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Acute Toxicity Sudy of Eladi Quatha: A Compound Ayurvedic Formulation

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Abstract

In the modern era, the Indian Ayurvedic system of Medicine is believed to treat diseases from the root cause. The ayurvedic system claims to have many indigenous plants and herbal preparations, which provide therapeutic benefit in the treatment of kidney diseases. ***Aim –*** *To study the benefits of herbal preparation Eladi quatha for kidney disease Urothalisis.* ***Method -*** *This study was undertaken to explore the effects of Eladi quatha in animals to treat kidney stone diseases. However, no safety profile for this formulation has been reported to date; thus, in this study, freshly prepared Eladi quatha was evaluated for acute toxicity. Acute toxicity test was evaluated as per OECD 423 guidelines with 5000 mg/kg as a limit test in Wistar strain Albino rats. Test formulations were administered to overnight fasted animals and parameters like body weight, behavioral changes, and mortality were assessed for 14 days. Hematological and biochemical parameters were assessed on the 14th day after administration.* ***Result -*** *No significant changes were noted in terms of behavioral changes, mortality, and body weight. The samples did not affect any of the hematological parameters. However, an increase in blood urea level was observed.* ***Conclusion-*** *This study concludes that freshly prepared Eladi quatha is relatively safe up to the dose of 5000 mg/kg. However, further chronic toxicity evaluation is necessary to establish its safety profile on chronic administration.*

Key words - *Eladi quatha, Urothalisis, ayurvedic, toxicity evaluation, Albino rats.*

1. Introduction

Kidney stones or urinary tract stones are known by a variety of names, including nephrolithiasis/urolithiasis[1], which is the formation of stones along the nephrolithiasis or urinary tract, specifically in the nephron or the kidneys. To further complicate the issue, kidney stones or urinary can also be called renal or urinary calculi. Calculi are stones[2]. However, these terms are used interchangeably, but they all refer to kidney stones. Overall clinical manifestations of this complaint are governed by the size and nature of crystals, whereas urinary chemistry is an important factor in determining the type of crystals formed and the nature of macromolecules included on the crystals' surface. As a result, studying urinary chemistry in relation to stone-forming minerals provides a good indication of the risk of stone formation.

The formation of urine stones is a multistep process that includes nucleation, aggregation, and growth. However, the underlying mechanism is not well understood. In general, urine becomes supersaturated with crystal-forming minerals, which precipitate in the urinary system. The volume and pH of urine vary during the physiological process of urine formation, which promotes mineral crystallization in urine. In most cases, the presence of inhibitor protein compounds in the urine prevents crystal formation [3].

The factors that increase the likelihood of developing a kidney stone a high protein diet [4], high salt diet, male, Caucasian, obesity, dehydration, medications such as antacids, carbonic anhydrase inhibitors, and other drugs

such as indinavir, acyclovir, sulfadiazine, and triamterene used to treat four different types of infections [5]. These can cause crystallization of your intake and the formation of stones. Medications containing sodium and calcium also increase the risk of developing kidney stones[6]. Crystal urea is also a risk factor, as is a family history. These risk factors will cause a number of effects. For starters, some of these risk factors will increase urinary solute concentrations, including calcium, uric acid, calcium oxalate, and sodium [7]. Renal stones can be caused by hypertension, obesity, or gout.

Kidney disease is still regarded as a global and recurring health problem. Urolithiasis is a complex condition caused by a series of physicochemical occurrences within the kidneys [8]. Through the passage of stones in the urinary tract system, stones can cause a variety of symptoms such as pain, obstruction, infection, and hemorrhage [9]. Treatment and management of renal stones relies on surgical techniques, such as extracorporeal shock \swave lithotripsy, percutaneous lithotripsy, and transurethral lithotripsy. These surgeries are complex and costly, and they have no effect on stone recurrence [10]. So, the study of herbal medicine has got importance.

Unfortunately, traditional or synthetic drugs used to treat kidney diseases have serious side effects. Different plants, plant extracts, and plant preparations are used to treat Urolithiasis in the Indian system of medicine [11]. Only a few plants, plant parts, and plant preparations used in traditional medicine have been pharmacologically evaluated for safety and efficacy. Due to a lack of scientific validation of efficacy and safety, most people overlook the Ayurvedic approach[12].

The Indian traditional medicine system is a rich source of valuable medicinal plants, but no scientific data has been reported to establish the activity of these plants. As a result, for drug development, these plants must be evaluated for biological efficacy and chemical constituents[13].

It is estimated that traditional medicine is used to treat 80 percent of the world's population. Medicinal plants have a long history of use and are far safer than synthetic drugs on a global scale. They are a trustworthy source for drug discovery [14]. Today, researchers are concentrating on drug discovery from medicinal plants. It is estimated that at least one-third of all medicinal products are derived from plants [15]. Medicinal plants are regarded as an acceptable, low-cost, readily available, and safe source of active compounds for pharmaceutical applications. Medicinal plants' therapeutic effects on kidney and urinary tract disorders have been studied extensively, and their efficacy has been demonstrated [16,17].

