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A COMPARATIVE PHARMACOGNOSTIC - EXPERIMENTAL STUDY ON *TAVAKSHEERI KANDA* (*Curcuma angustifolia Roxb*) AND *VARUNA TWAK* (*Crataeva nurvala, Buch-Ham*) FOR *ASHMARIGHNA KARMA* VIS-A-VIS ANTI-UROLITHIATIC ACTIVITY.

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ABSTRACT

Introduction- *Acharyas* have described various disorders related to *Mutravaha Srotas*, *Ashmari* is one among them, *Ashmaanaam raati dhadhati ya* which means the formation of hard stony structure is *Ashmari*. Ayurveda have described many drugs having *Ashmarihara karma, Varuna (Crataeva nurvala, Buch-Ham*) is one among the best drug having anti urolithiatic activity. *Tavaksheeri kanda* (*Curcuma angustifolia Roxb*) *Madhura rasa, Madhura vipaka, Sheeta virya* described as *Ashmari hara* in N*ighantu Ratnakara*, but it is not evaluated scientifically. Hence this experimental study is aimed to compare anti urolithiatic activity of *Tavaksheeri kanda* (*Curcuma angustifolia Roxb*) and *Varuna twak (Crataeva nurvala, Buch-Ham*).

Materials and Methods- The trial drugs were collected and authentified. Analytical study of the drugs was carried out as per standard protocol. In vivo study was carried out in Ethylene glycol induced urolithiasis model and statistical analysis was done. **Results -**The analytical study confirmed the genuinity of drugs. On HPLC *Tavaksheeri Kanda* showed the presence of Curcumin and *Varuna Twak* showed the presence of Quercetin which have anti-urolithiatic activity. Based on serum parameters, urine parameters and histopathological studies both the drugs found to have statistically significant effect on *Ashmari*. **Discussion and Conclusion-** In comparison *Varuna Twak* showed better anti- urolithiatic activity when compared to *Tavaksheeri Kanda. Tavaksheeri Kanda* had better diuretic activity when compared to *Varuna Twak.* Alcoholic extract of *Varuna Twak* had better effect on serum and urine parameters when compared to aqueous extract of *Varuna Twak*.

KEYWORDS: *Tavaksheeri; Ashmari, Varuna*; Ethylene glycol; Anti-urolithiasis**.**

INTRODUCTION

Renal calculi affect about 12 million people globally, and 1 in 10 individuals experience the condition at least once in their lifetime; around 2% have recurrent episodes. The prevalence is slightly higher in males (11%) than

in females (9%). Urolithiasis involves the aggregation of urinary crystalloids anywhere from the kidneys to the urinary bladder. [1]

Ashmari is one of the *Aṣṭamahāgada* that affects the *Mutravaha Srotas*. Classical texts and *Nighantus* mention several *Ashmarighna dravyas*, indicating the need to explore more effective remedies for renal calculi.[2]

Ethylene glycol is commonly used to induce urolithiasis in experimental models as it produces calcium oxalate crystals similar to humans. Potassium citrate, a clinically proven treatment, alkalinizes urine and reduces uric acid and cystine crystallization.[3]

Studies on *Varuna* (*Crataeva nurvala*) show that it increases urinary excretion of calcium and phosphorus and enhances urine output, thereby reducing stone formation. [4]

Tavaksheeri kanda (*Curcuma angustifolia Roxb*) described as *Ashmari hara* in N*ighantu Ratnakara*, but it is not evaluated scientifically.[5] As the part used of *Varuna* is the stem bark, its collection can lead to destruction of the tree and exploitation of the species, while *Tavaksheeri*, being perennial herb and a cultivated species, offers a more sustainable and eco-friendly alternative for medicinal use.
Thus, the present study aims to evaluate the anti-urolithiatic activity of *Tavaksheeri kanda* and compare it with *Varuna Twak*.

