EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *AMARANTHUS BLITUM* IN ETHYLENE GLYCOL INDUCED UROLITIASIS ON EXPERIMENTAL ANIMALS

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Abstract

The aim of the present study is to evaluate the anti-urolithiatic activity of ethonolic extract of *Amaranthus blitum* in ethylene glycol induced urolithiasis in rats. Urolithiasis is a condition that occurs when these stones exit the renal pelvis and move into the remainder of the urinary collecting system, which includes the ureters, bladder, and urethra.*Amaranthus blitum* linn belongs to the family Amaranthaceae is selected for the study due to its phytochemicals like flavonoids, carotenoids, β-carotene, vitamin C, phenolic compounds, and natural antioxidant . Healthy Male Wistar rats (150-200 gms) were selected and divided into five groups having six animals in each. Group-I served as normal and received regular rat food and drinking water adlibitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. Group-III received standard antiurolithiatic drug Cystone (750 mg/kg b. wt.). Group-VI and Group- V received ethanolic extract of *Amaranthus blitum* 200 mg/kg b. wt. and 400 mg/kg b. wt. for 28 days. Extracts were given once daily by oral route. Finally based on improvement in urine and serum parameters, antioxidant parameters, it is concluded that the ethanolic extract of leaves of *Amaranthus blitum* possess antiurolithiatic activity

Keywords: Urolithiasis, *Amaranthus blitum*, Ethylene glycol & Ammonium chloride

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Introduction

Urolithiasis is defined as Formation of stone in the urinary system, i.e. in the kidney, ureter, and urinary bladder or in the urethra [1] Urolithiasis epidemiology differs geographically in terms of incidence and prevalence, age and sex distribution, stone composition, and stone location. These differences have all been attributed to racial, dietary, and climatic factors. The prevalence, incidence, and distribution of urolithiasis according to age, sex, and type, as well as the location and chemical-physical make-up of the calculi, have all changed as a result of changing socioeconomic conditions. Epidemiological surveys have been previously reviewed showing that in economically developed countries the prevalence rate ranged between 4% and 20% [2]urolithiasis complicated and multifaceted process, the formation of kidney stones calculogenesis is influenced by both intrinsic such as age, sex, and inheritance and extrinsic such as geography, climate, food, mineral composition, and water intake. [3] Urolithiasis may exist asymptomatically, but it is often presented by excruciating pain that originates from the flank and radiates to the genitals.Pain is produced as a result of stone obstruction usually at uretro-pelvic junction, pelvic brim and vesico-ureteric junction. [4]. Investigating medical history, family history and dietary history of the patient also aids in diagnosis. Medical history may unveil the medical conditions the patient is suffering from or any medication or medical therapy the patient is recently adhering to or underwent that might be the predisposing factors for urolithiasis. Any family history of renal stone disease and food habits of the patient may further help in revelation of the disease and determining the potential cause [5]..Amaranth plant has been used in Ayurveda for the treatment of different diseases. [6]*Amaranthus blitum* linn belongs to the family Amaranthaceae is rich in phytochemicals like betacyanin, chlorophyll, betaxanthin, carotenoids, β-carotene, vitamin C, phenolic compounds, flavonoids and natural antioxidant which possess antiurolithiatic activity. These natural antioxidant phytochemicals protect many diseases, such as cancer, atherosclerosis, cataracts, cardiovascular diseases, retinopathy, arthritis, emphysema, and neurodegenerative diseases .This genus is adapted to abiotic stresses, such as salinity and drought [7]According to several studies, this leafy vegetable has an extensive capability to a broad range of medicinal properties such as analgesic and hyperglycemic activity [8]. hepatoprotective, antiradical, antibacterial, cancer, atherosclerosis, cataracts, cardiovascular diseases, retinopathy, arthritis, emphysema and neurodegenerative diseases [9].Ethylene glycol-

induced hyperoxaluria model was used to assess the antiurolithiatic activity in albino rats.[10] Cystone (750 mg/kg b.wt) was used as a standard drug in inhibiting urinary stones, due to its potent diuretic, spasmolytic and saluretic effect, which is beneficial in relieving crystalluria

MATERIALS AND METHODS:

Chemicals and reagents:

All reagents used in the present study was were of analytical grade.