Eladi quatha is an Ayurvedic compound formulation described in Chakradutta for the treatment of kidney diseases, particularly Urolithiasis [18]. This formulation is widely used by physicians to treat Urolithiasis. However, no safety profile for this formulation is currently available. As a result, biological assays, animal models, clinical trials, and chemical standardization are required as scientific proof for the formulation. In the current study, fresh samples of Eladi quatha were chosen for acute toxicity testing in order to assess the benefits in Urolithiasis.

Eladi quatha is a classical Ayurvedic formulation prepared from eight ingredients like:
Amomum subulatum Linn. (Zingiberaceae)
Piper longum Linn. (Piperaceae),
Madhuca longifolia Var. (Sapotaceae),
Bergenia ligulata Wall. (Saxifragaceae),
Vitex negundo Linn. (Labiatae),
Tribulus terrestris Linn (Zygophyllaceae)
Justicia adhatoda Linn. (Acanthaceae),
Ricinus communis Linn. (Euphorbeaceae)

The aim of the study was to evaluate Eladi quatha using biological methods. The first step in quality control is to ensure the quality of the ingredients used in the preparation of Ayurvedic medicine[19]. As a result, all of the ingredients used in the preparation of the quatha were authenticated by an approved authority by studying their morphology, anatomy, powder microscopy, physical constants, and phytochemical profile. Different batches of Eladi quatha were prepared using traditional methods and evaluated organoleptically and physically[20]. Eladi quatha was evaluated for its urolithiasis action in Albino Wistar rats.

2. Materials and Methods

Procurement and preparation of plant material

Collection of ingredients and authentication

The crude drugs mentioned in Chakradutta and Bhaishajya Ratnavali for the preparation of Eladi Quatha were purchased from the Department of Ayurveda, Parul University (Gujarat, India), and authentication was done by the Department of Botany, Parul Institute of Applied Sciences, Parul University (Gujarat, India). The foreign impurities were removed and then crude drugs were washed with water, sorted, and sun-dried under shade below 45°C. Dried drugs were stored in tightly closed containers.

Reparation of Eladi Quatha

The formulation of Eladi qwatha is mentioned in Bhaishajya Ratnavali and it contains the following drugs: Different parts of plants contain active constituents that have been tested for urolithiasis activity[21,22].

Table 1: Composition of Eladi quatha

Sr No.	Name of the drug (Part used)	(Part used)	Botanical Name	Family	Quantity taken in gm
1.	Ela	Fruit	*Amomum subulatum* Linn.	Zingiberaceae	3.75 gm
2.	Pippali	Fruit	*Piper longum* Linn.	Piperaceae	3.75 gm
3.	Madhuca	Seed	*Madhuca longifolia* Var.	Sapotaceae	3.75 gm
4.	Pashanabheda	Leaves	*Berginia ligulata* Wall.	Saxifragaceae	3.75 gm
5.	Nirgundi beeja	Seed	*Vitex negundo* Linn.	Lamiaceae	3.75 gm
6.	Gokshura	Fruit	*Tribulus terrestris* Linn.	Zygophyllaceae	3.75 gm
7.	Vasa	Leaves	*Justicia adhatoda* Linn.	Acanthaceae	3.75 gm
8.	Eranda	Seed	*Ricinus communis* Linn.	Euphorbeaceae	3.75 gm

Method of Preparation:

The Eladi quatha was prepared as per the standard process described in the quatha vidhi of Sarngadhara Samhita [23]. Three batches of the quatha were prepared. The dried crude drugs were taken as per mention in the formula. The herbs were ground and converted into a coarse powder. Then added with 4 parts of water and subjected to mild heat with infrequent stirring without covering the vessel. The reduction was done until the amount reduced to 1/4th of its original volume and contents were filtered through the double-folded clean cloth into a stainless-steel vessel and then the residue was discarded.

Standardization of Prepared Eladi quatha

Eladi quatha is subjected to numerous standardization parameters consistent with WHO Guidelines as follows[24]. The standardization of herbal drug is very vast and massively very high, but according to the. guidelines set by WHO [25] herbal drugs can be often summarized. Thus, the standardization of quatha was done as follows.

Qualitative analysis tests

The quatha formulation was evaporated on a water bath for the removal of water, the dried mass was refluxed with methanol three times and filtered. The methanol extract was subjected to preliminary phytochemical analysis. The ethanol extract was prepared in the same manner and phytochemical analysis was carried out. Dragendorff's test for Alkaloids, flavonoids, Ferric chloride test for tannins, Keller killiani test for glycosides, steroids, reducing sugars, starch and monosaccharides was carried out by using appropriate chemical reagents[26].

Acute toxicity study

a. Animals:

Albino Wistar rats weighing 180 - 200 g were used for the study in a group of six animals in each group (3 males and 3 females in each group). The experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee, Parul Institute of Pharmacy and Research, Parul University (Gujarat, India). The animals were housed in a group of three in a separate cage made up of polypropylene with stainless steel grill in an air-conditioned environment. The temperature of the environment was 25 ± 2 ºC and 40 to 60% humidity with a 12 h light-dark cycle. Pelleted standard rat food was given and drinking water was given ad. Libitum.

b. Acute oral toxicity study procedure

The acute oral toxicity study of freshly prepared Eladi quatha was conducted in compliance with OECD guideline 425.[27] Total of twelve animals were selected and each group (normal and control) has three animals (Wistar albino male and female rats) were fasted overnight (~12 h) and weighed. Water was provided during the fasting period[27-28]. Test doses of the sample was calculated for 5000 mg/kg administered via the oral route. The animals were observed continuously for 6 hours after the dosing for behavioral changes like increased or decreased motor activity, convulsions, fur color muscle spasm and relaxation, salivation, diarrhea. All the animals were continuously observed for mortality and weight change during the study period. The animals were constantly observed for 14 days [28-29].