OBJECTIVES

1. To evaluate experimentally *ASHMARIGHNA KARMA* (Anti-urolithiatic activity) of *Tavaksheeri Kanda (Curcuma angustifolia Roxb) & Varuna twak (Crataeva nurvala, Buch-Ham).*
2. To compare *ASHMARIGHNA KARMA* (Anti-urolithiatic activity) of *Tavaksheeri Kanda (Curcuma angustifolia Roxb)* and *Varuna Twak (Crataeva nurvala, Buch-Ham).*

MATERIALS AND METHODS

Collection and authentication of trial drugs

Varuna twak & *Tavaksheeri kanda* were collected from natural habitat Ernakulam, Kerala**,** and identity of the trial drugs was confirmed by botanist.

Preparation of Extract

Aqueous extracts of both drugs were prepared through Cold maceration method &ethanolic extracts of drugs were prepared through Soxhlet apparatus method.

Analytical Evaluation of the Trial Drug

- The Macroscopic characters (Sensory evaluation) of the trial drugs *Varuna twak (Crataeva nurvala (buch-ham)* and *Tavaksheeri kanda (Curcuma angustifolia Roxb)* were carried out.
- Pinch of powdered crude drug previously sieved was put on the slide and mounted in glycerin and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.
- The physico-chemical analytical tests viz. foreign matter, Moisture content (Loss on drying), total ash, acid insoluble ash, alcohol soluble extractive value, Water Soluble extractive value, pH values were carried out as per the Standard protocol.
- Chromatographic analysis: HPLC system equipped with dual Quaternary pump, manual/auto injector and photo diode array detector or UV detector supported by suitable software.

METHODOLOGY

ANIMAL MODEL

The required number of healthy adult Wister albino rats weighing between 180-250 grams of either sex was procured from Vertebrates Lab, Bangalore and Experimental study was carried out at PES College of Pharmacy, Bengaluru. The experimental procedure was carried out in accordance with the ethical guidelines for animals proposed by Government of India. Approval was obtained from Institutional Animal Ethics Committee (IAEC). Registration Number 2138/PO/RcBiBt/S/21/CPCSEA.

DOSE OF THE STANDARD AND TRIAL DRUGS

Dose was calculated as per OECD guidelines.

Dose of Induction drug Ethylene glycol along with drinking water	0.75% V/V Oral (0.75 ml ethylene glycol in 100ml water).
Dose of Standard drug Potassium citrate	50mg/kg oral
Aqueous extract of *Varuna*	500mg/kg bw
Alcoholic extract of *Varuna*	500mg/kg bw
Aqueous extract of *Tavaksheeri*	400mg/kg bw
Alcoholic extract of *Tavaksheeri*	400mg/kg bw

Grouping of Experimental animals

Group no	Number of animals	Group name	Treatment	Duration
1	5	Normal control	No medical intervention	Day 1 to 28 days
2	5	Disease control	Ethylene glycol in drinking water	Day 1 to 14days
3	5	Standard	Ethylene glycol in drinking water	Day 1 to 14 days
			Potassium citrate	Day 15 to Day 28
4	5	Trial Group 1	Ethylene glycol in drinking water	Day 1 to 14 days
			Aqueous extract of *Crataeva nurvala*	Day 15 to Day 28
5	5	Trial Group 2	Ethylene glycol in drinking water	Day 1 to 14 days
			Alcohol extract of *Crataeva nurvala*	Day 15 to Day 28
	5	Trial Group 3		Day 1 to 14 days

6			Ethylene glycol in drinking water	
			Aqueous extract of *Curcuma angustifolia*	Day 15 to Day 28
7	5	Trial Group 4	Ethylene glycol in drinking water	Day 1 to 14 days
			Alcohol extract of *Curcuma angustifolia*	Day 15 to Day 28

EXPERIMENTAL TRIAL PROPER

Animals were divided into seven groups having 5 Wistar albino rat in each group. All the experimental animal groups except normal control were induced urolithiasis with ethylene glycol for 14 days given along with drinking water. Standard and trial drugs were administered orally. Distilled water was added to the drugs to make a homogenous solution and administered orally once a day.

