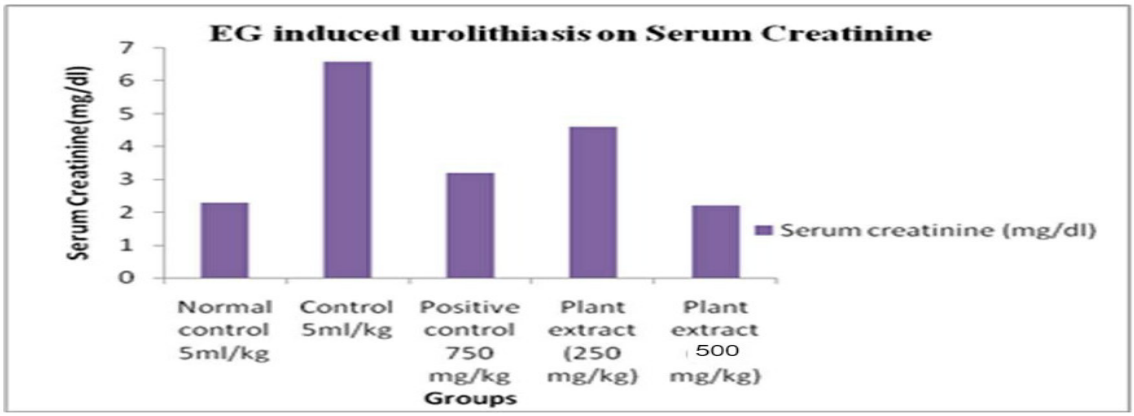

Figure No. 3. EG induced urolithiasis on Serum Creatinine

Serum uric acid

The levels of Serum uric acid on blood serum were indicated that the significantly (P<0.0001) increased in EG-induced group II animals. As the serum uric acid levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the serum uric acid levels was significantly (P<0.0001) decreased in both 200mg/ kg and 400 mg/kg groups. The plant extract 250 mg/ kg treated group of animals serum uric acid level was constantly less when compared to 500mg/ kg treated group of animals.[20]

Table. No.4 Effect ethylene glycol induced urolithiasis on uric acid

S. No	Treatment	Dose (mg/kg)	Uric acid (mg/dl)
1	Normal control	5ml/kg	2.4±0.05
2	Control	5ml/kg	6.8±0.07
3	Positive control	750	3.4±0.1
4	Plant extract	250	4.7±0.11
5	Plant extract	500	2.5±0.03

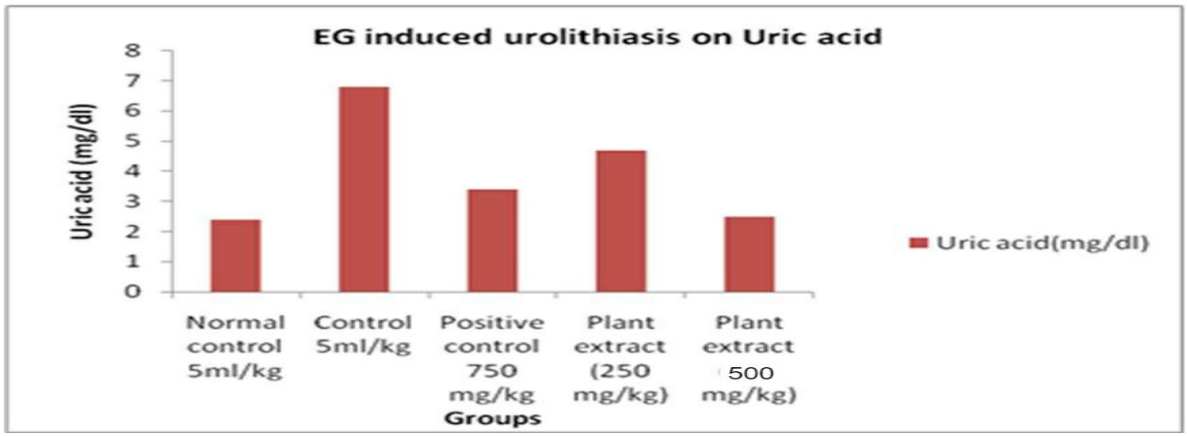

Figure No. 4. EG induced urolithiasis on Uric acid

Urine volume

Urine volume was significantly (P<0.0001) decreased in EG-induced group II animals. While the serum Urine volume levels was significantly (P<0.001) increased in cystone treated group III animals. However, the Urine volume levels was significantly (P<0.0001) decreased in both 250mg/ kg and 500 mg/kg groups. The plant extract 250 mg/ kg treated group of animals Urine volume level was constantly less when compared to 500mg/ kg treated group of animals.

