



Antiuro lithiatic Evaluation of α - Mangostin Fraction Isolated from *Garcinia mangostana* Pericarp through Computational, *in vitro* and *in vivo* Approach

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Authors' contributions

This work was carried out in collaboration between both authors. Both the authors have contributed equally. The idea was generated by author PS as a supervisor and the work was carried out and finished by author AK. Both authors read and approved the final manuscript.

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ABSTRACT

Background: The aqueous crude extract of *Garcinia mangostana* fruit pericarp was already proven to contain antiuro lithiatic property. Based on this previous study the current study was focused on analysing the anti-uro lithiatic property of α - mangostin, a xanthone polyphenol isolated from the fruit pericarp of *G. manostana*, which has not been tested for its anti-uro lithiatic property till now.

Objective: The aim of this present study is to evaluate the anti-uro lithiatic property of the isolated α - mangostin from *G. mangostana* fruit pericarp using *in silico*, *in vitro* and *in vivo* analysis.

Study Design: Antiuro lithiatic activity of α - mangostin through Molecular docking study \rightarrow *In vitro* S.S.M model study \rightarrow Animal studies.

Place and Duration: Department of Biotechnology, Sri Venkateswara College of Engineering, Post Bag No.1, Pennalur, Sriperumbudur Tk, Kancheepuram Dt, TN-602117, India.

Materials and Methods: *In silico* Molecular docking of α - mangostin with Kidney stone forming proteins- Xanthine dehydrogenase (Xdh), Oxalate oxidase and Tamm-Horesefall Protein (THP) were performed using AutoDock 4.0 and was visualised in Discovery studio software. *In vitro*

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Simultaneous Static flow Model (S.S.M) was performed to investigate its Anti-urolithiatic property against Calcium Oxalate (CaOx) and Calcium Phosphate (CaP) crystals. Based on the *in silico* and *in vitro* analysis, the study was extrapolated to Ethylene Glycol (EG) induced urolithiasis rat models. The animal study was performed with 36 Albino *Wistar* rats which were divided into 6 groups. All group except group I received EG (0.75% in drinking water) for the induction of Urolithiasis for 28 days under curative regimen. Group III was administered orally with Cystone (750 mg/kg) from 15th to 28th day. Group IV to VI was administered orally with GMPE (300 mg/kg, 500 mg/kg and 750 mg/kg) from 15th to 28th day.

Results: Molecular Docking studies showed an inhibitory interaction of α - mangostin with oxalate oxidase, Xdh and THP with binding affinity of -4.47, -4.00 and -3.41 Kcal/mol respectively. S.S.M showed 54.71% inhibition for CaOx crystals and 62.21% inhibition of CaP crystals. The animal studies showed significant results in reduction of serum calcium ($P<0.01$), serum phosphate ($P<0.01$), urine calcium ($P<0.001$) and urine phosphate ($P<0.01$).

Conclusion: Thus, α - mangostin proved to be potent Anti-urolithiatic agent by reducing and disintegrating the urinary crystals.

Keywords: α - mangostin; antiurolithiasis; ethylene glycol; S.S.M.

1. INTRODUCTION

Kidney stone disease is one of the common metabolic disorder which is termed scientifically as Urolithiasis (Uro = Urinary system; Lithiasis = stone formation) or Nephrolithiasis (Nephro = Kidney; Lithiasis = stone formation). The stone is formed mainly due to the accumulation of four main mineral compounds such as Calcium, Struvite, Uric acid and Cystine. Based on the composition of stone formed they are classified as Calcium stone, Struvite stone, Uric acid stone and Cystine stone [1]. Urine Supersaturation of these minerals followed by series of process including Nucleation of minerals, Growth & Aggregation of crystals and retention within renal tubules lead to the formation of calculi in the kidney [2]. Based on the position of the stone formed they are classified as Kidney stone, Ureteral stone and bladder stone [3]. Kidney stone disease is one of the diseases that appears recurrently in 70-81% male and 47-60% female in the total affected population [4]. By this factual report it is evident that males are more prone to kidney stone than females. And this is mainly because women typically excrete more citrate and less calcium than men [3]. Currently the treatment undertaken for Kidney stone disease and its major side effects are 1) Allopathy medication - renal injury and stone recurrence 2) Extracorporeal Shock Wave Lithotripsy (ESWL) - traumatic effects, acute renal injury, stone recurrence, possibility of infection 3) Open renal Surgery – Unusual [5]. Thus, phyto-therapy or herbal-therapy is predominantly favored and preferred now a days

by most of the specialist since it has reduced, or no side effects.

Till now many fruits and vegetables are known to dissolve the renal stones. But the base and reason to carry this work was to use the waste inedible part (here rind) of the fruit (*Garcinia mangostana*) which is rich in polyphenolic content to treat urolithiasis. In this current study a polyphenol named α - Mangostin was isolated from the inedible rind of the fruit and analyzed for its efficiency in treating urolithiasis. In such a way the waste biomass which possess antioxidant, antimicrobial, antiinflammatory, ACE-inhibition and diuretic properties could be utilised to formulate it as a potent anti-urolithiatic drug.

It was reported that among the 68 different xanthenes that has been identified in various parts of the *G. mangostana* plant, 50 are present in its pericarp. The most bountiful xanthone present in the rind of mangosteen fruit is α - mangostin. Others than α - mangostin, the other xanthenes present in the rind are γ , β -mangostin, gartanin, 8-deoxygartanin, garcinones A, B, C, D and E, mangostinone, 9-hydroxycalabaxanthone and isomangostin [6]. α - mangostin is greatly answerable for its therapeutic properties like antimicrobial agent, anti-oxidant, anti-proliferation, antiinflammatory, antiobesity and actuates apoptosis [7].

The current antiurolithiatic study started with molecular docking work, then moved to *in vitro* S.S.M analysis and then finally studied on animals (Fig. 1).