Parameters of the study:

- Blood parameters: creatinine, calcium, phosphorus, uric acid on 28th day
- Urine parameters: creatinine, calcium, phosphorus, uric acid on 28th day
- Urine volume- on 1st, 14th ,28th day
- Body weight on 1st ,14th & 28th day
- Histopathology of kidney: on 29th day

Collection of Urine:

The Wister albino rats are administered with 2ml of normal saline kept in metabolic cage and sample is collected after 4hrs.

Collection of blood:

The blood was collected from retro- orbital sinus by anesthetizing rats with chloroform soaked in cotton and administered as inhalation. 2ml of blood was collected in sterile test tube from each rat and the serum was separated by centrifuging at 3000rpm for 15 min. Then serum was stored in deep freezer at -20C, and used for different investigations.

Sacrificing of Animals (Euthanasia):

Animals were sacrificed as per CPCSEA guidelines excessive induction of anesthetic.

Histopathology Procedure

- Kidneys are dissected and fixed in neutral formalin (10% solution) for at least 3 days, then washed with running water.
- This was followed by dehydration with alcohol of increasing strength (70%, 80% and 90%) for 1 hour each.
- Cleansing was done by using xylene. After cleansing, the organ pieces were subjected to paraffin infiltration automatic tissue processing unit. The organ pieces were washed under running water to remove formalin completely.
- Then the sectioning and staining (Hematoxylin) was done.

STATISTICAL ANALYSIS

All the data were compiled using one way ANOVA followed by Post hoc turkey's test. P value <0.05 were considered as statistically significant. Mean values and standard error of mean were calculated and all the values were expressed as Mean ± SD.

OBSERVATIONS AND RESULTS

Observation during preparation of extracts of *Varuna Twak* and *Tavaksheeri kanda*

Observation	Aqueous extract of *Varuna*	Ethanolic extract of *Varuna*	Aqueous extract of *Tavaksheeri*	Ethanolic extract of *Tavaksheeri*
Quantity of drug	250 gm	250 gm	250 gm	250 gm
Extract Obtained	27.5 gm	25.5 gm	14.5 gm	10 gm
Yield of extract	11%	10.1%	5.8%	4%
Consistency	Semisolid, sticky	Semisolid, sticky	Semisolid, sticky	Semisolid, Sticky
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Astringent	Astringent	Sweet, bitter	Sweet, bitter
Colour of Extract	Blackish brown	Blackish brown	Pale yellowish	Yellowish brown

Physical Constituents of *Varuna Twak* and *Tavaksheeri Kanda*

Parameters	Findings *(Varuna)*	API Standard *(Varuna)*	Findings *(Tavaksheeri)**
Foreign matter	Nil	Not more than 2%	Nil
Loss on Drying**	9.2%	-	11.03%
Total ash	8.090%	Not more than 13%	0.43%
Acid insoluble ash	0.015 %	Not more than 1%	0.073%
Alcohol soluble extractive value	8.9%	Not less than 1%	2.13%
Water soluble extractive value	17.13%	Not less than 8%	1.57%
pH value	6.01	-	6.68

*All the parameters of *Tavaksheeri* were done by triplicate method as standard values were not available in API.

