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Phytochemical Investigation and In Vivo Antiurolithiatic Activity of Merremia Emarginata (BURM.F.) Hallier F.

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ABSTRACT:
Kidney stone disease, also known as nephrolithiasis or urolithiasis. A solid piece of material develops in the urinary tract brings about pathological condition. The main aim of the work done by the authors was to carry out the phytochemical investigation and In vivo Antiurolithiatic activity of Merremia emarginata in experimental animal models. Phytochemical investigation of Merremia emarginata aqueous extract disclosed the presence of Principle constituents Flavonoids, Phenols, Tannins, Alkaloids and Saponins. One or more chemicals may be responsible for the distinct pharmacological activity. Ethylene glycol (0.75% v/v) induced Urolithiasis models were used for current study. Group I (normal control) served with regular diet and drinking water ad libitum. Groups II-V fed Ethylene glycol (0.75%) in drinking water throughout the experimentation period (28 days) to induce lithiasis. Group II (disease control) left for induction of calculus. Group III served with Standard, Cystone (750mg/kg Body weight) from day 14 to day 28. Groups IV and V served with test samples of AQME extract (250 and 500 mg/kg, respectively) once in a day orally from day 1 to day 28. As a part of experimental analysis both urine and serum samples were collected on day 14 and day 28. Serum samples were analyzed for calcium, Phosphate, uric acid, urea, magnesium contents. Whereas urine samples were analyzed for oxalate, uric acid, citrate, creatinine, calcium, magnesium and Phosphate. Results stated that AQME (250 and 500 mg/kg) and Cystone (700 mg/kg) group exhibited significant ($p < 0.001$) rise in urine output, urinary pH & considerable reduction in stone size as compared to disease group on 28th day of experimentation. AQME at both 250 and 500 mg/kg showed significant dose-dependent Antiurolithiatic activity (considerable reduction in stone size).

Key words: AQME-Aqueous extract of Merremia emarginata, In vivo antiurolithiatic activity, Cystone

I INTRODUCTION

Kidney stones typically form in the kidney and leave the body in the urine stream. A small stone may pass without causing key symptoms. If a stone grows to more than 5 millimeters, it can cause blockage of the ureter, resulting in severe pain in the lower back or abdomen. A stone may also result in blood in the urine, vomiting, or painful urination. Stone formation is also caused due to imbalance between promoters and inhibitors. The rate of occurrence is three times higher in men than women, because of enhancing capacity of testosterone and inhibiting capacity of estrogen in stone formation [1]. Stone formation is affected by various factors such as metabolic disorders, stress and certain medication. **U**rine when supersaturated with Calcium oxalate which means that it will contain Calcium oxalate crystals that form spontaneously called nucleation of Calcium oxalate crystals. Their size must be controlled to prevent retention in ducts and the eventual development of lithiasis. This is achieved in part by specific inhibitory effect on crystal growth [2]. Throughout history, every civilization in the world used plants or their derivatives for treatment or prevention of diseases. In many countries throughout the world, herbal medicines are broadly used in the treatment of wide range of diseases including urological diseases [3]. Merremia emarginata Burm.f, belonging to the family Convolvulaceae is a perennial, much branched Creeper [4]. Merremia is a genus of flowering plants in the morning glory family. Members of this genus are commonly known as wood roses [5]. It is a perennial, prostrate plant with slender stems 30-75cm long that usually form roots at the nodes. The plant was studied for diuretic, laxative and purgative properties & is used in the treatment of

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rheumatism, neuralgia and headache. Various studies were conducted for Antioxidant[6] , Nephroprotective [7,8] , Antibacterial[9] , Antidiabetic[10] , Analgesic[11] , Anti-cancer activities[12]. However, Literature survey indicated that no published reports on the Merremia emarginata against urolithiasis in animal models. Hence, the experiment was designed with an aim to narrate the protective effect of Merremia emarginata in kidney stone prevalence. The current study substantiate the ethnobotanical potential of Merremia emarginata against urolithiasis.