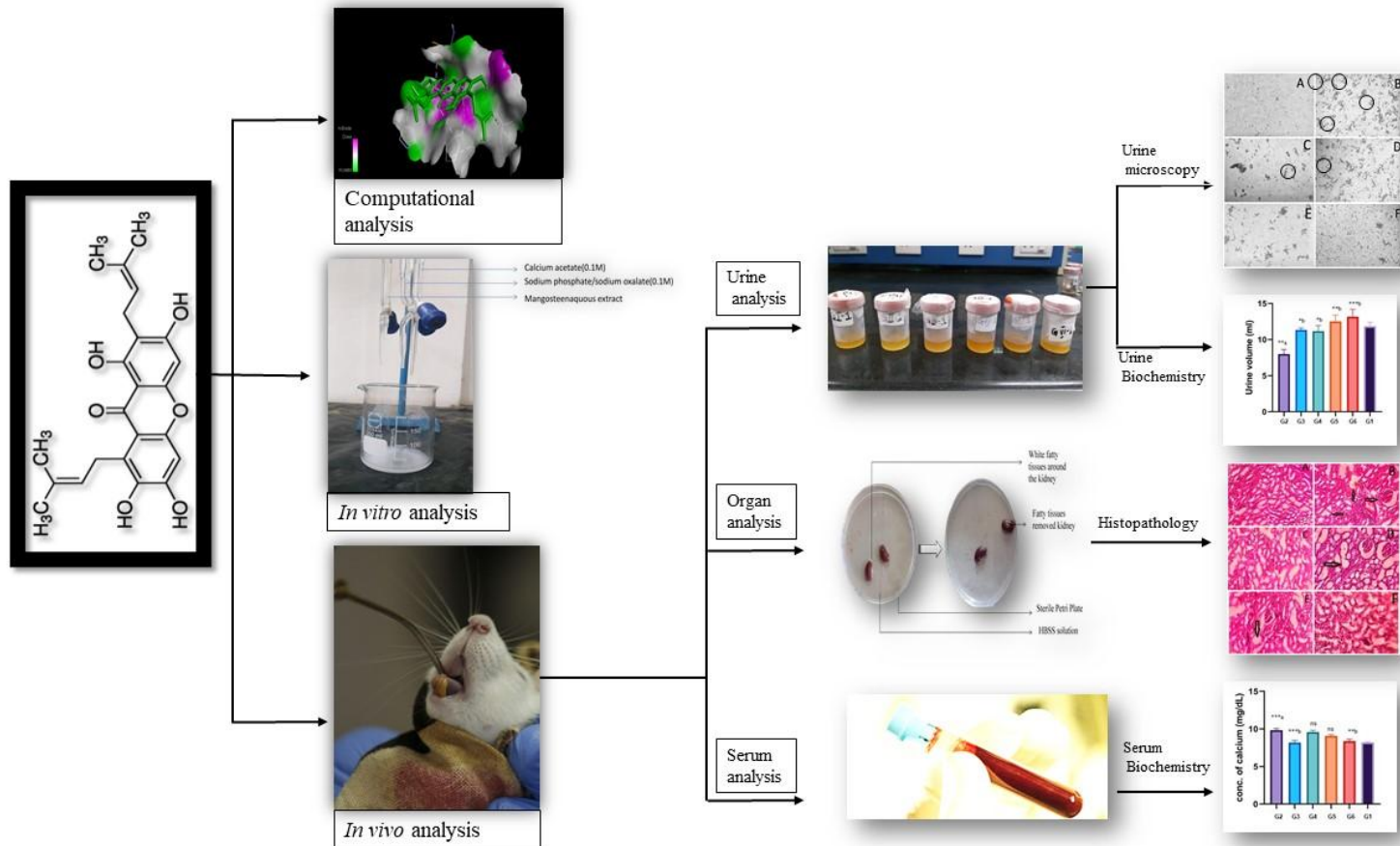


Fig. 1. The overall workflow

Xanthone polyphenols are generally reported to have antioxidant and diuretic property. As antioxidants, polyphenols were reported to inhibit Angiotensin-Converting Enzyme (ACE) and suppress *Renin-Angiotensin-Aldosterone System* (RAAS) in renal cells. The above property helps in avoidance and desolution of renal stone. As diuretics it builds the amount of liquid going through Kidney and flushes out all atomic level crystals [8,9]

Though, many standard drugs like Cystone, Calcuri and Chandraprabha bati, were globally marked as polyherbal anti-urolithiatic formulation [10], this study was focused on determining the anti-urolithiatic potential of single compound effect of isolated α - Mangostin rich fraction.

2. METHODOLOGY

2.1 Chemicals Used

Calcium chloride dihydrate, Sodium oxalate, Sodium phosphate, Benzene, Methanol, Pentobarbitone sodium and TFA were purchased from Sigma-Aldrich Ltd. Cystone® was purchased from Himalaya Drug Company

2.2 Software Used

The software used in this study were AutoDock Tool (Autodock 4) and Biovia Discovery Studio 2020 client.

2.3 Preparation of Crude Methanol Extract

300 g of Dried *G. mangostana* fruit pericarp extract powder was extracted with 1500 ml of methanol using Soxhlet apparatus to obtain the liquid crude extract E1. E1 was then subjected to vacuum roto-evaporator at 65°C under 500 mm Hg to evaporate the solvent and the crude extract E2 was collected. The yield % was calculated [11].

2.4 Purification of α - Mangostin Fraction

E2 crude extract was then washed with Demineralized water in the ratio of 1:1 (w:v) with constant stirring and left undisturbed for 1 to 3 H for settling which is then filtered using Whatmann No. 1 filter paper. This process separates the settled crude extract from the water. The water is discarded and the extract was dried under vacuum which gives water washed crude extract

E3. 50 g E3 was dissolved in 50 ml Benzene, heated at 50°C and then filtered again. The filtrate collected was then subjected to vacuum roto-evaporator at 50°C under 500 mm Hg to evaporate the solvent and dry. The pale yellow colored shiny powder obtained was again dried in low temperature and powdered in mortar. This dried and purified α - Mangostin rich Fraction (AMF) was then characterized using HPLC and was used for the further studies in this research work [11].

2.5 HPLC Characterization of α -Mangostin

The purified fraction containing α - Mangostin was subjected to Analytical HPLC (RP-C18, 150X4.6 mm, SS, Exsil ODS 5 μ M particle size). The mobile phase consisted of 0.1% TFA in water (Eluent A) and 0.1% TFA in methanol (Eluent B). A flow rate of 1.0 ml/min was fixed. 20 μ l of the fractionate sample was introduced through the rotary injection valve and UV detection was recorded at 242 nm [11].

2.6 Prediction of Drug Likeness of α -Mangostin

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) property and the druglikeness of α - mangostin was determined by Lipinski's rule of 5. The SMILES of α - Mangostin obtained from PubChem (PubChem CID: 5281650) was loaded on SwissADME (<http://www.swissadme.ch/>) and various physicochemical properties of α - Mangostin was tabulated and verified for any objection from Lipinski's rule of 5 which enlists certain limitations for a compound to be an effective oral drugs [12].

2.7 Molecular Docking of α - Mangostin on Kidney Stone Forming Proteins

Molecular docking was carried out with α -Mangostin (Ligand- PubChem CID: 5281650) and Xdh [13] (PDB ID: 1JRP), THP [14] (PDB ID: 4WRN) and Oxalate oxidase [15] (PDB ID: 2ETE) (Receptors) in Autodock Tools. Ligand structure was downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and the receptors structure were downloaded from PDB (<https://www.rcsb.org/pages/help/advancedsearch/pdbIDs>). Native ligands of these selected proteins such as Oxypurinol (PubChem CID: 135398752) for Xdh and acetylglucosamine (PubChem CID: 24139) for Oxalate oxidase and

THP were chosen as control. Protein molecule and ligand molecules were prepared separately and used in "PDBQT" for further docking process in AutoDock Tool. In Autodock 4, Kollaman charges were added to the protein structure using AutoDock tool. The Grid for docking calculation was centered to cover the protein-binding site residues and accommodate ligand to move freely. During the docking procedure, a Lamarckian genetic algorithm (LGA) was used for flexible ligand rigid protein docking calculation. The docked complex was visualized in Biovia Discovery Studio 2020 client Visualizer software. Binding energy, Number of Hydrogen bond formations, interacting atoms and distance of bond formation were noted, tabulated and compared with positive control.

2.8 In Vitro Antiurolithiatic Study-Simultaneous Static Flow Model

The inhibition of CaOx and CaP mineralization by AMF was evaluated by Simultaneous flow Static Model (S.S.M) [16]. Different test concentration of AMF (100 mg/ml, 200 mg/ml, 300 mg/ml and 500 mg/ml), Calcium acetate (0.1 M) and Na₂C₂O₄ (0.1 M) (for calcium oxalate) or sodium phosphate (for calcium phosphate) were filled (25 ml) in three separate burettes and were allowed to fall simultaneously into an empty clean beaker with a slow and steady pace. For 10 min, the mixture was kept in hot water bath and then cooled to room temperature. Supernatant was discarded and the precipitate formed was collected into a pre-weighed Petri plate. The precipitate collected in plates were dried in a hot air oven at 120°C, cooled and weighed. Weight of the dry precipitate was recorded. Then percentage inhibition was calculated by following formula:

$$\% \text{ Inhibition of CaOx/CaP} = \frac{(\text{Weight of ppt. in blank set} - \text{Weight of ppt. in experimental set} \times 100)}{\text{Weight of ppt. in blank set}}$$

2.9 Animals Maintenance

Male *Wistar* albino rats with 150 to 250 g body weight were used in this study. They were housed in well ventilated polypropylene cages with an air conditioning system and regulated temperature at 24°Celsius and humidity at 50 to 60% on 12 hrs day and night cycle using artificial lights. Animals were fed with commercial feed pellets for Rats and distilled water *ad libitum*.