**As API Standards was not available for loss on drying of *Varuna* it was done by triplicate methods.

HPLC OF TRIAL DRUG *VARUNA*

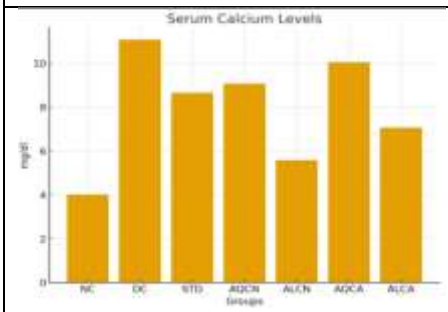
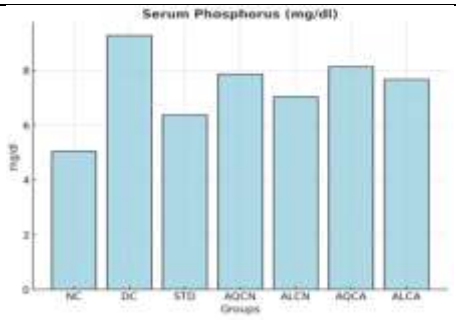
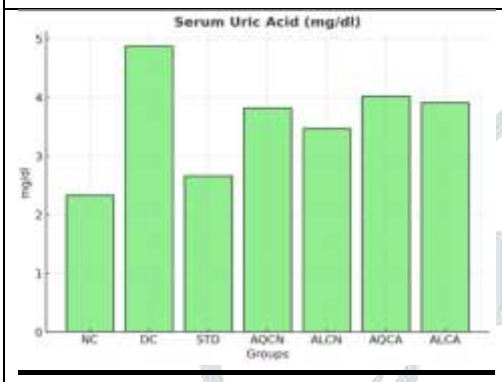
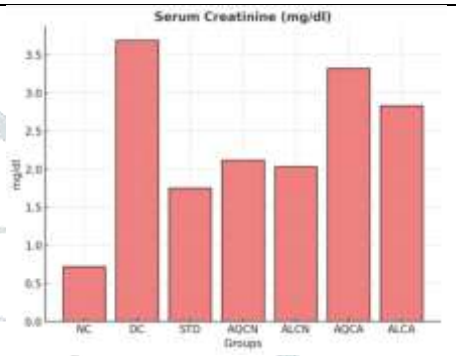
Test Sample	Reten. Time (min)	Area (mV.s)	Amount (w/w% of sample)
Standard Quercetin	4.457	17894.490	-
AQCN	4.473	84.680	0.5
ALCN	4.430	2254.878	12.6

HPLC OF TRIAL DRUG *TAVAKSHEERI*

Test Sample	Reten. Time (min)	Area (mV.s)	Amount (w/w% of sample)
Standard Curcumin	6.633	12656.562	-
AQCA	6.683	23.510	0.2
ALCA	6.697	74.479	0.6

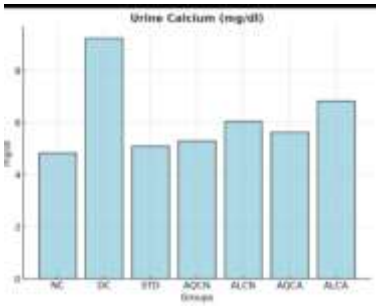
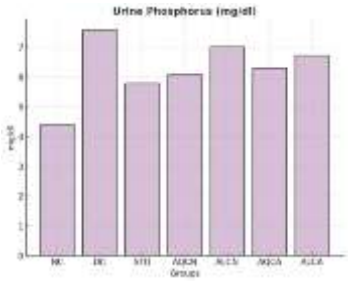
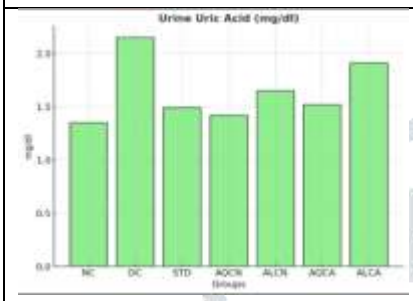
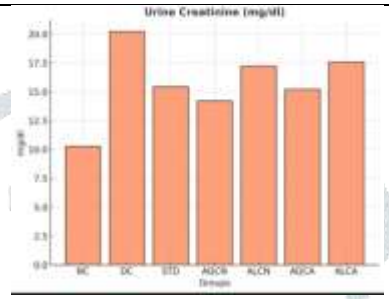
Serum Parameters of Experimental Study