On the 14th day, blood was collected for estimation of hematological and biochemical parameters. The hematological parameters such as Hemoglobin, WBC count, RBC count, Platelets counts, MCV, MCHC, Neutrophils, Lymphocytes, Eosinophils, and Monocytes were observed [30]. The biochemical parameters measured were blood sugar, serum creatinine, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, serum albumin, and serum globulin, serum protein [31,32].

On the 15th day of the study, one rat from each group was sacrificed by as per CPCSEA guideline. Necropsy and gross examination on internal organs (liver, heart, kidney, lungs, and spleen) were carried out at Histo VCL Laboratories (Vadodara, Gujarat) Identified organs were fixed in 10% buffered formalin solution and then histopathological examinations performed. Organs were preserved in paraffin wax for histological examination. Histology sections at a thickness of 4 µm were obtained using a microtome. They were stained with hematoxylin and eosin and observed under a light microscope (400x Magnification). A consultant histopathologist recorded pathological changes of the tissue sections in test groups in comparison to the control group and corresponding photomicrographs were taken.[33,34].

c. Statistical Analysis

All the qualitative data were expressed as mean ± Standard Error of Mean (SEM). Every statistical analysis was performed with one-way analysis of variance (ANOVA) followed by the t-test using GraphPad Prism 8.0.0 for Windows. Statistically significant differences were accepted at $p \leq 0.05$.

3. Results and Discussion

Evaluation of Eladi quatha

Physical analysis of each batch of the formulation was performed. The organoleptic characteristics were found same for all the batches the organoleptic evaluation of prepared Eladi quatha was summarized in the following table:

Table 2: Organoleptic evaluation of three batches Eladi quatha

Sr No.	Parameters	Observation		
		EQ1	EQ2	EQ3
1	Color	Dark brown	Dark brown	Dark brown
2	Odor	Characteristics	Characteristics	Characteristics
3	Taste	Bitter	Bitter	Bitter
4	Appearance	Clear	Clear	Clear

EQ1 = Eladi Quatha batch no. 1, EQ2 = Eladi Quatha batch no. 2, EQ3 = Eladi Quatha batch no.

Qualitative analysis of formulation

Table 3: Qualitative analysis of three batches Eladi quatha

Phytoconstituents	Test	Observations		
		EQ1	EQ2	EQ3
Alkaloids	Dragendorff's test	+	+	+
	Mayer's test	+	+	+
Tannins	Ferric chloride test	+	+	+
Flavonoids	Shinoda test	+	+	+
Carbohydrates	Fehling test	+	+	+
Glycoside	Keller–killiani test	+	+	+
Steroids	Salkowski test	-	-	-
Reducing sugar	Benedict test	+	+	+
	Fehling test	+	+	+
Starch	Iodine test	+	+	+

Monosaccharides	Barfoed's test	-	-	-

Acute Study

Table 4: Mean body weight of test and control group of rats

Groups	Initial body weight (g)	Final body weight (g)	Actual change (g)
Control	203 ± 7.73	211.4 ± 5.55	8.4 ± 2.18
Test	204.00 ± 11.20	210.40 ± 10.80	6.4 ± 0.4

Data =Mean +/- SEM, * P < 0.05 unpaired T Test ANOVA (p): < 0.0001 when compared with control group and Test group.