2.10 Grouping of Animals

Animals were divided into six different groups containing six animals each. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups except for negative control group till the 28th day to induce urolithiasis. In this curative regimen study, all the groups except negative control group received AMF at different concentrations (300 mg/kg, 500 mg/kg and 750 mg/kg once in a daily by oral gavage) from 15th day to 28th day.

- Group – I: Negative Control
- Group – II: Ethylene glycol (0.75%) in drinking water (positive control)
- Group – III: Ethylene glycol (0.75%) in drinking water + Cystone (750 mg/kg) → 15th to 28th day (standard control)
- Group – IV: Ethylene glycol (0.75%) in drinking water + Test concentration 1 AMF (300 mg/kg) → 15th to 28th day
- Group – V: Ethylene glycol (0.75%) in drinking water + Test concentration 2 AMF (500 mg/kg) → 15th to 28th day
- Group – VI: Ethylene glycol (0.75%) in drinking water + Test concentration 3 AMF (750 mg/kg) → 15th to 28th day

2.11 Measurement of Physical Parameters

The physical parameters such as change in body weight, water intake and weight of the kidney were measured.

2.12 Urine Biochemistry

Animals were caged in separate metabolic cages and the urine was collected separately. Urine volume was measured using the measuring cylinder and expressed in mL. The acidity of the urine was tested using a pH meter. Urine was then analyzed for calcium, phosphate, and magnesium in the collected urine samples using separate commercial kits and the results were expressed in mg/dl.

2.13 Microscopic Analysis of Urine

1 ml of urine sample was taken from each group of experimental animals and was centrifuged at 10,000 rpm for 15min. The supernatant were discarded, and the collected pellets were suspended in 1 ml of PBS

buffer. The microscopic slide was wiped properly with ethanol using cotton. 1 drop of PBS containing suspended crystals were taken for microscopic analysis and viewed under 10X light microscope and observed the presence and nature of CaOx and CaP crystals. The images of crystals were photographed and recorded.

2.14 Biochemical Analysis of Serum

On the 30th day after the collection of urine, blood was collected from the animals after anaesthetising with Pentobarbitone sodium (35 mg/kg body wt, i.p.). The animals were lied front side down and upper front side was cut open using sterile forceps and scissors to draw 5 ml of blood from heart by cardiac puncture method. From the collected blood, serum was separated by centrifugation at 10,000 rpm for 10 min and was analyzed for biochemical parameters such as creatinine, urea nitrogen and uric acid using separate commercial kits. The results were expressed in mg/dl. Then both the kidneys were identified and removed from the animals for histo-pathological studies.

2.15 Histo-Pathological Analysis of Kidney Tissues

Both the isolated kidneys from each animal were washed with Hanks' Balanced Salt Solution (HBSS). The fatty layer around the organ were cut and removed carefully without damaging the tissue. Then they were fixed in 10% neutral-buffered formalin. The stored tissue was cut into 5 μ m thin sections, stained with Hematoxylin-Eosin (H & E) and observed under optical light binocular microscope for histopathological examination. Then the carcass of the experimental animals was disposed immoderately by handing over to the bio-waste management firm.

2.16 Statistical Analysis

The results were expressed as mean \pm SEM. Statistical significance between means was analyzed by One Way Analysis of Variance followed by "Dunnett's test" where $P < 0.05$ was considered less significant, $P < 0.01$ was considered as significant, $0.01 < P < 0.001$ was

considered as moderately significant, $P < 0.0001$ was considered statistically highly significant.

3. RESULTS AND DISCUSSION

3.1 HPLC Characterization of α -Mangostin

The yield % was about 30% for 300 g of dried pericarp powder. The AMF fraction obtained through series of process that was subjected to HPLC showed the presence of α -mangostin in it. It was characterized by the peak produced at retention time 23.982 min at 242 nm (Fig. 2.B). This was confirmed by comparing it with the peak produced by standard α -mangostin which showed peak at 23.312 min at 242 nm (Fig. 2.A). This was also in accordance with Pradeep et al. [11]. Thus, the purified AMF extract fraction confirmed the presence of α -mangostin.

3.2 Prediction of Drug Likeness of α -Mangostin

It was reported that the drug should satisfy at least to some extent among the basic molecular features like molecular weight (130- 725), hydrogen bond donor (>5), hydrogen bond acceptor (>10), and Octanol-water coefficient ($\log P > 5$) [12]. From Table 1, it was found that the various molecular features of α -mangostin where satisfying Lipinski's rule of 5 except hydrogen bond donor (3) and hydrogen bond acceptor (6). Moreover, it was reported that the Bioavailability score should be 0.55 for a neutral organic compound that satisfy Lipinski's rule to act as a good oral drug [17]. The Bioavailability score (0.55) showed it to be a good enduring compound for Gastro-intestinal absorption through oral injection. Therefore, α -Mangostin proved to act as a good oral drug.

Table 1. Molecular properties of α -Mangostin

Molecular Weight (g/mol)	410.46
miLogP	6.32
No. of H-Bond Acceptor	6
No. of H-Bond Donor	3
No. of Rotatable Bonds	5
Bioavailability Score	0.55