Groups	**Serum Calcium**	**Serum phosphorus**	**Serum Uric acid**	**Serum creatinine**
Normal control	4.01 ± 0.75	5.04 ± 0.80	2.33 ± 0.33	0.72 ± 0.15
Disease Control	11.08 ±0.46	9.28 ± 0.56	4.88± 0.42	3.69 ± 0.16
STD	8.65 ± 0.67	6.37± 0.63	2.66 ± 0.18	1.75 ± 0.23
AQCN	9.07 ± 0.07	7.87 ±0 .38	3.82± 0.29	2.12 ± 0.41
ALCN	5.58 ± 0.28	7.04 + 0 .75	3.47 ± 0.17	2.03± 0.22
AQCA	10.05 ± 0.65	8.67± 0.42	4.02± 0.37	3.32 + 0.32
ALCA	7.06 ± 0.05	8.13 ± 0.52	3.91± 0.13	2.83 ± 0.42

SERUM CALCIUM	SERUM PHOSPHORUS
	
SERUM URIC ACID	**SERUM CREATININE**
	

Urine parameters of Experimental Study

Groups	Calcium	Phosphorus	Uric acid	Creatinine
Normal control	4.83 ± 0.38	4.42 ±0.03	1.35 ± 0.13	10.26 ± 0.26
Disease Control	9.24± 0.42	7.58 ± 0.47	2.15± 0.23	20.21± 1.78
STD	5.09 ± 0.45	5.79± 0.33	1.49± 0.12	15.43± 0.63
AQCN	5.29+ 0.16	6.08 + 0.56	1.42 ± 0.56	14.20 ± 2.01
ALCN	6.05 + 0.24	7.02 + 0.49	1.65 + 0.26	17.21± 1.78
AQCA	5.63+ 0.29	6.30 ± 0.35	1.52 + 0.37	15.21+ 0.34
ALCA	6.83+ 0.37	6.73 + 0.66	1.91± 0.14	17.58+ 0.81

URINE CALCIUM	URINE PHOSPHORUS
	
URINE URIC ACID	**URINE CREATININE**
	

Volume of Urine

Groups	Day 1st (ml)	Day 14th (ml)	Day 28th (ml)
Normal Control	4.5± 0.23	5.6±0.27	5.1± 0.36
Disease Control	3.7±0.34	1.5±0.06	2.5± 0.51
STD	4.8± 0.06	2.2±0.13	3.4 ± 0.26
AQCN	3.34±0.56	1.8±0.17	4.6± 0.33
ALCN	3.8± 0.40	2.3±0.52	3.7 ± 0.22
AQCA	4.3±0.02	1.67±0.34	4.8 ± 0.34
ALCA	4.65±0.52	1.93±0.42	4.2 ± 0.21

- Based on observations diuretic effect of trail drugs found to be AQCA > AQCN>ALCA>ALCN.

Body Weight of Experimental Animals

Days	Mean Value						
	NC	DC	STD	AQCN	ALCN	AQCA	ALCA
1ST day	34.7 ± 0.4	33.4 ± 0.2	31.2 ±0.6	36.8 ± 0.2	36.0±0.2	37.5 ±0.8	37.9± 0.6
14th day	34.3 ±0.8	33.5± 1.0	30.6± 0.8	36.6 ±1.1	35.8±1.2	38.3 ±1.2	37.3±1.1
28th day	35.1±0.4	32.5	31.4 ± 0.2	35.5 ±1.3	34.6±1.4	37.1 ±0.4	36.2±0.1

In comparison Significant weight gain was observed in AQCA when compared to other treatment groups.