Collection and authentication of leaves:

Fresh plant leaves of *Amaranthus blitum* was collected from local areas of Kadapa, Andhra Pradesh and authentified by Dr. A. Madhusudhana Reddy, Professor, Department of Botany, Yogi vemana University, kadapa, Andhra Pradesh, India. Voucher specimens (No: EC- 1619) for these plants has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Preparation of extract:

Leaves *Amaranthus blitum* of was taken, powdered in a grinder-mixer to obtain a coarse powder and then passed through 40 mesh sieves. About 200 gms of powder was extracted by using ethanol by Soxhlet apparatus process up to 24hrs. The solution was filtered through Whatman filter paper and the resultant filtrate was distilled under reduced pressure for recovery of solvent. The dried extract thus obtained was kept in desiccators and used for further experiments.

Experimental animals:

Wistar albino rats of male sex (150-230 gm) procured from venkateswara nterprises (Bangalore), were used in the present study. The animals were housed in the clean propylene cages and maintained under standard conditions (25±2ºC, relative humidity 44 – 56 % and 12-hours light and dark cycles respectively) and fed with standard rat diet (M Mysore feeds, Bangalore) and purified drinking water *ad* libitum for 1 week before and during the experiments. Animals were handled with human care. Institutional Animal Ethical Committee (IAEC) of P. Rami Reddy Memorial College of Pharmacy (1423/PO/Re/S/11/CPCSEA/2022) approved the present study.

EXPERIMENTAL DESIGN:

The experimental design used to carry out the antiurolithiatic activity of ethanolic extract of *Amaranthus blitum* in ethylene glycol induced urolithiasis in rats.

Healthy Male Wistar rats (150-200 gms) were selected and divided into five groups having six animals in each. Group-I served as normal and received regular rat food and drinking water adlibitum. Ethylene glycol (0.75%) & Ammonium chloride (1%) in drinking water was fed in Group-II to Group-V for induction of renal calculi for 28 days. Group-III received standard antiurolithiatic drug Cystone (750 mg/kg b. wt.). Group-VI and Group- V received ethanolic extract of *Amaranthus blitum* 200 mg/kg b. wt. and 400 mg/kg b. wt. for 28 days. Extracts were given once daily by oral route .

Collection of blood samples

The blood samples were collected from the retrorbital venous plexus of rats without any coagulant for the separation of serum, at the regular intervals of the treatment. After collecting the blood in ependroff tubes they were kept for 1 h at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters. [11]

Statistical analysis

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way analysis of variance ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

Results and discussion:

Effect of EEAB on urinary output-:

There was a significant decrease in the urine levels on 28th day in rats treated with ethylene glycol (EG) (G-II) when compared to the normal group (G-I). The group (G-III) rats treated with standard drug cystone showed a significant increase in urine levels on 28thday when compared to control (G-II).

Groups IV and V receiving EEAB (200 mg/kg and 400 mg/kg) showed significant increase in urine levels on 28th day when compared to control group suggesting to treat kidney stones by our extract. The results of urine levels were shown in above Table .No 1 signifies that the high dose of EEAB was effective when compared to low dose of EEAB.

Table .No: 1: Effect of EEAB on urinary output in ethylene glycol induced urolithiasis

Groups	Treatment	urinary output (ml/day)
I.	Normal (Received 1 % water)	24.04 ± 0.12
II.	Control (Received EG in distilled water)	13.03 ± 0.48[a]
III.	Standard (Received EG in distilled water + cystone)	21.43 ± 0.62[c]
IV.	Test-1 (Received EG in distilled water + low dose of drug)	19.42 ± 0.53[b]
V.	Test-2 (Received EG in distilled water + high dose of drug)	20.33 ± 0.15[c]

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control

Effect of EEAB on urine calcium levels, phosphate levels and oxalate levels

There was a significant increase in the calcium levels , on 28th day in rats treated with ethylene glycol (EG) (G-II) when compared to the normal group (G-I). The group (G-III) rats treated with standard drug cystone showed a significant decrease in calcium, phosphate& **oxalate** levels on 28th day when compared to control (G-II).