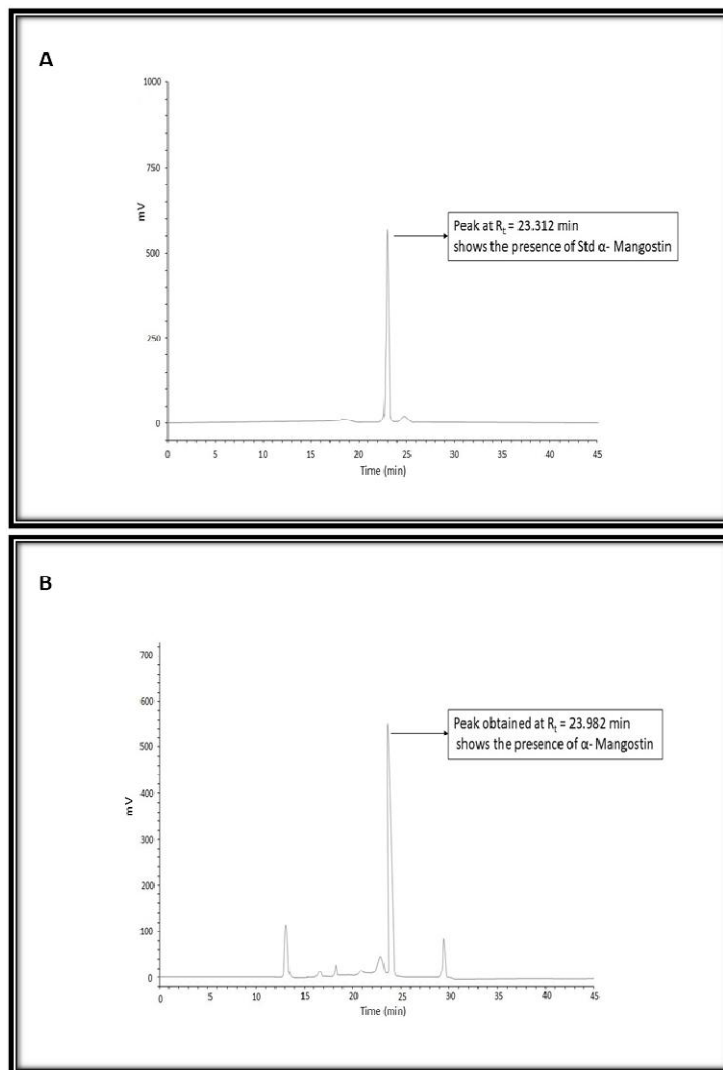


Fig. 2. HPLC characterisation of α -Mangostin in AMF

3.3 Molecular Docking of α -Mangostin with Kidney Stone Forming Proteins

The molecular docking study with α -Mangostin as inhibitor of proteins involved in Kidney Stone forming helps us understanding the potential of α -Mangostin as Anti-urolithiatic agent. It was reported that APRTase is involved in nucleotides recycling. But Xdh was reported to degrades the APRTase, inducing kidney stone by formation of DHA which is one of the key components in kidney stone formation. Molecular docking of α -Mangostin with Xdh was done to predict the inhibition of mutated Xdh [13]. It was reported that THP of stone formers have an abnormal molecular structure that promotes crystal

aggregation [18]. THP was reported to be a promoter of CaOx aggregation in the confined urine of intermittent stone former which may be due to the inclination to self-aggregation, which eliminates its communication with CaOx monohydrate stones [14]. Oxalate oxidase was reported to be an accountable factor in correspondence for urolithiasis both in humans and animals [15]. Docking results showed a better inhibitory effect of α -Mangostin on these proteins thus inhibiting stone formation.

α -Mangostin was docked with major Kidney stone forming proteins using AutoDock Tools and the H bonding results were recorded and tabulated in Table 2.

Table 2. Molecular docking results of α - mangostin with kidney stone forming proteins

S. No	Receptor	Ligand	Binding Energy (Kcal/mol)	No. of H Bonds formed	Atom of Ligand involved Interaction	Chain: Residues of Receptor involved in interaction	Atom of the Receptor's residue involved in interaction	Distance of the H Bond (Å)
1	Xanthine Dehydrogenase, Chain B	α - mangostin	-4.00	2	H	B:ALA125	O	4.46
					H	B:GLU42	OE2	3.96
		Oxypurinol	-4.77	5	H	B:ARG21	O	2.42
					H	B:ARG223	O	2.91
					O	B:LEU23	O	2.71
					H	B:CYS194	O	1.90
O	B:HIS193	HD1	2.24					
2	Oxalate oxidase, Chain A	α - mangostin	-4.47	1	H	A:PRO91	O	4.98
					O	A:SER1	HT2	1.81
		acetylglucosamine	-2.72	4	H	A:ASP4	OD2	2.31
					H	B:SER1	O	2.20
					H	B:ASP4	O	2.65
3	Tamm-Horsfall protein, Chain A	α - mangostin	-3.41	3	H	A:GLU398	O	4.56
					H	A:GLU399	O	4.75
					O	A:ARG90	HH12	5.91
		acetylglucosamine	-3.48	3	O	A:PHE687	HN	2.32
					H	A:SER683	OG	2.15
					H	A:THR681	O	1.85

The B chain of Xdh interacts with α - mangostin by two H bonds formed with Oxygen Atom of ALA125 with binding energy of -4.00 Kcal/mol (Fig. 3.A).

The A chain of THP interacts with α - mangostin by three H bonds with binding energy of -4.00 Kcal/mol. Bond between Oxygen Atom of GLU398 & H atom of α - mangostin, Oxygen Atom of GLU399 & H atom of α - mangostin and H atom at H12 position of ARG90 & O of α - mangostin were formed (Fig. 3.B).

The A chain of Oxalate oxidase interacts with α - mangostin by one H bonds formed with Oxygen Atom of PRO91 with binding energy of -4.00 Kcal/mol (Fig. 3.C).

The docking study with native ligands as the standard control was already performed and analysed in our previous study [19].

The other interactions such as *van der waals*, Alkyl, Pi-Sigma and Pi-Alkyl interactions were also formed between the receptors and ligand.

In the case oxalate oxidase, even though acetyl glucosamine showed better H-bonds, the

Binding energy with α - mangostin was less when compare to acetyl glucosamine. The molecular docking study with Tamm-Horsfall protein was found to be nearly similar both in respect to H-Bond and Binding energy. In case of Xdh interaction with Oxypurinol was found to be better than α - mangostin. On an average it could be concluded that α - mangostin could act as a better anti-urolithiatic agent on basis of Molecular docking.

3.4 *In Vitro* Antiurolithiatic Activity Study-Simultaneous Static Flow Model

In vitro S.S.M model has been widely used to analyse and test the anti-urolithiatic effect of plant extracts. The nucleation of the kidney stone crystals could be inhibited through the adsorption of the compounds present in the extract on the surface of the crystals [20]. It was observed that AMF at 100 mg/ml, 200 mg/ml, 300 mg/ml and 500 mg/ml was found to inhibit calcium phosphate formation with % inhibition of about 15.93%, 25.41%, 48.78% and 54.71% respectively. Whereas AMF at 100 mg/ml, 200 mg/ml, 300 mg/ml and 500 mg/ml was found to inhibit calcium phosphate formation with % inhibition of about 20.69%, 31.61%, 55.59% and 62.21% respectively (Fig. 4).