Figure No5: Images of Experimental Study

Animal cage

Urine collection

Urine samples

Oral gavaging

Anesthesia

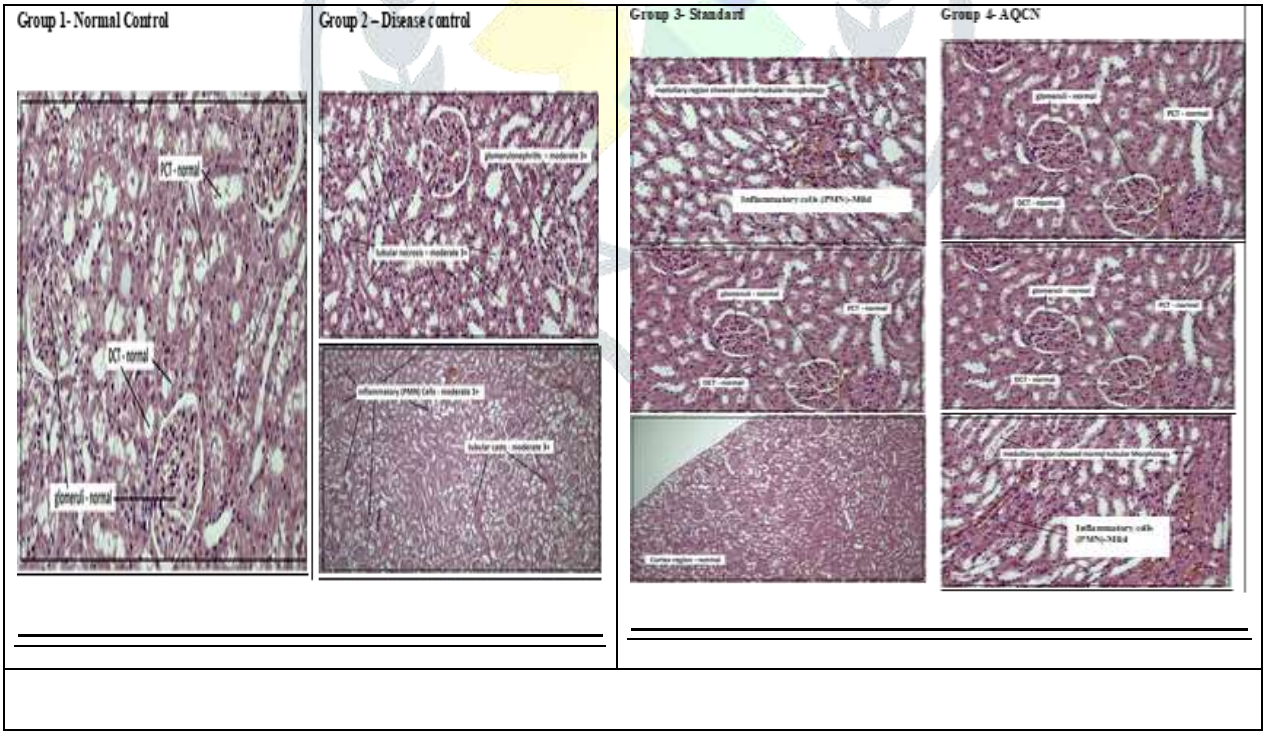
Retro-orbital blood collection

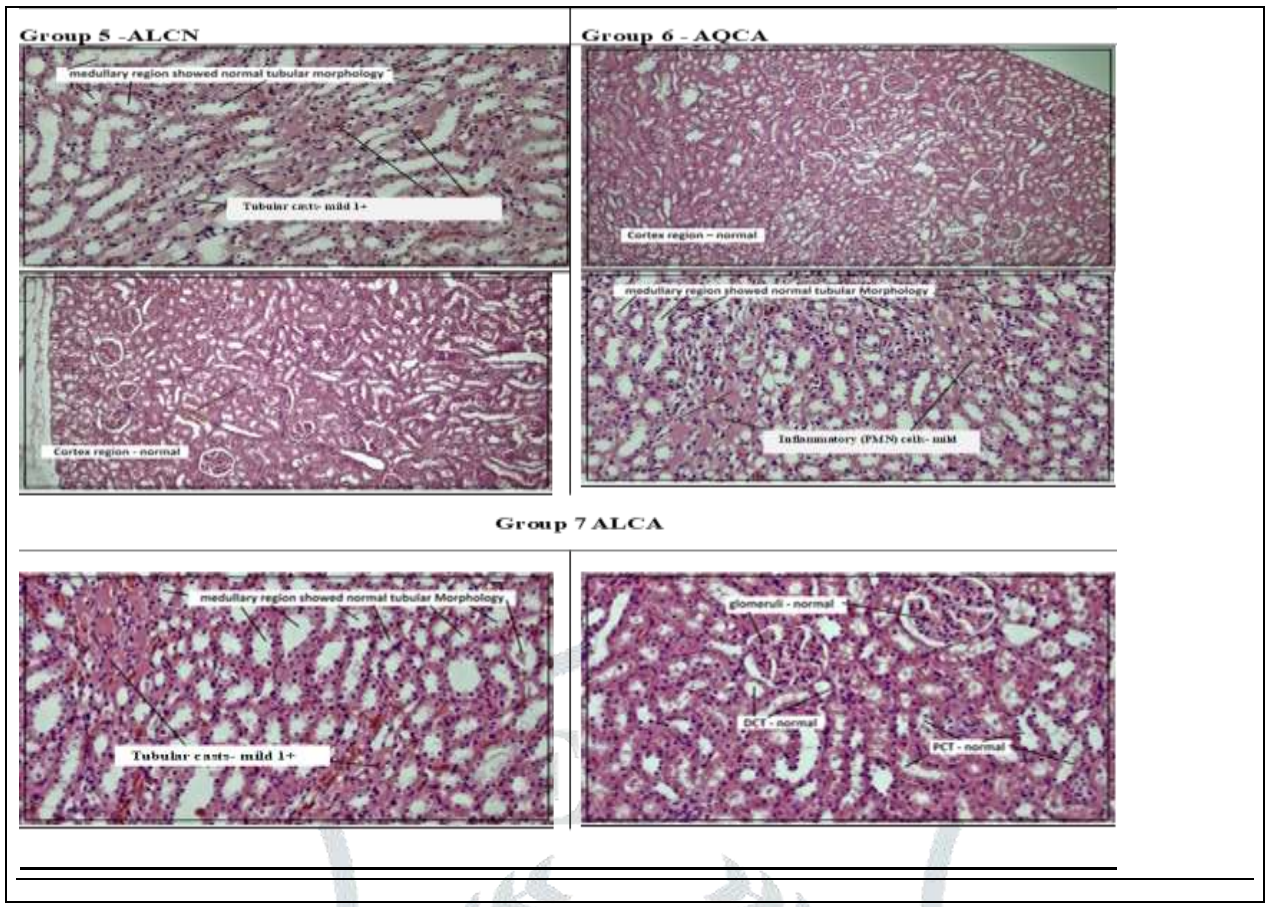
Dissection

Excised Kidneys

Histopathology of Experimental Animals

Groups		Observations
Group 1	Normal Control	Histopathological studies show normal cortex, glomeruli, distal convoluted tubule and proximal convoluted tubule.
Group 2	Disease Control	Histopathological studies show **tubular necrosis moderate-3+, glomerulonephritis – moderate -3+, tubular casts- 3+ &inflammatory cells**.
Group 3	Standard	Histopathological studies show normal medullary morphology and **mild inflammatory cells**.
Group 4	AQCN	Histopathological studies show **mild inflammatory cells**, & normal glomeruli, distal convoluted tubule and proximal convoluted tubule
Group5	ALCN	Histopathological studies show **mild tubular casts**; normal cortex & medullary morphology.
Group 6	AQCA	Histopathological studies show **mild inflammatory cells;** cortex& medullary region appears to be normal.
Group 7	ALCA	Histopathological studies show **mild tubular casts.**





DISCUSSION

In this study *Tavaksheeri* was compared with *Varuna* with respect to its *Ashmarighna karma.* The powder microscopy of *varuna* showed the presence of stone cells, fragments of lignified fibres& prismatic cells which confirms its identity& *Tavaksheeri* showed the presence of abundant starch grains, parenchymatous cells& fragments of lignified fibres which confirms its identity.