Table. No.5. Effect ethylene glycol induced urolithiasis on urinary volume

S. No	Treatment	Dose (mg/kg)	Urinary volume (ml)
1	Normal control	5ml/kg	33.6 ±2.3
2	Control	5ml/kg	16.26±1.46
3	Positive control	750	30.66±3.22
4	Plant extract	250	19.26±2.66
5	Plant extract	500	30.46±3.22

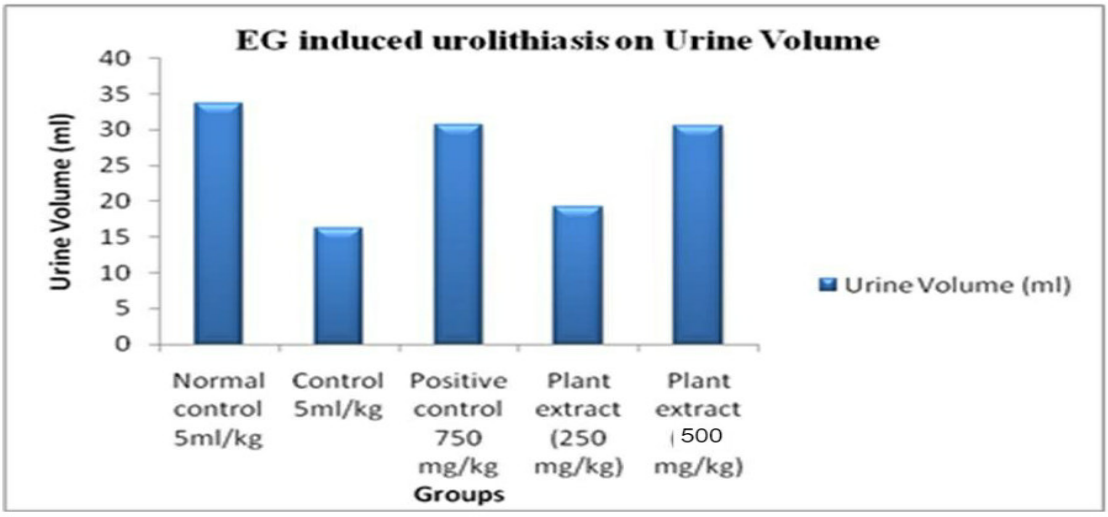

Figure No. 5. EG induced urolithiasis on Urine Volume

Kidney weight

Weight variation of kidney results were clarified that the significantly (P<0.0001) increased in EG-induced group II animals. While the Kidney weight levels was significantly (P<0.001) decreased in cystone treated group III animals. However, the Kidney weight levels was significantly (P<0.0001) decreased in both 250mg/ kg and 500 mg/kg groups. The plant extract 250 mg/ kg treated group of animals Kidney weight level was constantly less when compared to 500mg/ kg treated group of animals.[21]

Table. No.6. Effect ethylene glycol induced urolithiasis on Kidney weight

S. No	Treatment	Dose (mg/kg)	Kidney weight (mg)
1	Normal control	5ml/kg	34.6 ±2.2
2	Control	5ml/kg	56.26±3.4
3	Positive control	750	39.66±3.2
4	Plant extract	250	49.26±2.6
5	Plant extract	500	39.76±3.2

