

# Evaluation Of Antiuro lithiatic Activity Of Methanolic Seed Extracts Of *Persea Americana* Against Calcium Oxalate Induced Urolithiasis In Rats

Smitha Grace S. R<sup>1\*</sup>, Manasa B. Y<sup>2</sup> & Jyoti Bala Chauhan<sup>3</sup>

<sup>1</sup>\*Research Scholar Department of Biotechnology, PRIST University, Vallam, Thanjavur, Tamil Nadu, India,  
<sup>2,3</sup>Department of Studies in Biotechnology, Pooja Bhagavat Memorial Mahajana Education centre, Post graduate wing of SBRR Mahajana First Grade College, Metagalli, K.R.S Road, Mysuru 16, Karnataka

\*Corresponding Author: Smitha Grace S. R

\*Research Scholar Department of Biotechnology, PRIST University, Vallam, Thanjavur, Tamil Nadu, India, Email: Smithagrace13@gmail.com, +91- 9945165301.  
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## Abstract

**Objective:** *Persea Americana* methanolic seed extracts are considered for the present study to access its efficacy, urolithiasis is induced by administering the male rats with calcium oxalate.

**Methods:** Calcium oxalate urolithiasis is induced in male rats by means of hyperoxaluria model by administering ethylene glycol of 0.75% v/v, with 1% w/v ammonium chloride supplemented in drinking water for 3 days followed by 0.75% v/v of ethylene glycol for 25 days. The PAMSE is administered to urolithiasis induced test group at dose of 150mg/kg respectively for 28 days.

**Results:** As anticipated after 28 days the calcium oxalate is deposited significantly in the kidney which is associated with the increase of Urinary oxalate, Urine calcium & magnesium levels, serum uric acid, creatinine in urolithiasis control groups. ACP, ALP, AST & ALT well known biochemical parameters are seen to be increased with decreased level of LDH assessed using kidney homogenate, copiously confirming the induction of urolithiasis, its promising to know that the PAMSE intubation to the test group daily decreased the quantity of formation of calcium oxalate and all the biochemical changes induced by urolithiasis is reverted.

**Conclusion:** Results from this study shows that PAMSE is effective inhibitor of crystallization of calcium oxalate comparable to the standard Cystone in dose dependent mode. Therefore indicating PAMSE's significant antiuro lithiatic activity.

**Keywords:** Urolithiasis, Calcium oxalate, ethylene glycol, Urolithiasis, PAMSE (*Persea Americana* methanolic seed extract)

## INTRODUCTION:

Urolithiasis is a common problem plagued for many centuries with high recurrence. The certainty and observations concerning traditionally used medicinal plants, has increased the attention of the human population to practice natural medicine for health care needs. Dietary interventions and wide range of medicinal plants seems to be promising methods for curing as well as prophylactic agent for urolithiasis. The formation of the kidney stones is seen more in industrialized countries with 10% to the males and only 3% to women this wide spread diseases happening to the urinary tract also shows high relapse rate, the reason for the occurrence is dependent on multiple factors related to genetics, the diet intake and low-slung activity commonest of the stones are the Calcium-containing kidney stones, the process starts with aggregation of crystals in the urinary, associated with development of insoluble particles and nucleation posing discomfort when they pass through the tract, obstruction which builds up the pains, followed by recurrent infection and hemorrhage. Extracorporeal shock wave lithotripsy, percutaneous lithotripsy, transurethral lithotripsy are available surgical procedure which are complex, complicated, expensive with cases of reoccurrence reported (5-10%). The widely used medications are basically diuretics thiazide and alkali-citrate inhibiting hypercalciuria and hyperoxaluria but they seem to be less affective, researchers are in colossal search for safe drugs and therapies, it's well known since millennia the medicinal plants possess antioxidant activities exerting inhibition on crystallization, nucleation, aggregation of crystals making them beneficial for treatment of urolithiasis, however there is no well reputable data indorsing the phytonutrients in the prevention of kidney stones.