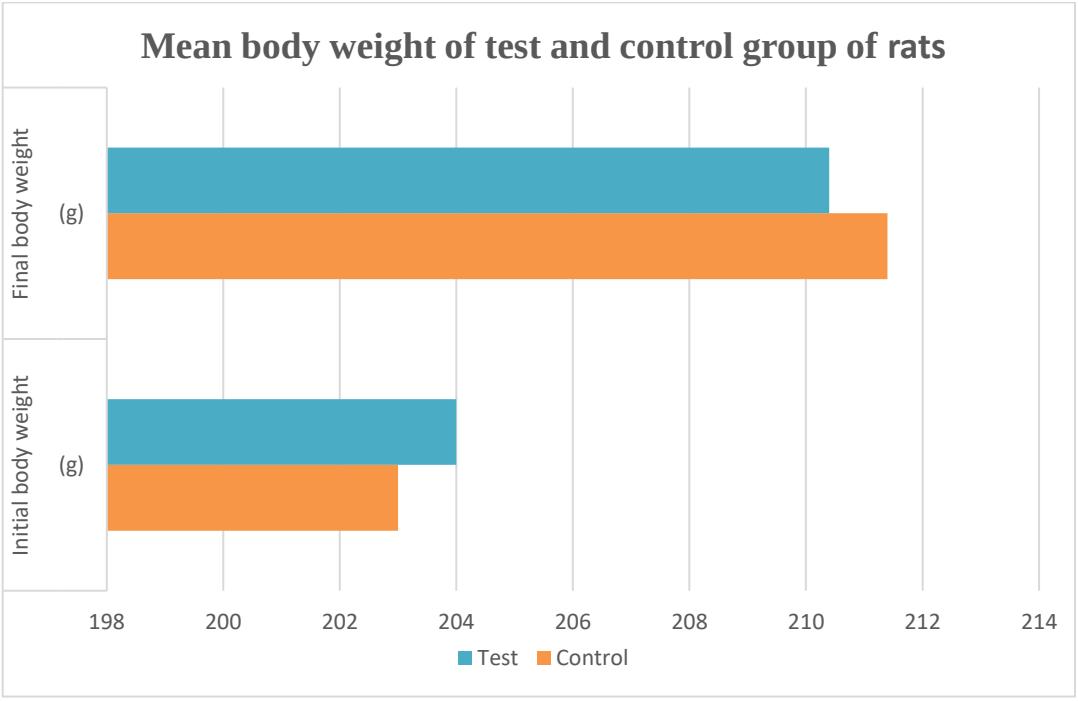

Fig. 1 - Mean body weight of test and control group of rats

Hematological parameters of male and female rats are shown in the following tables.

Table 5: Hematological analysis of Male & Female rats

Parameter/ Units	Control (Mean ± SEM) Male	Control (Mean ± SEM) Female	Test (Mean ± SEM) Male	Test (Mean ± SEM) Female	P Value
Hemoglobin g/dL	13.77± 0.25	12.98± 0.30	14.4± 0.22	12.4± 0.22	< 0.0001 when compared with control group and Test group
RBC Million/µL	8.05± 0.24	6.05± 0.22	7.11± 0.05	7.35± 0.05	
WBC g /µL	7766.6±720.3	7653.6± 340.6	8963.3± 3127.4	8963.3± 2724.4	
Neutrophils %	18.18± 1.63	5.1± 0.25	30.8± 0.64	5.1± 0.25	
Eosinophils %	2.1± 0.25	2.12± 0.18	2.5± 0.25	2.0± 0.20	
Lymphocytes %	63.05±2.2	61.21± 0.14	62.7± 3.4	61.18± 0.12	
Monocytes %	2.21± 0.15	2.17± 0.09	2.18± 0.16	2.19± 0.06	
Platelets mm^3	130.64± 0.13	54.4± 0.17	105.72± 0.21	56.12± 0.26	
MCV fL	54.4± 0.17	21.73± 0.21	56.12± 0.26	24.32±0.27	
MCHC %	21.73± 0.21	45.05± 3.4	24.32±0.27	34.7± 4.4	

Data of Male & Female Rat – Mean +/- SEM P < 0.05 unpaired T Test
ANOVA (p): < 0.0001 when compared with control group and Test group
RBC = Red Blood Cell, WBC = White Blood Cell, MCV = Mean Corpuscular Volume, MCHC = Mean Corpuscular Hemoglobin Concentration.