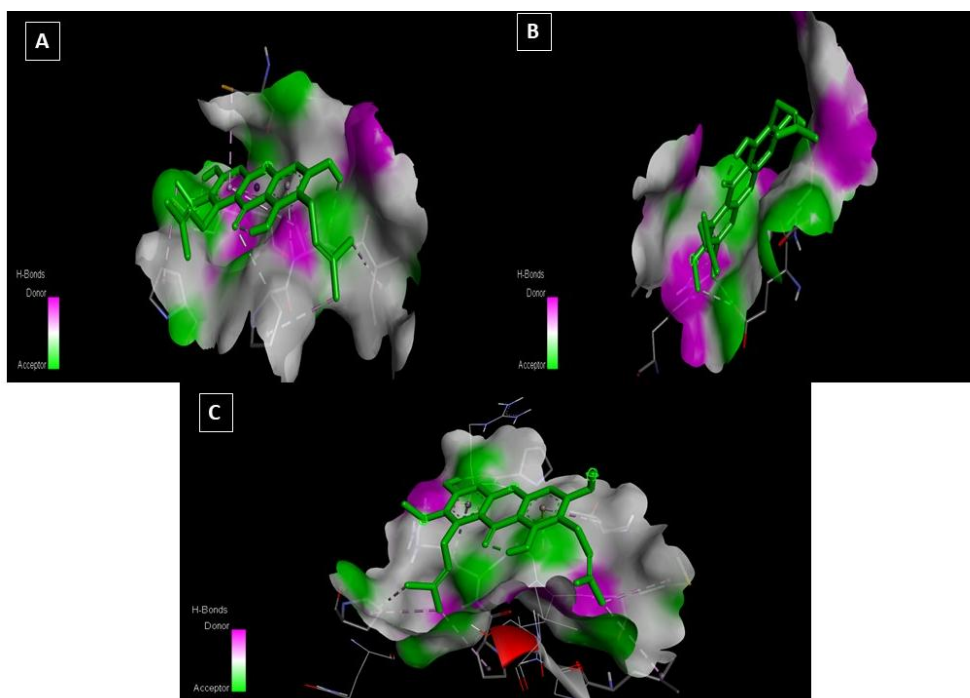


Fig. 3. Molecular docking of α - mangostin with kidney stone forming proteins

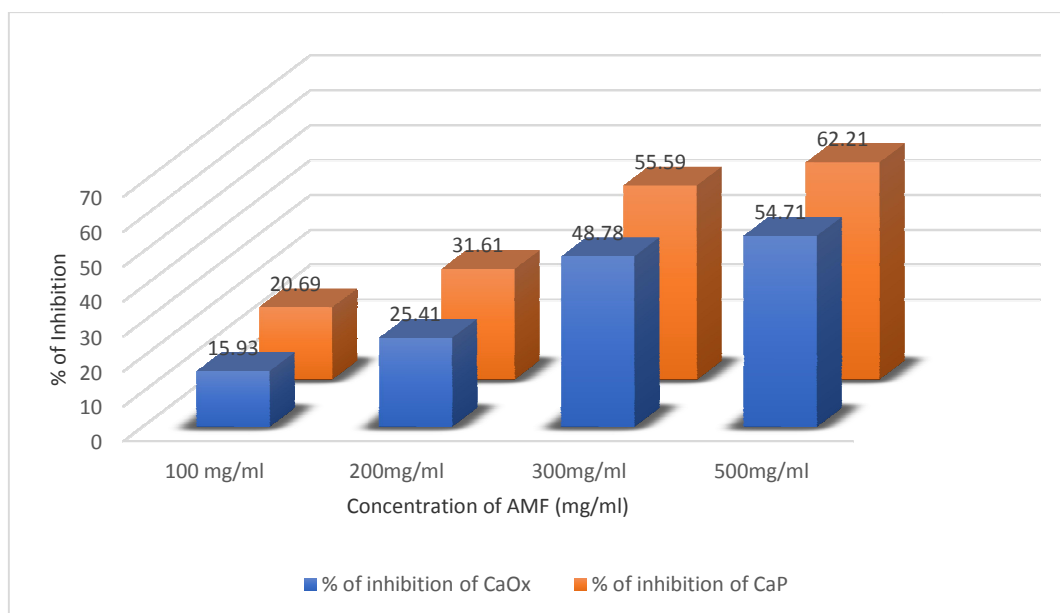


Fig. 4. *In vitro* antiurolithiatic efficiency of AMF by S.S.M

3.5 *In Vivo* Animal Study

Since *in silico* and *in vitro* study of α -Mangostin showed significant results as an antiurolithiatic agent, *in vivo* studies were done keeping *in vitro* studies as the background. EG, sodium oxalate, ammonium oxalate, and glycolic acid are some of the chemicals used for inducing urolithiasis [21]. Here in this study of testing the antiurolithiatic activity of AMF, EG is used to induce lithiasis in *Wistar* albino rats. Studies have been made that proves the potency of ethylene glycol in inducing urolithiasis in *Wistar* Albino rats. Oxalic acid, a main component of the kidney stone, is formed in the body by the accumulation of glycolic acid, a metabolite of EG [22].

3.6 Analysis of Physical Parameters

The observed physical parameters of the experimental animals are recorded as in Fig. 5. The calculi-induced group was found to show reduction in body weight and water intake when compared to animals in negative control group. The reduction in the quantity and frequency of food intake was found to be the major factor of weight loss. Physiological imbalance and/or mental stress may be the other factors influencing weight reduction [23]. A significant ($P < 0.01$) gain in body weight in animals treated with 750 mg/kg of AMF compared to calculi-induced group was noted. But it was noted that

there was reduction ($P < 0.0001$) of body weight in Group-II animals compared to Group-I. A decrease ($P < 0.0001$) in volume of water intake by group-II was noted when compared to Group-I. Along the co administration of 750 mg/kg dried aqueous AMF a significant ($P < 0.001$) increase in volume of water intake was also noted. Ethylene glycol administration also leads to hypertrophy of renal papilla and increased kidney weight probably by inflammation and fluid accumulation [24]. This may be due to the weight added by the calculi in the kidney. The weight of the kidney of Group-II was high when compared to control group while on administration with 750 mg/kg of AMF, a significant ($P < 0.01$) reduction in weight of the kidney was observed.

3.7 Biochemical Analysis of Urine

The analysed urine biochemistry was recorded as in Fig. 6. Decreased urine output is also one of the causes of urolithiasis [25]. Administration of AMF showed increased water intake which further gave an increased urine output. A significant ($P < 0.001$) increase in urine volume was observed in Groups-VI compared to animals in Group-II. Among various types of kidney stones based on stone composition, Uric acid stones were reported predominant in highly acidic urine, whereas calcium oxalate and calcium phosphate stones occur in highly alkaline urine [26,27]. The urine pH was found to be reduced significantly ($P < 0.01$) in Group-VI

compared to animals in Group-II. Increased concentration of urinary calcium and phosphate were a factor favouring the growth of calcium oxalate or calcium phosphate crystals [26]. Supplementation with AMF which served as curative regimen showed reduction in urinary calcium concentration. Studies have proved that low level of Magnesium promotes the nucleation of stone crystals and retention of calcium in renal tubule. This is because magnesium binds to oxalate and forms a soluble complex. Thus, it reduces the concentration of oxalic acid to be available to form calcium oxalate [28]. Calcium, phosphate and uric acid level in urine was found to be increased in Group-II compared to negative control, AMF and Cystone treated group. Calcium ($P<0.001$), Phosphate ($P<0.01$) and Uric Acid ($P<0.001$) levels were found to be lowered significantly in Group-VI. Magnesium excretion was reduced in EG treated group compared to control group. 750 mg/kg AMF treated group was found to have significantly ($P<0.001$) increased magnesium level compared to Group-II.

3.8 Biochemical Analysis of Serum

Due to the obstruction to the outflow of urine by stones in the urinary system or due to the damage of renal parenchymal cells, Glomerular filtration rate may get decreased and results in accumulation of the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid in the blood [29]. The analysed serum biochemistry was recorded in Fig. 7. The serum levels of calcium, phosphate, creatinine, uric acid and BUN were found to be elevated, whereas total protein was found to be decreased in Group-II animals compared to Group-I, indicating impaired renal function. 750 mg/kg AMF treated group was found to have reduced BUN ($P<0.01$), creatinine ($P<0.001$), uric acid ($P<0.001$), calcium ($P<0.01$) and phosphate

($P<0.01$) levels and elevated total protein ($P<0.0001$) level compared to Group-II.

3.9 Microscopic Analysis of Urine

Microscopic analysis of urine samples of animals showed various calculi- crystals such as Caox, struvite and uric acid as shown in Fig. 8. Microscopic examination of urine of Group-I animals showed the absence of calculi crystals, whereas Group-II showed Aggregated forms of calculi crystals. Group III showed very a smaller number of rectangle shaped calcium oxalate and calcium phosphate crystals and most of the aggregates were almost dissolved. Group-V animals showed only few aggregate crystals when compared to Group-IV. Group-VI animals showed almost dissolved crystal. Thus, the efficiency of AMF in dissolving Calculi was found to be in the order Group-VI < Group-VII < Group-VIII.