II MATERIALS & METHODS

2.1 Collection of Plant material & authentication:

Merremia emarginata herb was collected in December month in rice crop fields of Narakoduru Village, Guntur district Andhra Pradesh in India. The plant was authenticated by authenticated by Dr. M. Raghu ram, Assistant professor of Department of Botany& Microbiology, Acharya Nagarjuna University, and Guntur. The plant material was thoroughly washed with water to clean all earthen debris, shade dried, ground into powder using a mixer and placed in a desiccator until further use.

Fig. no. 1: Merremia emarginata (burm.f.) Hallier f.

2.2 Physicochemical examination:

2.2.1 Determination of Moisture Content (Loss on Drying):

A method commonly used for the determination of moisture content is the loss-on-drying method, in which a substance is heated until it is completely dry[13].Approximately 1 g of plant sample is taken separately in an evaporating dish and placed in hot air oven, heated at 105°C for 1 hour. The moisture content is determined by weighing the sample before and after drying and determining the difference. Loss on drying is expressed in percentage.

2.2.2 Determination of Total Ash [14, 15]:

Accurately weighed powdered sample (3 g) was placed in a separate tarred silica crucible. Sample was ignited gradually to increase the temperature up to 450°C until carbon free ash is obtained. The charred mass was exhausted with hot water, filtered to collect the residue on an ash less filter paper. The residue along with filter paper was incinerated, cooled and weighed. Finally, the percentage of total ash was calculated with reference to air-dried drug.

2.2.3 Determination of Acid Insoluble Ash [14, 15]:

The ash thus obtained above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid. The insoluble particles were collected using Gooch crucible, washed with hot water ignited to constant weight. Further the percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

2.2.4 Determination of Alcohol Soluble Extractive value [14, 15]:

A known quantity (5 g) of coarsely powdered plant material was macerated with 100 ml of alcohol in a closed flask for 24 hrs, shaking frequently during six hours and allow it to stand for 18 hrs. The above was filtered, 25 ml of the filtrate is evaporated to dryness in a tarred porcelain dish. Dried at 105ºC, cooled and weighed. The percentage of alcohol-soluble extractive value is calculated with reference to the air-dried drug.

2.2.5 Determination of Water Soluble Extractive value [14,15]:

The same procedure was carried out for the determination of water-soluble extractives, using chloroform water instead of Alcohol. The

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percentage of water-soluble extractive value is calculated with reference to the air-dried drug.

2.2.6 Detection of any foreign organic matter [14, 15]:

The whole plant material selected for the study was initially examined for the presence of any foreign debris like stones, mud, rodent fecal matter, insects or any noxious substance by visual examination before making it in to powder form. Further a small portion of the powder was spread on a clean glass slide and examined in microscope. The results were illustrated in Table 1.

2.3 Extraction: Coarsely powdered plant material was extracted with different solvents based on polarity index 7:3 (27- 30°C) on an orbital shaker (Remi, India) for 48 hrs. The extracts thus obtained were dried under reduced pressure using a rotary evaporator. Stored in a desiccator for further analysis. The percentage yield of aqueous extract of Merremia emarginata (AQME) was high when compared to alcoholic extract of Merremia emarginata (ALME). The results were illustrated in Table 2.

2.4 Phytochemical Screening:

Aqueous extract of Merremia emarginata was subjected to Phytochemical screening of various secondary metabolites using already established standard protocols [16, 17].

Preliminary phytochemical screening revealed that the aqueous extract of Merremia emarginata showed positive for the presence of Flavonoids, Phenols, Tannins, Alkaloids, and Saponins. The results were illustrated in Table 3.

III EXPERIMENTAL PROTOCOL

3.1 Acute oral toxicity studies:

Acute toxicity tests can be used to assess the safe dose of an extract. The procedure used in this study was based on OECD standards 423 (Organization for Economic Cooperation and Development) (Acute toxic class method). The acute toxic class method is a step-by-step procedure that involves three animals of the same sex in each step. The protocols were officially recognized by Institutional ethical committee [18, 19].The results of the same were illustrated in Table 4.