Groups IV and V receiving EEAB (200 mg/kg and 400 mg/kg) showed significant decrease in

Calcium, phosphate and oxalate levels on 28th day when compared to control group suggesting to treat the kidney stones by our extracts.The results of were shown in above Table .No:2, signifies that the high dose of seeds extract was effective when compared to low dose of EEAB.

Table No: 2 Effect of EEAB on urine calcium levels , phosphate levels , and oxalate levels in ethylene glycol induced urolithiasis On 28th day

Groups	Treatment	Calcium (mg/dl)	Phosphate (mg/dl)	Oxalate (mg/dl)
I	Normal (Received 1 % water)	3.134 ±0.412	3.234 ± 0.421	3.321 ±0.163
II	Control (Received EG in distilled water)	8.632 ± 0.618[a]	9..232 ± 1.612[a]	7.643 ±0.324[a]
III	Standard (Received EG in distilled water + cystone)	3.542 ±0.298[c]	4.721 ± 0.315[c]	4.142 ±0.612[c]
IV	Test-1 (Received EG in distilled water + low dose of drug)	5.132 ±0.349[b]	6.236 ± 0.592[b]	5.876 ±0.542[b]
V	Test-2 (Received EG in distilled water + high dose of drug)	4.965 ±0.783[c]	5.231 ± 0.276[c]	4.342 ±0.162[c]

Effect on serum calcium, serum creatinine, and uric acid

Administration of ethylene glycol induced a significant increase in , serum calcium , creatinine , and uric acid levels, on 28th day in control group (G-II) when compared to normal group (G-I). On treatment with cystone induced a significant reduction in calcium levels on 28th day in standard group (G-III) when compared to control group (G-II).

Groups IV and V receiving EEAB of (200 mg/kg and 400 mg/kg) showed significant decrease in, serum calcium levels, , creatinine , and uric acid on 28th day when compared to control group.The results of serum calcium , creatinine , and uric acid levels were shown in above Table.No:3 signifies that the high dose of EEAB was effective when compared to low dose of EEAB.

Table No: 3 Effect of EEAB on calcium levels , serum creatinine and Effect on uric acid in ethylene glycol induced urolithiasis On 28th day

Grouops	**Treatment**	**Calcium (mg/dl)**	**Creatinine (mg/dl)**	**Uric acid (mg/dl)**
I.	Normal (Received 1 % water)	6.426 ± 0.132	0.927 ±0.362	4.439 ±0.256
II.	Control (Received EG in distilled water)	17.213 ± 0.427^{c}	3.464 ±0.135^{c}	9.726 ±0.126^{c}
III.	Standard (Received EG in distilled water + cystone)	8.126 ± 0.436^{c}	1.267 ±0.623^{c}	5.213 ±0.431^{c}
IV.	Test-1 (Received EG in distilled water + low dose of drug))	10.521 ± 0.232^{b}	1.339 ±0.425^{b}	6.781 ±0.264^{b}
V.	Test-2 (Received EG in distilled water + high dose of drug)	9.254 ± 0.131^{c}	1.295 ±0.634^{c}	5.967 ±0.366^{c}

Effect of EEAB on urea

Administration of ethylene glycol induced a significant increase in urea levels on 28th day in control group (G-II) when compared to normal group (G-I). On treatment with cystone induced a significant reduction in urea levels on 28th day in standard group (G-III) when compared to control group (G-II). Groups IV and V receiving EEABof (200 mg/kg and 400 mg/kg) showed significant decrease in urea levels on 28th day when compared to control groupas shown in fig.6.3. .The results of urea levels were shown in above Table .No:4signifies that the high dose of EEAB was effective when compared to low dose of EEAB

Effect of EEAB on urea

Administration of ethylene glycol induced a significant increase in urea levels on 28th day in control group (G-II) when compared to normal group (G-I). On treatment with cystone induced a significant reduction in urea levels on 28th day in standard group (G-III) when compared to control group (G-II).