Total ash values of *varuna and tavaksheeri* were 8.09 % & 0.43% respectively[6]. Acid insoluble ash values of *varuna and tavaksheeri* (triplicate method) were 0.015 % & 0.073% respectively, which are within the standards, confirms the genuinity. The Retention time of Aqueous extract and alcoholic extract of *Varuna are* 4.473 and 4.430 respectively corresponds to standard quercetin i.e. 4.457, Quercetin has proven antioxidant, anti-inflammatory and anti-urolithiatic activity[7]. The retention time of Aqueous extract and alcoholic extract of *tavaksheeri* are 6.683 and 6.697 respectively corresponds to standard curcumin i.e. 6.633, Curcumin also has proven antioxidant, anti-inflammatory and anti-urolithiatic activity. Model selected for this study is ethylene glycol induced urolithiasis.[8]

Ethylene glycol can be easily administered through drinking water. Also, urolithiasis can be induced within 2-4 weeks and have similarity to human pathology (mainly calcium oxalate will be formed). [9]

Varuna due to its *Kshaya, Madhura, Tikta rasa, Laghu -Ruksha guna, Ushna virya & Bhedana karma* act as lithotripsic as well as healing renal tissue damage. It is also known as *Ashmarighna*[10]*. Tavaksheeri* due to its *Madhura Rasa, Laghu-Snigdha Guna & Vrana ropana karma* act as lithotripsic as well as healing renal tissue damage.[11]

Tavaksheeri found to have statistically significant effect on serum and urine parameters also in histopathological studies, so it can be used clinically as *Ashmarighna Dravya. Tavaksheeri* found to be more *mutrala* (diuretic) and *bhrumana* (nourishing) when compared to *Varuna.*

In case of serum parameters alcoholic extract of *Tavaksheeri* showed better effect than its aqueous extract. In case of urine parameters aqueous extract of *Tavaksheeri* showed better effect than its alcoholic extract. In case of serum parameters alcoholic extract of *Varuna* showed better effect than its aqueous extract. In case of urine parameters aqueous extract of *Varuna* showed better effect than its alcoholic extract.

In comparison *Varuna* have better Anti-urolithiatic activity when compared to *Tavaksheeri (*also have statistically significant Anti-urolithiatic activity*) and Tavaksheeri* have better diuretic activity when compared to *Varuna.*

CONCLUSION

The study confirmed the authenticity and quality of both *Varuna* and *Tavaksheeri* through pharmacognostic, physicochemical, and phytochemical analyses. HPLC quantification further validated the presence of key bioactive markers—quercetin in *Varuna* and curcumin in *Tavaksheeri*. Based on OECD guidelines, the selected doses of 500 mg/kg for *Varuna* and 400 mg/kg for *Tavaksheeri* were found to be safe and effective. Both drugs produced significant improvements in serum and urine parameters, with *Varuna* exhibiting comparatively superior anti-urolithiatic activity. *Tavaksheeri,* however, demonstrated a more pronounced diuretic effect, as it has statistically significant anti-urolithiatic activity supporting its traditional claim as *Ashmari hara*. Histopathological findings also revealed notable regenerative changes in renal tissues in both treatment groups. Overall, the study suggests that while Varuna remains more potent in correcting biochemical alterations, *Tavaksheeri* offers a promising and sustainable alternative with beneficial diuretic and protective effects in urolithiasis.

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