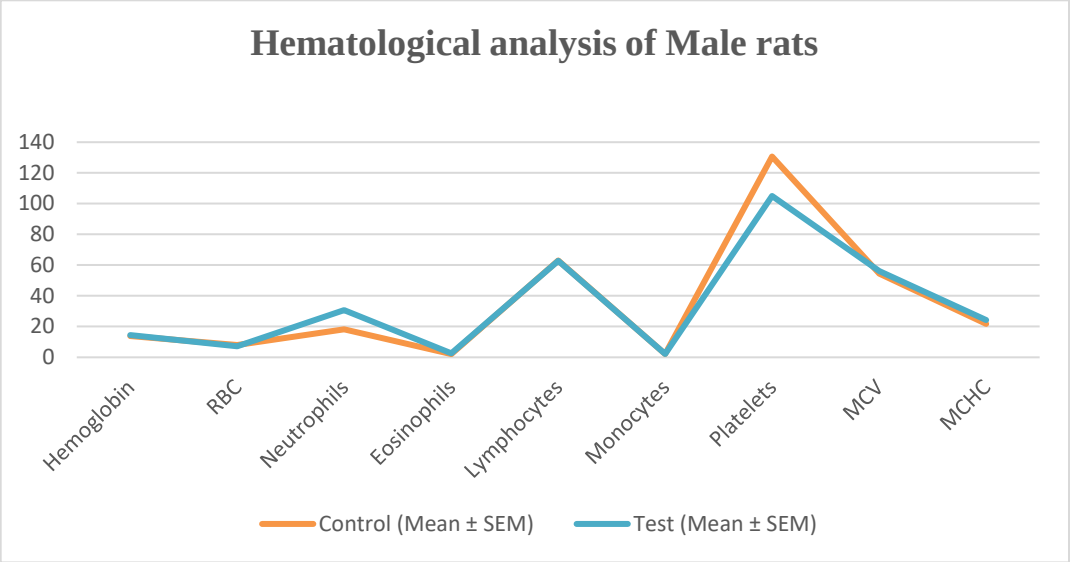

Fig. 2 - Hematological analysis of Male rats

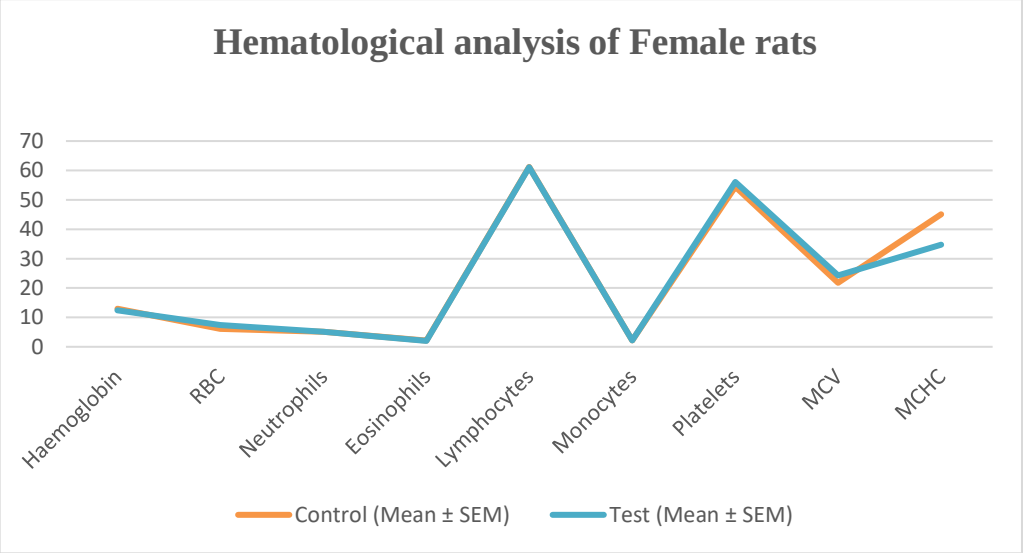

Fig. 3 - Hematological analysis of Female rats

Standardization of prepared formulation is a very important aspect to maintain and prove the quality, purity, and efficacy. The WHO specifies the guidelines for the assessment of the safety, efficacy, and quality of herbal medicines [23]. The aim of the study was to analyze a classical Ayurvedic formulation, Eladi quatha by pharmacogenetic, phytochemical, and chromatographic analyses and evaluate its anti-urolithiasis potential. In the preparation of Eladi quatha, the classical method was used. The data of standardization of this quatha is not available to date. Therefore, in the present study standards for the preparation and evaluation of Eladi quatha were developed.

The raw materials were evaluated and compared to standards for assurance of the quality of raw materials. Three formulations as per Ayurveda were prepared and evaluated. The results generated from these studies are discussed.

The Eladi quatha was prepared and evaluated in three batches. All three formulations are having characteristic color, odour and taste. Qualitative analysis of formulation revealed the presence of alkaloids, tannins, flavonoids, carbohydrates, glycoside, starch, reducing sugar, and non-reducing sugar may be due to the presence of many herbal drugs. The presence of important constituents confirms the therapeutic activity of the formulation.

No mortality and/or signs of morbidity were observed for 14-days of acute toxicity study. Any pernicious change in body weight, biochemical, and hematological parameters along with relative organ weight were not

observed for the Eladi quatha treated group. Change in body weight is an important factor to monitor the health of an animal. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No salivation, diarrhea, lethargy or unusual behaviors were observed. There were no other toxic effects observed for both male and female rats within the study period. After analysis of blood, no significant variation was observed in the test group about hematological and biological parameters when compared to the control group. The graphical presentation also makes it clear that no significant variation in the results.

Similar Acute toxicity study done by **Kalpu N. Kotecha et al in 2013** [35] had reported similar results. , in test drug administered groups like control group, gain in body weight was observed and the magnitude of body weight gain was comparatively high in market sample of administered group. Furthermore, at 5 000 mg/kg, sample did not produce any observable toxic effects during entire duration of study and all animals survived 14 days of observation.

The doses of 1000, 1500, and 2000 mg/kg were safe. As there was no mortality recorded for all the doses, the LD50 value was assumed to be greater than the limit test dose of 2000 mg/kg, body weight. Hence 125, 250 and 500 mg/kg, oral doses were selected to evaluate sub-acute toxicity study.

Histopathological comparison of vital internal organs revealed no abnormalities in the tissue structure and cell structure in the test group compared to the normal control group. Light microscopic examination of organs included liver (Fig. 4), heart (Fig. 5), lung (Fig. 6), kidney (Fig. 7), and spleen (Fig. 8) showed unnoticeable differences in the histological and cellular structures of all organs. In the liver, the cellular structures of hepatocytes, sinusoids, and central veins were similar to those in the normal control group. In the heart, the cellular structures of cardiac muscle cells were normal. In the lung, the cellular structures of the bronchiole, alveoli, alveolar duct, and blood vessel were normal. Similarly, no abnormalities were observed in the spleen and kidney of the rats of the test group compared to the normal control group.

In the similar study done by **Basavaraj M. Dinnimath et al. 2017** [36] on Antiurolithiatic activity of natural constituents reported similar hepatological results like they had observed no significant tubular damage, hemorrhage, disrupted brush border and tubular congestion in the kidney sections (cortex) of the rats.