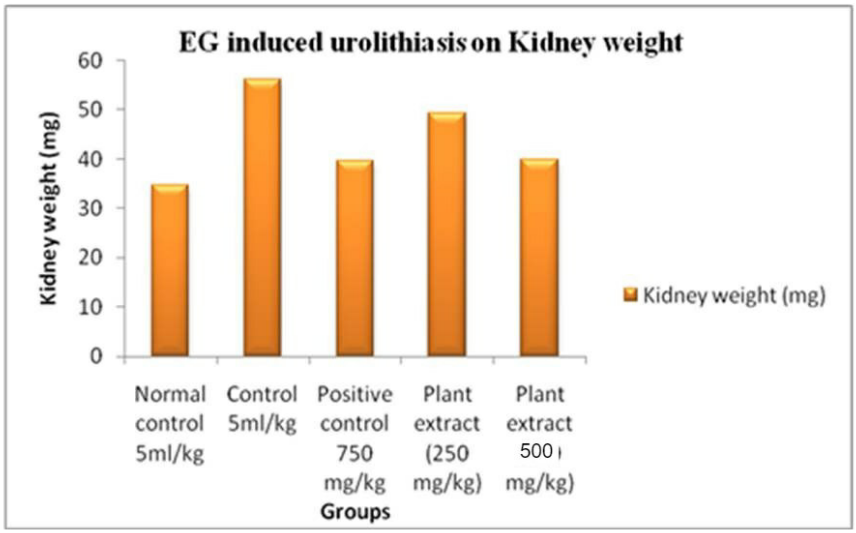

Figure No. 6. EG induced urolithiasis on Kidney weight

RESULTS AND DISCUSSION

The present work deals with the Pharmacognostical, Phytochemical and Pharmacological Investigations on the leaves of *Amoora cucullata*.

Pharmacognostical Investigations

Amoora cucullata (Roxb.) It belongs to the family Meliaceae. The species occurs in lowland forest and along tidal riverbanks. A mangrove associate, A. cucullata is a small to medium-sized tree with plank buttresses and pneumatophores. Amoora cucullata is a tree with a broad, rounded crown of arching branches. it usually grows up to 15 metres tall.The microscopic characters for the leaves of *Amoora cucullata* were studied.

The leaf has thick midrib and smooth and even lamina. The midrib has wide and short conical adaxial part with gradually sloping lateral sides: the abaxial part in broad and semicircular.

Thin plate of several, elongated narrow cells united laterally forming star like scales are very frequent in the powder. Long, narrow worm like fibres are seen in the powder.Thin plate of several, elongated narrow cells united laterally forming star like scales are very frequent in the powder.4.

Prismatic calcium oxalate crystals are abundant in the leaf fragments.

The quantitative microscopy for the leaves of *Amooracucullata*like vein islet & vein termination, stomatal number & stomatal index and palisade ratio were estimated.

The results of quantitative microscopy of leaves of *Amooracucullata* showed the maximum vein islet number.

The standards liked total ash, water soluble ash, acid insoluble ash, sulphated ash, loss on drying and fibre content were also estimated.

Phytochemical Investigations

The powder drug was analysed (fluorescence analysis) with various reagentsto see the colour change that were produced in day and UV light.

The powder drug was also subjected to elemental analysis. It contains organic carbon, nitrogen, phosphorus, potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum, but free of heavy metals.

The coarsely powdered dried leaves of *Amooracucullata* powder was extracted with petroleum ether, chloroform, acetone, ethanolic and aqueous extracts, and their colour and consistency were studied. The preliminary phytochemical analysis clearly indicated the presence of alkaloids, carbhohydrates, terpenoids, tannins, aminoacids, flavanoids, gums, and mucilage.

The ethanolic extract of *Amoora cucullata* subjected to column chromatography for the separation of compounds. The ethanolic extract yielded the compound in the fraction chloroform : acetone (80:20).

Pharmacological Investigations

Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Stone formation in ethylene glycol – fed rats is caused by hyperoxaluria, which cause increased renal retention and excessive excretion of oxalate in urine. Urinary lithiasis is generally the results of an imbalance between inhibitors and promoters in the kidneys.

Rats are the most frequently used animals in models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Rat models of calcium oxalate urolithiasis induced by either EG alone or in combination with other drugs such as AC, are often used to study the pathogenesis of kidney crystal deposition. Using the accelerated model, in the present study rats were treated with 0.75% EG 24 days. All positive control rats developed calcium oxalate depositions during that time.In this study, calcium excretion was increased in calculi – induced animals. An increase in urinary phosphate is also observed in calculi induced rats. Increased urinary phosphate excretion along with oxalate stress seems to provide phosphate crystals, which induce calcium oxalate deposition. Treatment with the test extracts of *Amoor acucullat a*re stored the phosphate level, thus reducing the risk of stone formation.

CONCLUSION

The present study, *Amoora cucullata* has been scientifically validated in terms of Pharmacognostical, Phytochemical and Pharmacological aspects.Using the Pharmacognostical and Phytochemical standards, the plant *Amoora cucullata* can be authenticated, identified and differentiated from other related species. Also these Pharmacognostic parameters may help in the detection of adulteration in commercial.The Pharmacological screening of Diuretics, Anti urolithiatic activity and Anthelmintic activity of *Amoora cucullata* showed significant results.

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