3.2 In vivo Antiurolithiatic Activity [20]**:**

Ethylene glycol induced urolithiatic model was chosen to examine the antiurolithiatic activity in male Wistar rats on Merremia emarginata aqueous extract (250 &500 mg/kg body weight) and dose was selected based on acute toxicity studies. The animals were randomly divided into five groups, each comprising of 6 rats.

Group I: Control rats were received normal rat food and drinking water ad libitum (make available at all time).

Group II to V were administered ethylene glycol orally in drinking water from day 1- day28.

Group II: served as the Positive control where calcium oxalate stones were induced but not treated.

Group III: received Standard antiurolithiatic drug, Cystone (750 mg/kg body weight) from day 14 to day 28.

Group IV: received Merremia emarginata aqueous extract (250 mg/kg body weight).

Group V: received Merremia emarginata aqueous extract (500 mg/kg body weight) from day 1to day 28.

At the end of the 28-day cycle, serum samples were obtained, and biochemical parameters were analyzed. Further the kidneys were isolated, cleaned and subjected to histopathological examinations [21-24]. The results were illustrated in table 5&6.

IV RESULTS & DISCUSSION

Table 1: Physicochemical analysis of leaf, of Merremia emarginata

Name of the test (%w/w)	Leaf
Loss on drying	13.52±0.03
Total Ash value	9.34±0.04
Acid insoluble Ash value	1.6
Water soluble extractive value	9.46
Alcohol soluble extractive value	2.45
Foreign matter	<2

All values are given as mean of 3 results (n=3); SEM=Standard error of mean

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Table 2: Merremia emarginata Plant extracts:

S.no	Plant	Percentage Dry weight	Colour	Consistency
1	Aqueous exctract of Merremia emarginata (AQME)	9.45 %	Greenish brown	Semi solid
2	Alcholic extract of Merremia emarginata (ALME)	2.4 %	Greenish brown	Semi solid

Table 3: Preliminary Phytochemical screening of AQME plant extract

S.No.	Chemical constituents	AQME	ALME
1.	Flavoniod	Present	Present
2.	Phenolics	Present	Present
3.	Tannins	Present	Present
4.	Alkaloids	Absent	Present
5.	Glycosides	Absent	Absent
6.	Saponins	Present	Present

Table 4: Effect of Acute toxicity studies on AQME extract

S.No.	Groups (n=3)	Dose (mg/kg) b.w	Lethality
1.	I	300	no
2.	II	2000	no

Table 5: Effect of AQME plant extract on Urinary parameters in ethylene glycol induced urolithiasis in rats.

	Serum parameters					
	No. of Days	**Control**	**Disease (+ve) control**	**Standrad (Cystone)750mg/kg**	**AQME 250 mg/kg**	**AQME 500 mg/kg**
Calcium (mg/dl	14	10.08±0.11	12.97±0.32	12.42±0.31	12.72±0.56	12.32±0.36
	28	10.25±0.14	14.12±0.29	10.76±0.21	11.67±0.23	10.94±0.23
Phosphate (mg/dl)	14	5.02±0.18	7.34±0.15	7.42±0.024	7.62±0.29	7.69±0.31
	28	5.04±0.16	8.12±0.16	5.24±0.16	5.62±0.37	5.21±0.21
Uric acid (mg/dl)	14	2.74±0.12	6.75±0.32	6.53±0.32	6.64±0.46	6.72±0.42
	28	2.84±0.10	6.98±0.42	3.21±0.47	3.75±0.23	3.33±0.84
Urea (mg/dl)	14	14.64±1.23	27±2.12	28.32±3.2	27.2±1.23	28.42±2.12
	28	14.72±1.78	35±3.21	16.32±1.25	22±2.31	18±3.12
Magnesium (mg/dl	14	3.14±0.02	1.64±0.04	1.92±0.23	1.82±0.16	1.94±0.12
	28	3.1±0.03	1.84±0.02	2.75±0.12	2.64±0.12	2.93±0.16

All the values are mean SEM (n ¼ 6), one-way ANOVA followed by Dunnett's test. **P < 0.001 and *P < 0.05 versus Normal group. ##P < 0.001 and #P < 0.05 versus disease control group.