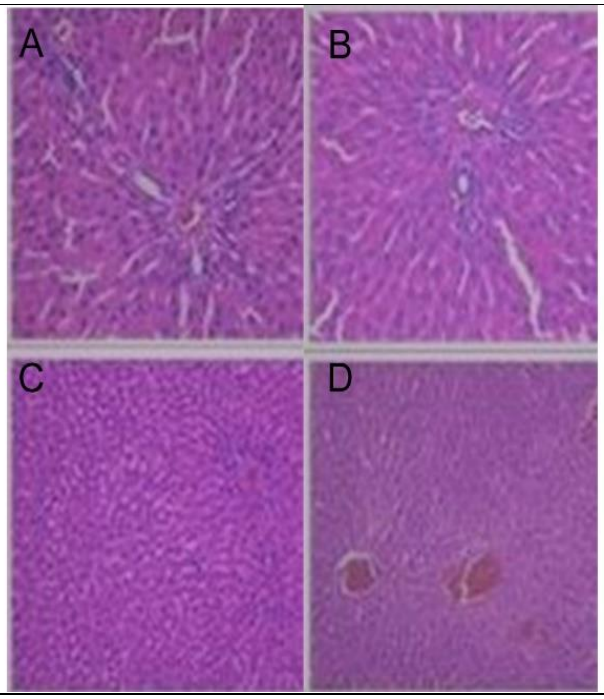

Fig. 4: Histopathology of Liver
(A - Control, male; B – Test, male; C – Control, female; D – Test, female)

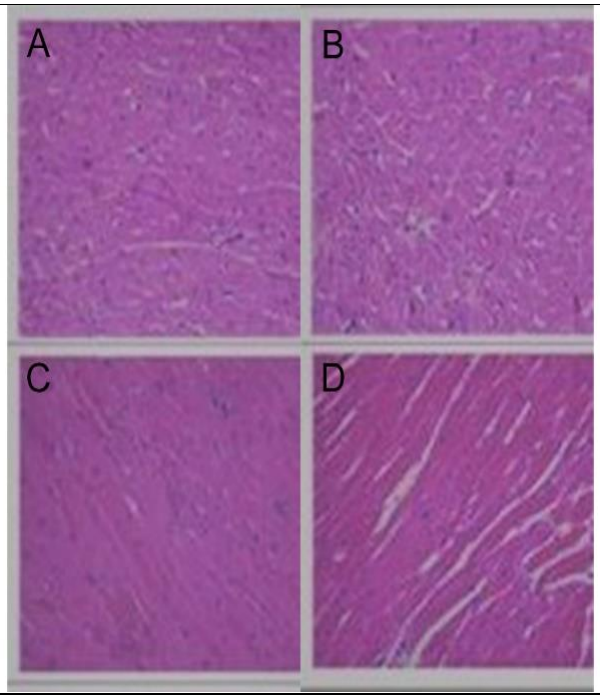

Fig. 5: Histopathology of Heart
(A - Control, male; B – Test, male; C – Control, female; D – Test, female)

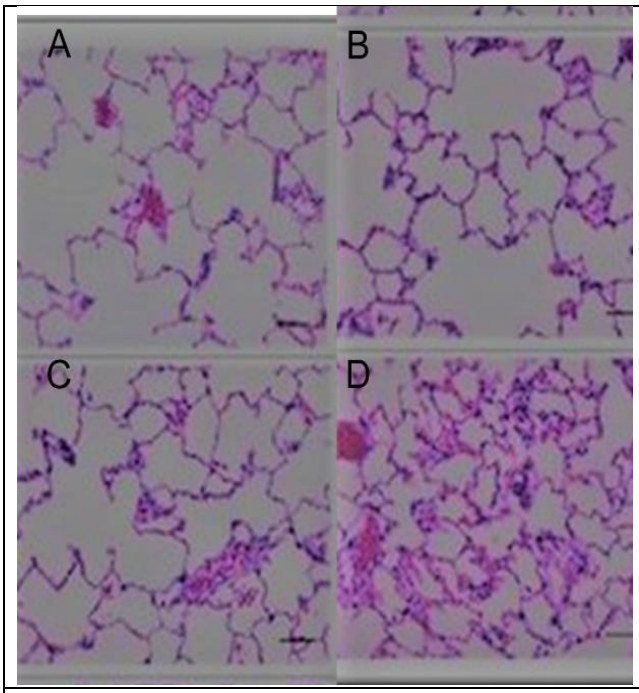

Fig. 6: Histopathology of Lung
(A - Control, male; B – Test, male; C – Control, female; D – Test, female)

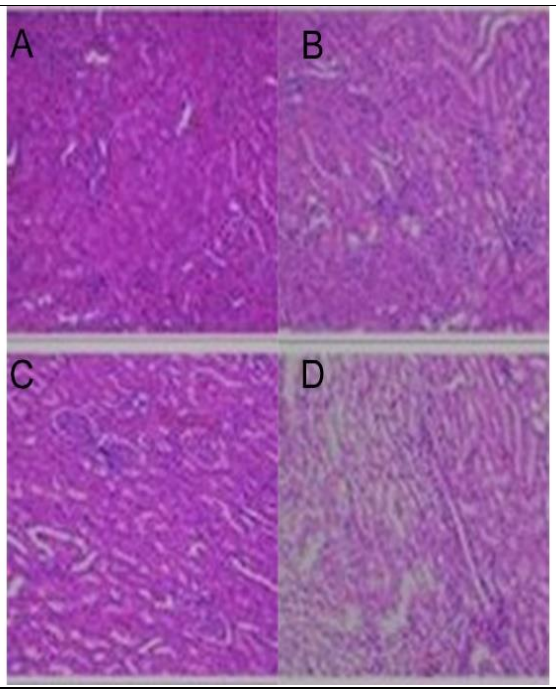

Fig. 7: Histopathology of Kidney
(A - Control, male; B – Test, male; C – Control, female; D – Test, female)