3.10 Histo-pathological Analysis of Kidney Tissue

Glomerular changes were predominant such as shrunken, variable sized, haemorrhagic, detached and loss of glomeruli with mild degeneration of tubular epithelial cells and moderate dilation of the tubules were appreciated mainly in the junction between cortex and medulla in Group-II animals. Mild focal areas of interstitial nephritis with infiltration of predominantly mononuclear cells were observed along with congestion of blood vessels were noted in calculi induced group. There were no abnormalities or calculi deposits in group-I. Administration of AMF (750 mg/kg) (Group-VI) and Cystone (750 mg/kg) (Group-III) showed decreased renal injury and calcium deposits in the kidney which are identified by the arrow marks in Fig. 9.

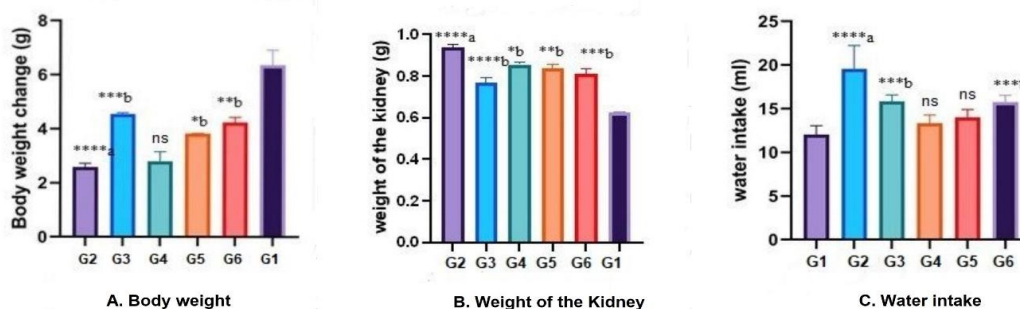


Fig. 5. Physical parameters recorded from test animals

Values are expressed in mean \pm SEM (n=6), * $P<0.05$, ** $P<0.01$, *** $0.001<P<0.0001$, **** $P<0.0001$; a significant compared with control group, b significant compared with calculi induced group, ns not significant

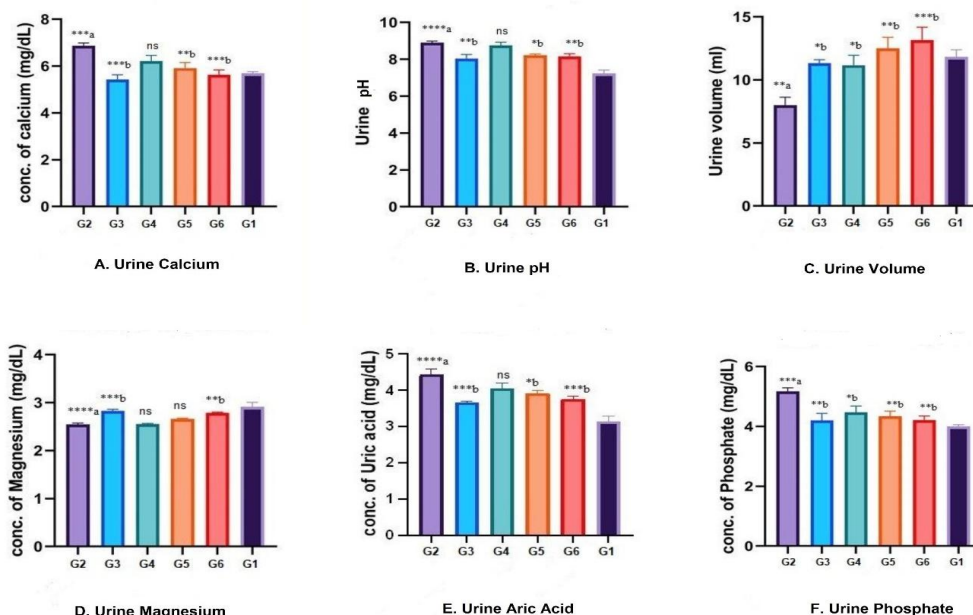


Fig. 6. Urine parameters recorded from test animals

Values are expressed in mean \pm SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant

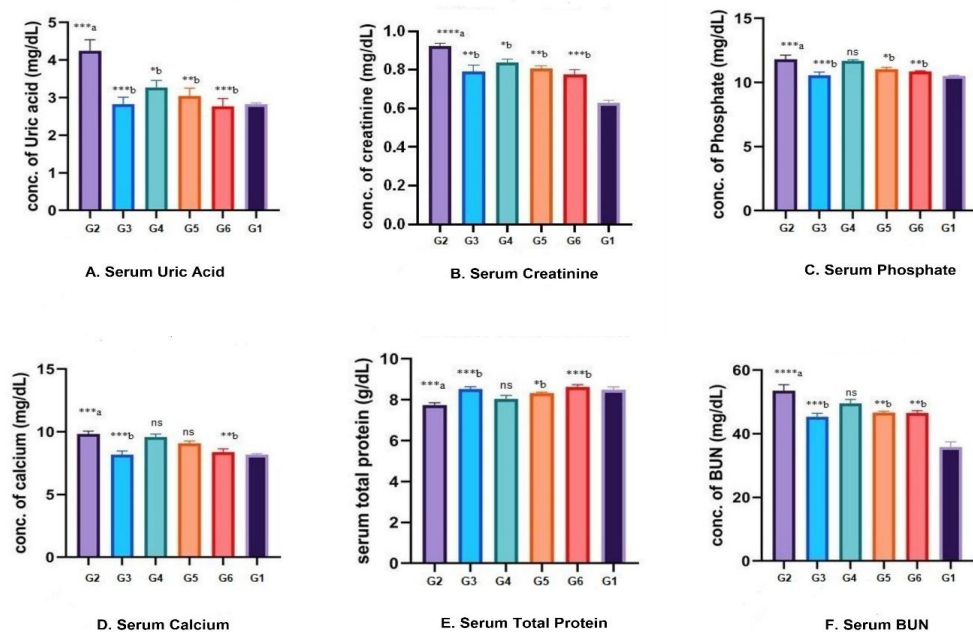


Fig. 7. Serum parameters recorded from test animals

Values were expressed in mean \pm SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant

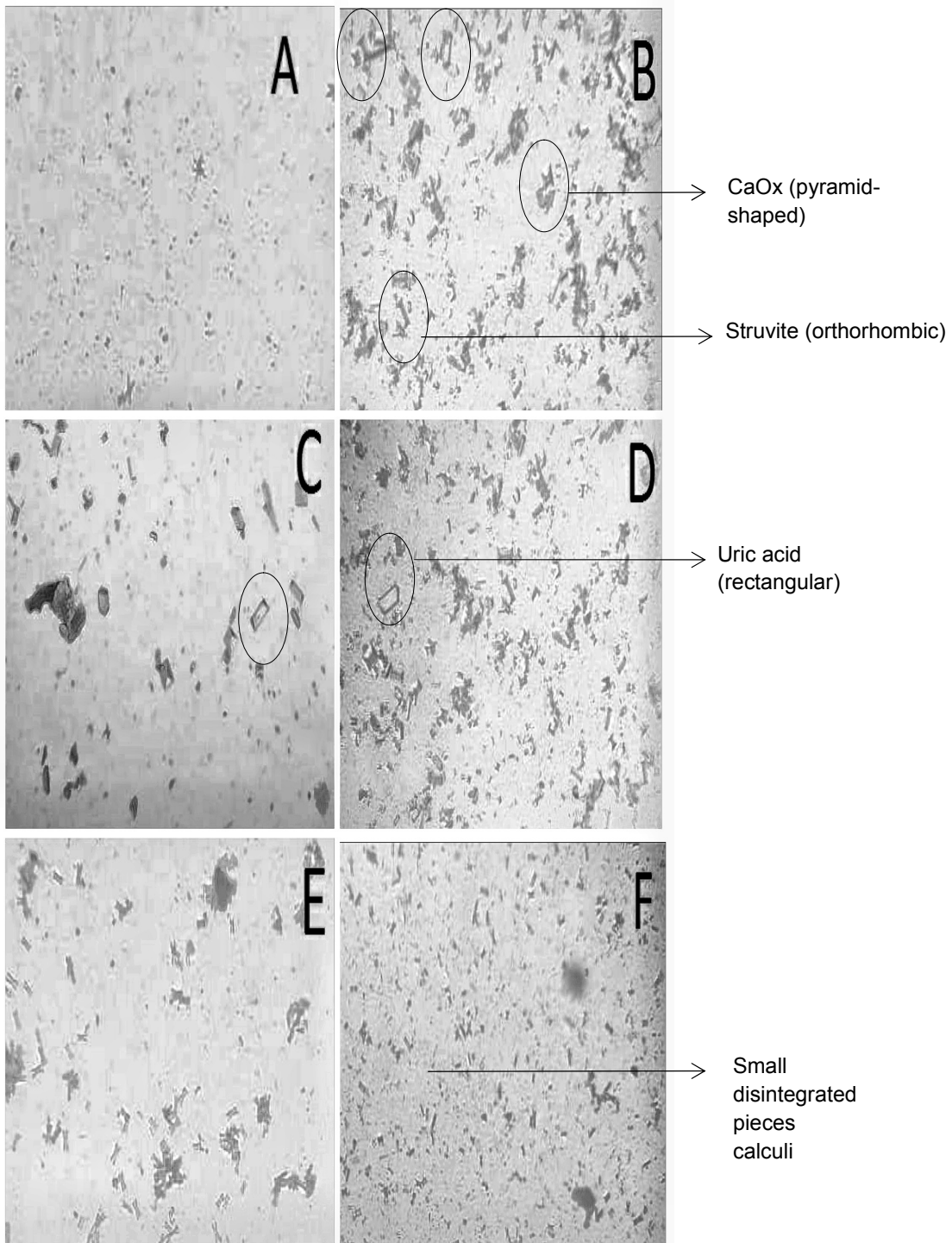


Fig. 8. Microscopy picture of urine in rats (x10)
(a) Untreated healthy control, (b) Lithiatic control, (c) Rats treated with Cystone 750 mg/kg body weight, (d) Rats treated with AMF 300 mg/kg body weight, (e) Rats treated with AMF 500 mg/kg body weight, (f) Rats treated with AMF 750 mg/kg body weight

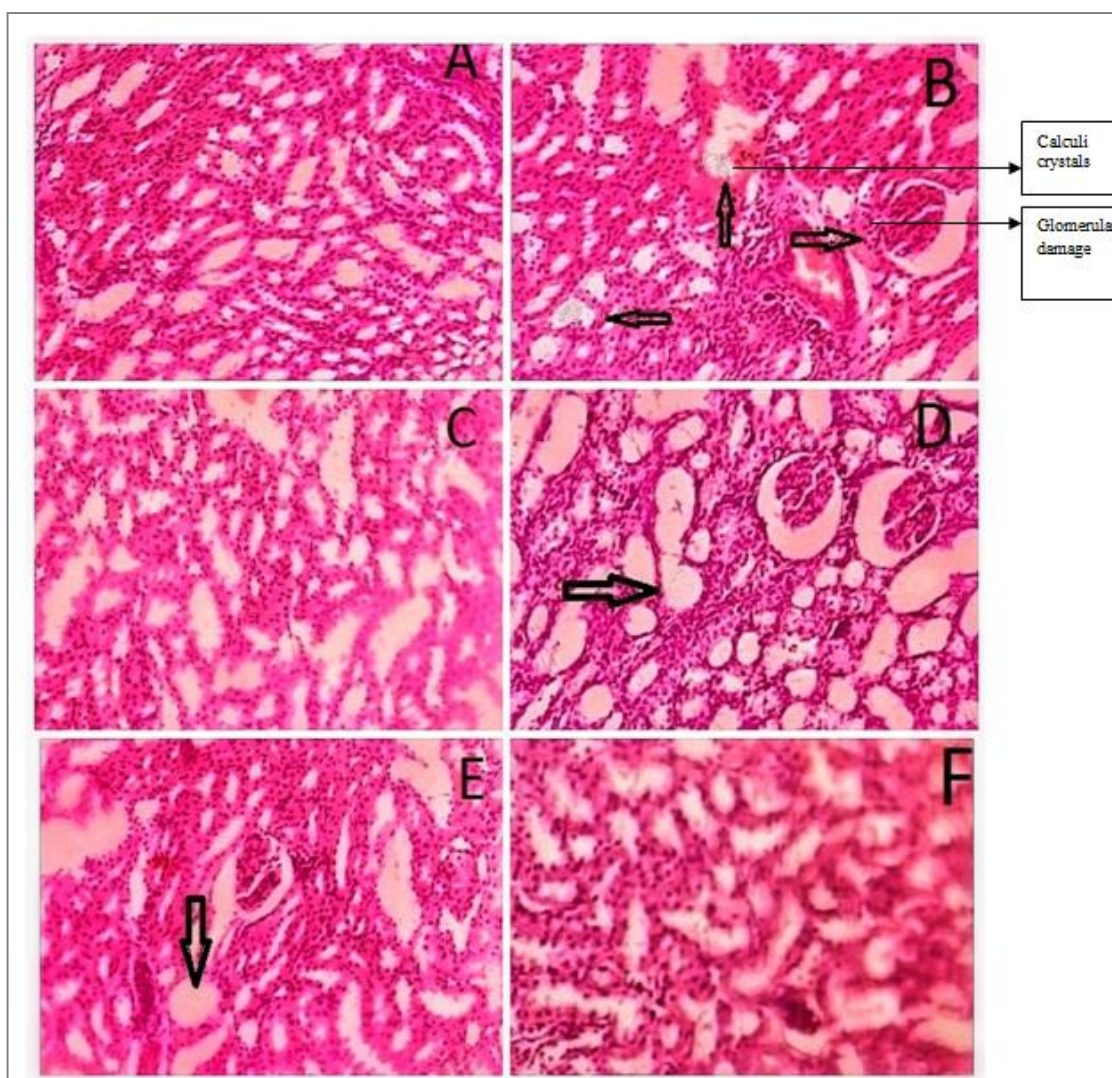


Fig. 9. Histology of kidney section stained with H and E stain (x100)

(a) Untreated healthy control, (b) Lithiatic control, (c) Rats treated with Cystone 750 mg/kg body weight, (d) Rats treated with AMF 300 mg/kg body weight, (e) Rats treated with AMF 500 mg/kg body weight, (f) Rats treated with AMF 750 mg/kg body weight.