Groups IV and V receiving EEAB of (200 mg/kg and 400 mg/kg) showed significant decrease in urea levels on 28th day when compared to control group.The results of urea levels were shown in above Table .No:4 signifies that the high dose of EEAB was effective when compared to low dose of EEAB

Table No: 4 Effect of EEAB on serum urea level in ethylene glycol induced urolithiasis

Groups	Treatment	urea (mg/dl)
I.	Normal (Received 1 % water)	8.026 ± 0.313
II.	Control (Received EG in distilled water)	23.271 ± 0.821^{c}
III.	Standard (Received EG in distilled water + cystone)	10.543 ± 0.231^{c}
IV.	Test-1 (Received EG in distilled water + low dose of drug)	12.642 ± 0.236^{b}

V.	Test-2 (Received EG in distilled water + high dose of drug)	11.241± 0.361[c]

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control

Effect of EEAB on tissue parameters:

Effect on SOD and CAT levels:

A significant decrease in the SOD& **CAT** levels on 28th day was observed in rats treated with ethylene glycol (G-II) when compared to the normal group (G-I). The group (G-III) rats treated with standard drug cystone showed a significant increase in SOD levels on 28th day when compared to control (G-II)

Groups (IV and V) receiving EEAB(200 mg/kg and 400 mg/kg) showed significant increase in SOD& **CAT** levels on 28th day when compared to control group suggesting to treat the kidney stones by our extract. The results of SOD & **CAT** were shown in Table .No:5 signifies that the high dose of EEAB was effective when compared to low dose of EEAB.

Table No : 5 Effect of EEAB on SOD and CAT levels

Groups	**Treatmenst** _	**SOD (U/mg protein)**	**CAT (µM H2O2 consumed/mg protein)**
I.	Normal (Received 1 % water)	13.076 ± 0.257	47.821 ±0.072
II.	Control (Received EG in distilled water)	4.284 ± 0.861[c]	20.518±0.672[c]

III.	Standard (Received EG in distilled water + cystone)	12.516 ± 0.321[c]	45.342±0.792[c]
IV.	Test-1 (Received EG in distilled water + low dose of drug)	10.353 ± 0.428[b]	42.213±0.272[b]
V.	Test-2 (Received EG in distilled water + high dose of drug)	11.128 ± 0.521[c]	43.351±0.976[c]

Data represents the Mean ± SEM values (n=6). Statistical significance: [a]P<0.05, [b] P<0.01, [c] P<0.001 with respect to Disease control on 28th day by One way ANOVA followed by Dunnetts: Compare all columns vs. Disease control

Conclusion

Urolithiasis is a condition that occurs when these stones exit the renal pelvis and move into the remainder of the urinary collecting system, which includes the ureters, bladder, and urethra. The prevalence rate ranged between 4% and 20% in economically developed countries. The prevalence of kidney stones varied greatly between geographic locations, ranging from 8% to 19% in males and from 3% to 5% in females in Western countries. Our work aims to study the antiurolithiatic activity of the methanolic extract of leaves of *Amaranthus blitum* against 0.75% ethylene glycol and 1% ammonium chloride for induction of renal calculi by using cystone (750 mg/kg) as a standard lithiatic drug for 28 days. There is a significant restoration of urine and serum parameters, antioxidant parameters exhibiting antiurolithiatic activity of these plants. In support to this study, histopathological results also show significant activity of the plant.

Finally based on improvement in urine and serum parameters, antioxidant parameters, it is concluded that the ethanolic extract of leaves of *Amaranthus blitum* possess antiurolithiatic activity.Most of these studies were preliminary, carried out in animals and are not sufficient for the development of a pharmaceutical product. Still, intensive preclinical and clinical studies are required to evaluate the efficacy and toxicity of the ethanolic extract of leaves of *Amaranthus blitum* . Further, chemical studies are needed to isolate the active principles and investigate them

in order to identify a promising lead compound the ethanolic extract of leaves of *Amaranthus blitum*

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