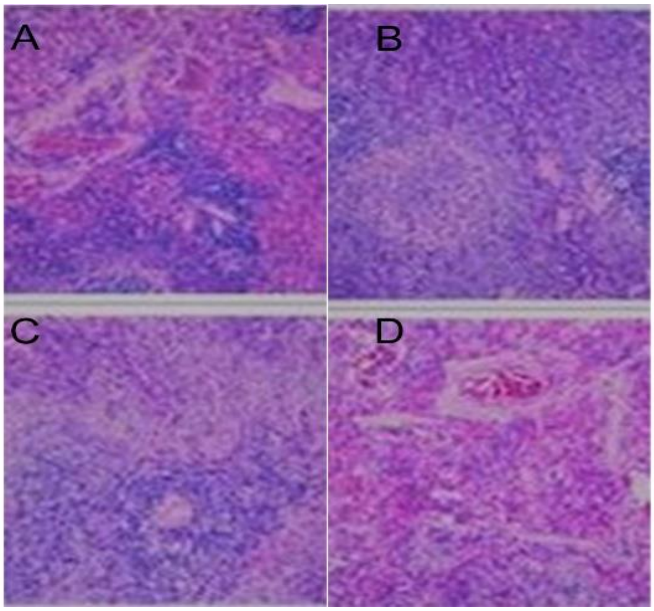

Fig. 8: Histopathology of Spleen
(A - Control, male; B – Test male; C – Control female; D – Test female)

4. Conclusion

The results indicated no toxic effect of Eladi quatha in acute dose administration with good and safety window for single-dose administration. Moreover, there were no changes in any hematological, biochemical, physical, or behavioral changes. Eladi quatha is showing good results with no mortality hence, supporting for therapeutic use in Urolithiasis. Further study needs to do for the determination of dose of administration for repeated dosing and subsequent dose optimization.

Declaration of interest

The authors declare that there is no conflict of interest.

References:

1. Leveridge, M., D'Arcy, F.T., O'Kane, D., Ischia, J.J., Webb, D.R., Bolton, D.M., et al. Renal Colic: Current Protocols for Emergency Presentations. Eur J Emerg Med. 2016; 23 2-7 .
2. Goldfarb, D.S., Avery, A.R., Beara-Lasic, L., Duncan, G.E. and Goldberg, J.A Twin Study of Genetic Influences on Nephrolithiasis in Women and Men. Elsevier Kidney Int Rep. 2019; (4) 535-540.
3. Keddis MT, Rule AD. Nephrolithiasis and loss of kidney function. Curr Opin Nephrol Hypertens, 2013;22:390–396.
4. Siener, R. Dietary Treatment of Metabolic Acidosis in Chronic Kidney Disease. Nutrients, 2018 (10) 4 512.
5. Maalouf, N.M., Moe, O.W., Adams-Huet, B. and Sakhaee, K. Hypercalciuria Associated with High Dietary Protein Intake Is Not Due to Acid Load. J Clin Endocrinol Metab, 2011; 96(12):3733-40
6. Li, K., Wang, X.-F., Li, D.-Y., Chen, Y.-C., Zhao, L.-J., Liu, X.-G., et al. The Good, the Bad, and the Ugly of Calcium Supplementation: A Review of Calcium Intake on Human Health. Clin Interv Aging, 2018 pg. no. 2443-2452.
7. Carbone, A., Al Salhi, Y., Tasca, A., Palleschi, G. et al. Obesity and Kidney Stone Disease: A Systematic Review. Minerva Urologica e Nefrologica, 2018 ; 70 (4)pg. no.393-400.
8. Prajapati R, Kalariya M, Parmar S and Sheth N. Phytochemical and pharmacological review of Lagenaria sicereria, J of Ayurveda and Inte Med 2010; 1(4): 266-72.
9. Mandavia, D.R.; Patel, M.K.; Patel, J.C.; Anovadiya, A.P.; Baxi, S.N.; Tripathi, C.R. Anti-urolithiatic effect of ethanolic extract of Pedalium murex linn. fruits on ethylene glycol-induced renal calculi. Urol. J. 2013; 10, 946–952. [PubMed]
10. Miller, N.L.; Lingeman, J.E. Management of kidney stones. Br. Med. J. 2007; 334, 468.
11. Amos T. N., Bashir L., Saba S. E., et al. Phytochemicals and acute toxicity profile of aqueous and methanolic extracts of Crateva adansonii leaves in swiss albino rats. Asian Journal of Biochemistry. 2015;10(4):173–179.
12. Viegas C., Jr., Bolzani S. V., Barreiro J. E. Os produtos naturais e a química medicinal moderna. Química Nova. 2006;29(2):326–337.
13. Suman. Herbs: an alternative approach in nephroprotection. Res J Pharmacognosy Phytochem 2013;5:15e21.
14. Mohsenzadeh A, Ahmadipour SH, Ahmadipour S, Asadi-Samani M. A review of the most important medicinal plants effective on cough in children and adults. Der Pharmacia Lettre. 2016;8:90–6.
15. Saki K, Bahmani M, Rafieian-Kopaei M. The effect of most important medicinal plants on two important psychiatric disorders (anxiety and depression)-a review. Asian Pac J Trop Med. 2014;7:34–42.
16. Asadbeigi M, Mohammadi T, Rafieian-Kopaei M, Saki K, Bahmani M, Delfan B. Traditional effects of medicinal plants in the treatment of respiratory diseases and disorders: an ethnobotanical study in the Urmia. Asian Pac J Trop Med. 2014;7:S364–8.
17. Gupta A, Chaphalkar SR. Anti-inflammatory and immunosuppressive activities of some flavonoids from medicinal plants. J HerbMed Pharmacol. 2016;5:120–4.
18. Chakrapani dutta, Chakra Dutta Reprint edition, Choukhambha Sanskrit Bhavan; 2010 Varanasi.
19. Benzie I. F. F., Wachtel-Galor S. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd. Boca Raton, FL, USA: CRC Press, Taylor & Francis; 2011.
20. Birdi T., Daswani P., Brijesh S., et al. Newer insights into the mechanism of action of Psidium guajava L. leaves in infectious diarrhoea. BMC Complementary and Alternative Medicine. 2010;10(33):1–11.
21. Nagal A, Singla RK. Herbal resources with antiurolithiatic effects: a review. Indo Glob J Pharm Sci 2013 vol 3, pg 114.
22. Patel PK, Patel MA, Saralai MG, Gandhi TR. Antiurolithiatic activity of saponinrich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl.(Solanaceae) against ethylene glycol induced urolithiasis in rats.J Ethnopharmacol 2012;144:160e70.
23. Gopalapilla S.A. Sarngadhara samhita with hridayapriya interpretation. (Malayalam) 1998, chapter 2, 5th ed. Vol. 133. 1998. verse 143-145.
24. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems, World Health Organization Geneva 2004.
25. Barbalho M. S., Farinazzi-Machado V. M. F., Goulart A. R., et al. Guajava (guava): a plant of multipurpose medicinal applications. Medicinal and Aromatic Plants. 2012;1(4):1–6.
26. Sanda K. A., Grema H. A., Geidam Y. A., Bukar-Kolo Y. M. Pharmacological aspects of Psidium guajava*:*