4. CONCLUSION

Based on this above *in silico*, *in vitro* and *in vivo* studies, it is concluded that α -Mangostin acts as a potent Antiurolithiatic agent by reducing and disintegrating the urinary stones such as CaOx and CaP. The most significant outcome achieved from this research was to use the waste biomass in formulating therapeutical drugs with less side effects. And even though the standard drug cystone (polyherbal), was able to show significant stone reduction than α -mangostin, it is evident that being a mono-herbal drug (single compound) α -mangostin was found to have a potent antiurolithiatic activity. The mechanism

behind α -Mangostin to act as an antiurolithiatic agent could be due to its ACE-inhibition, diuretic, anti-oxidant, anti-microbial and anti-inflammatory properties. Yet, further clinical study has to be carried out to evaluate the anti-urolithiatic efficiency of α -Mangostin.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animals approval for Protocol-8/Phase-1/2019 was sanctioned in 10th IAEC meeting held on

17.05.2019 at Sri Venkateswara College of Engineering (Reg.No:1398/PO/Re/S/10/CPCSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Vyas BA, Vyas RB, Joshi SV, Santani DD. Antiuro lithiatic activity of whole-plant hydroalcoholic extract of Pergularia daemia in rats. *J Young Pharm.* 2011;3(1):36-40. DOI:10.4103/0975-1483.76417
- Ratkalkar VN, Kleinman JG. Mechanisms of stone formation. *Clin Rev Bone Miner Metab.* 2011;9(3-4):187-97. DOI:10.1007/s12018-011-9104-8
- Colella J, Kochis E, Galli B, Munver R. Urolithiasis/nephrolithiasis: What's it all about? *Urol Nurs Off J Am Urol Assoc Allied.* 2005;25(6):427-8.
- Tiwari A, Soni V, Londhe V, Bhandarkar A, Bandawane D, Nipate S. An overview on potent indigenous herbs for urinary tract infirmity: Urolithiasis. *Asian J Pharm Clin Res.* 2012;5(1):7-12.
- Begun FP, Knoll CE, Gottlieb M, Lawson RK. Chronic effects of focused electrohydraulic shock waves on renal function and hypertension. *J Urol.* 1991;145(3):635-9. DOI:10.1016/S0022-5347(17)38410-0
- Gutierrez-Orozco F, Failla ML. Biological activities and bioavailability of mangosteen xanthenes: A critical review of the current evidence. *Nutrients.* 2013;5(8):3163-83. DOI:10.3390/nu5083163
- Ibrahim MY, Hashim NM, Mariod AA et al. α -Mangostin from *Garcinia mangostana* Linn: An updated review of its pharmacological properties. *Arab J Chem.* 2016;9(3):317-29.. DOI:10.1016/j.arabjc.2014.02.011
- Ahmed S, Hasan MM, Khan H, Mahmood ZA, Patel S. The mechanistic insight of polyphenols in calcium oxalate urolithiasis mitigation. *Biomed Pharmacother.* 2018;106:1292-9. DOI:10.1016/j.biopha.2018.07.080
- Khan SR. Reactive oxygen species as the molecular modulators of calcium oxalate kidney stone formation: Evidence from clinical and experimental investigations. *J Urol.* 2013;189(3):803-11. DOI:10.1016/j.juro.2012.05.078
- Nagal A, Singla RK. Herbal Resources with Antiuro lithiatic Effects: A Review. *Indo glob. J. pharm.* 2013;3(1):6-14.
- Pradeep kumar SV, Puranik SB, Nandini BN. Industrially feasible method for the extraction , purification and quantification of mangostin (Alpha-Mangostin) from *G . mangostana* Fruit Hulls. *Ijpr. Human.* 2016;7 (3):5-21.
- Mahmud S, Rahman E, Nain Z et al. Computational discovery of plant-based inhibitors against human carbonic anhydrase IX and molecular dynamics simulation. *J Biomol Struct Dyn.* 2020:1-7. DOI:10.1080/07391102.2020.1753579
- Sharma N , Bisht SS, Gupta S, Rana M, Kumar A PR. In-silico study for antiuro lithiatic activities of compounds extracted from horse gram (*Macrotyloma uniflorum*) collected from Uttarakhand (India) as xanthine dehydrogenase inhibitor by molecular docking. *Int J Recent Sci.* 2019;10(7):33630-33634.
- Vijayakumari B, Sasikala V, Radha SR, Rameshwar HY. In silico analysis of aqueous root extract of *Rotula aquatica* Lour for docking analysis of the compound 3-O-acetyl-11-keto- β -boswellic acid contents. *Springerplus.* 2016;5(1):1486. DOI:10.1186/s40064-016-3134-0
- Dinnimath BM, Jalalpure SS. In silico antiuro lithiatic screening of *aerva lanata* (L) isolated constituents. *Indian J Pharm Educ Res.* 2015;49(1):1-8. DOI:10.5530/ijper.49.2.8
- Mandavia DR, Patel MK, Patel JC, Anovadiya AP, Baxi SN, Tripathi CR. Anti-uro lithiatic effect of ethanolic extract of *Pedaliium murex* Linn. Fruits on ethylene glycol-induced renal calculi. *Urol J.* 2013; 10(3):946-52. DOI:10.22037/uj.v10i3.1530
- Martin YC. A bioavailability score. *J Med Chem.* 2005;48(9):3164-70. DOI:10.1021/jm0492002

18. Benković J, Furedi-Milhofer H, Hlady V, Čvorišćec D, Stavljenić-Rukavina A. Effect of Tamm-Horsfall Protein on Calcium Oxalate Precipitation. Clin Chem Lab Med. 1995;33(10):705-710. DOI:10.1515/ccim.1995.33.10.705
19. Kumaran A, Sukumaran P. In silico and in vitro analysis of antiurolithiatic activity of Garcinone E isolated from Garcinia magostana pericarp extract. Plant Cell Biotechnol Mol Biol. 2020;21(37-38): 121-135.
20. Phatak RS, Hendre AS. In-vitro antiurolithiatic activity of Kalanchoe pinnata extract. Int J Pharmacogn Phytochem Res. 2015; 7(2):275-9.
21. Khan SR. Animal models of kidney stone formation: An analysis. World J Urol. 1997;15(4):236-43. DOI:10.1007/BF01367661
22. Breljak D, Brzica H, Vrhovac I et al. In female rats, ethylene glycol treatment elevates protein expression of hepatic and renal oxalate transporter sat-1 (slc26a1) without inducing hyperoxaluria. Croat Med J. 2015;56(5):447-59. DOI:10.3325/cmj.2015.56.447
23. Morley JE, Levine AS, Rowland NE. Minireview. Stress induced eating. Life Sci. 1983;32(19):2169.
24. De Water R, Noordermeer C, Van der Kwast TH et al. Calcium oxalate nephrolithiasis: Effect of renal crystal deposition on the cellular composition of the renal interstitium. Am J Kidney Dis. 1999;33(4):761-71. DOI:10.1016/S0272-6386(99)70231-3
25. Chavada KS, Chavada KS, Fadadu KN, Patel K V, Patel KG, Gandhi TR. Effect of flavanoid rich fraction of citrus medica linn. (rutacea) on ethylene glycol induced urolithiasis in rats. J Drug Deliv Ther. 2012;2(4):109-116. DOI:10.22270/jddt.v2i4.222
26. Low RK, Stoller ML. Uric acid-related nephrolithiasis. Urol Clin North Am. 1997;24(1):135-48. DOI:10.1016/S0094-0143(05)70359-1
27. Ebrahimpour A, Perez L, Nancollas GH. Induced crystal growth of calcium oxalate monohydrate at hydroxyapatite surfaces. The influence of human serum albumin, citrate, and magnesium. Langmuir. 1991;7(3):577-83. DOI:10.1021/la00051a028
28. Grases f, Genestar C, Conte A, March P, Costa-Bauza A. Inhibitory effect of pyrophosphate, citrate, magnesium and chondroitin sulphate in calcium oxalate urolithiasis. Br J Urol. 1989;64(3): 235-7. DOI:10.1111/j.1464-410X.1989.tb06004.x
29. Ghodkar PB. Chemical Tests in Kidney Disease. Text Book of Medical Laboratory Technology. Mumbai: Bhalani Publishing House; 1994;118.

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