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Table 6: Effect of AQME plant extract on Serum parameters in Ethylene glycol induced urolithiasis in rats.

	Urinary parameters					
	No. of Days	**Control**	**Disease (+ve) control**	**Standard (Cystone) 750mg/kg**	**AQME 250 mg/kg**	**AQME 500mg/kg**
Urine Volume (ml/24 h)	14	9.16±0.52	6.12±0.46	6.42±0.42	6.14±0.32	6.94±0.24
	28	9.18±0.25	5.42±0.19	15.12±1.14	9.12±0.21	13.24±0.61
Urinary pH	14	6.74±0.25	5.45±0.29	5.6±0.32	5.72±0.31	5.64±0.46
	28	6.72±0.42	5.22±0.41	6.32±0.41	6.42±0.24	6.62±0.21
Oxalate (mg/24 h)	14	4.62±0.12	10.32±1.2	7.12±0.12	8.42±1.32	7.62±0.92
	28	4.68±0.14	11.98±1.34	5.64±0.34	6.42±0.27	5.94±0.84
Uric acid (mg/24 h)	14	1.98±0.15	4.51±0.41	3.50±0.24	4.32±0.64	4.12±0.64
	28	2.10±0.21	5.12±0.12	2.15±0.38	3.34±.56	3.00±0.24
Citrate (mg/24 h)	14	21±1.32	7.94±0.49	12±1.2	9.42±1.2	10.94±1.2
	28	22.12±2.1	8.31±0.12	19±2.6	17.25±2.3	19.12±2.1
Creatinine Clearance (mg/24 h)	14	37.45±3.12	14.32±2.76	25.2±4.9	18.42±2.4	23±2.1
	28	40.23±4.2	10±2.2	52.4±3.4	35.24±3.2	45±3.2
Calcium (mg/24 h)	14	3.12±0.12	5.69±0.32	5.42±0.62	5.64±0.14	5.12±0.42
	28	3.10±0.01	6.64±0.52	3.16±0.23	4.97±0.27	4.01±0.74
Magnesium (mg/24 h)	14	3.26±0.18	1.29±0.12	1.94±0.18	1.64±0.34	1.42±0.01
	28	3.24±0.09	1.21±0.14	3.02±0.17	2.14±0.21	2.94±0.23
Phosphate (mg/24 h)	14	4.97±0.64	7.6±0.41	7.12±1.02	6.94±1.2	7.02±0.32
	28	5.10±0.52	8.12±0.74	5.45±1.41	6.12±0.49	5.91±0.42

Ethylene glycol administrated to rats for 14 days caused considerable ($p < 0.001$) decline in urine output in all groups as compared to the control group. On 28th day in disease group urine volume was decreased as compared the 14th day urine volume, while AQME (250 and 500 mg/kg) and Cystone (700 mg/kg) group exhibited significant ($p < 0.001$) rise in urine output as compared to disease group on 28th day. Moreover, a significant ($p < 0.001$) decline in urinary pH was observed in the disease group, which was significantly ($p < 0.001$) increased in AQME (250 and 500 mg/kg) and Cystone (700 mg/kg) group. In urine analysis, calcium oxalate crystals were absent in control group animals whereas large size and more number of crystals were observed in disease control group animal urine. In AQME and Cystone treated animals urine showed very less number and small size of calcium oxalate crystals.

V SUMMARY & CONCLUSION

After examining the test, and it has to be noticed that Merremia emarginata has progressed all the physicochemical parameters as per the

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guidelines. The percentage yield of aqueous extract of Merremia emarginata (AQME) was high than alcoholic extract (ALME). Subsequently the phytochemical screening unfold that the material was found to contain Flavonoids, Phenols, Tannins, Alkaloids and Saponins as secondary metabolites. Therefore, the concluding factor is that one or more constituents will be responsible for the specified pharmacological activity. The plant extract was safe up to 2000 mg/kg, as illustrated in the acute toxicity studies. At the present study, it has revealed that the plant draws out significant Anti urolithiatic effect. So, finally the optimistic results, holds up the available Ethnobotanical data. Thus, further clinical studies required for narrating the exact mechanism of actions of the plant constituents.

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