an update. International Journal of Pharmacology. 2011;7(3):316–324.
27. OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method.
28. Ishtiaq S, Akram M, Kamran SH, Hanif U, Afridi MSK and Rehman Su: Acute and sub-acute toxicity study of a Pakistani polyherbal formulation. BMC Complementary and Alternative Medicine 2017; 17(387): 1-13.
29. Upadhyay P, Shukla R, Mishra SK: Acute and sub-acute toxicity study of hydroalcoholic leaves extract of Reinwardtia indica in rats. Biomedicine and Pharma-cotherapy 2019; 111: 36-41.
30. Dongmo OLM, Epoh NJ, Tadjoua HT, Yousuf S, Telefo PB and Tapondjou LA: Acute and sub-acute tocicity study of the aqueous extarct from the stem bark of Tetrapleura tetrapteura Taub (Fabacae) in mice and rats. Journal of Ethnopharmacology 2019; 236: 42049.
31. Schlottfeldt Fdos, S.; Fernandes, S.M.; Martins, D.M.; Cordeiro, P.; Fonseca, C.D.; Watanabe, M.; Vattimo Mde, F. Prevention of amphotericin B nephrotoxicity through use of phototherapeutic medication. Rev. Esc.Enferm. USP **2015**, 49, 74–79.
32. Noorafshan, A.; Karbalay-Doust, S.; Karimi, F. Diosmin reduces calcium oxalate deposition and tissue degeneration in nephrolithiasis in rats: A stereological study. Korean J. Urol. **2013**, 54, 252–257.
33. Prabhu, V.V.; Sathyamurthy, D.; Ramasamy, A.; Das, S.; Anuradha, M.; Pachiappan, S. Evaluation of protective effects of diosmin (a citrus flavonoid) in chemical-induced urolithiasis in experimental rats.Pharm. Biol. **2016**, 54, 1513–1521.
34. Lima Rd, Guex CG, da Silva ARH, Lhamas CL, Moreira KLdS and Casoti R: Acute and subacute toxicity and chemical constituents of the hydroethanolic extract of Verbena litoralis Journal of Ethnopharmacology 2018; 224: 7684.
35. Kalpu N. Kotecha, B. K. Kotecha,[1] Vinay J. Shukla,[2] Pradipkumar Prajapati et al Acute toxicity study of Vasaguduchyadi Kwatha*:* A compound Ayurvedic formulation Ayu. 2013 Jul-Sep; 34(3): 327–330.
36. Basavaraj M. Dinnimath et al Antiurolithiatic activity of natural constituents isolated from Aervalanat, Journal of Ayurveda and Integrative Medicine 2017 Volume 8, Issue 4 Pages 226-232