Lauraceae is a family of trees and shrubs known to mankind since cretaceous periods, these genera is known to contains essential oils, chemical constituents making them to be used for pharmaceutical use, these make them a source of solution for the future among this group is our fruit of investigation *Persea americana* of west Indian race localized in Karnataka,

Kerala and Maharashtra, they are known to contain the wide variety of phytoconstituents, it's rich in dietary fibre compared to other fruits. It is also high in manganese, phosphorous, iron and potassium, but low in sodium, and also contains vitamin E, vitamin C,  $\beta$ -carotene, thiamin, riboflavin, nicotinic acid and folate (Rainey et al, 1994) is a good source of the essential linoleic acid (Bergh, 1992), and the carbohydrate content is expectably low. Even though *Persea Americana* plants is documented for different pharmacological activities, the seed extracts is not explored. In light of this the present study is conceptualized to evaluate "Antirolithiatic activity of *Persea americana* seed methanolic extract in rats induced with urolithiasis using Calcium oxalate".

## MATERIALS AND METHODS:

**Collection, preparation of extract, purification, characterization :** The fresh *Persea americana* fruits of West Indian race is purchased from the local markets of Mysore district, Karnataka and authenticated by the institutional botanist as west Indian race, the seed is removed from the fruit, cut into small pieces, sun dried and grounded into fine powder and subjected to methanol solvent extraction using Soxhlet apparatus, flash evaporated, purified using column chromatography with solvent mixture (chloroform-ethyl acetate-methanol) and finally the column is washed with methanol of high polarity. The fractions showing the maximum peaks is pooled and subjected to preparative thin layer chromatography with the solvent system of ethyl acetate-methanol-water and R<sub>F</sub> values were calculated taking standard DBA, gallic acid, the fractions are stored for further analysis, initially to start with the characterization is partially done using UHPLC is performed at PBMMPG wing, Mysore, with the following procedure, Methanol and Water are HPLC grade and purchased from MERCK, Germany, the UHPLC system is comprised of a Shimadzu Pump, A Shimadzu PD and UV detectors, win 7 software. The detection wavelength was set at 280nm. A Delta peak c4 was used for the chromatographic separation. The mobile phase used for the separation was Water (solvent A) and Methanol (solvent B) in the ratio 60:40 at flow rate of 1ml/min at ambient.

**2.2 Phytochemical screening:** The *Persea americana* seed sample is subjected to phytochemical screen as explained by Prashanth Tiwari et al., with slight modifications, the detection included alkaloids, glycosides, Saponins, phytosterols, phenols, tannins, diterpenes, terpenoids, and basic biomolecules like proteins and amino acids.

### Antirolithiatic activity of *Persea Americana* seed methanolic extract against Calcium oxalate induced Urolithiasis in Rats:

The reagents and chemicals is procured from approved chemical suppliers and analytical grade the experiment is designed using the male wistar rats weighing 120-150gms, they are housed in the animal facility room, it's taken care that the humidity is maintained at  $25 \pm 2^\circ \text{C}$  relative humidity of 45 to 55% and the animals are acclimatized for a standard conditions of 12 hr. light 12 hr. dark cycle, with free access to food of the brand Chakan Oil Mills, Pune, India and water ad libitum, All experiments are done as per the guidance of CPCSEA, New Delhi and institutional animal ethical committee. IAEC (Ref. No-IAEC/FCP/05/2017).

**Experimental design for assessing antirolithiatic activity:** Urolithiasis is induced in the male wistar rats using the hyperoxaluria model, Ethylene glycol and ammonium chloride are used to promote the formation of calcium oxalate, a total number of 25 animals are grouped in five of four groups and the experimental protocol is listed in the table below.

Name of the group	Experimental protocol
Group I (Normal Group)	Distilled water 1ml/kg
Group II-IV: the experimental animals received calculi inducers along with their respective intubation samples for a span of 28 days. The Calculi inducers contained the combined mixtures of v/v 0.75% ethylene glycol, w/v of 1% ammonium chloride for only three days followed by intubation of ethylene glycol of 0.27% for remaining 25 days.	
Group II (control group)	Distilled water 1ml/kg.
Groups III (test group)	PAMSE at a dose of 150mg/kg,
Group IV (standard drug)	Cystone at a dose of 200mg/kg

**Table 1:** Experimental design

**Urine analysis:** After the completion of 28 days of treatment, the experimental animals is kept in metabolic cages for a span of 24hrs to collect the urine sample for further assay. The parameters are as follows.

- Urine volume:** All the 25 experimental animals which are placed in the metabolic cages after 24<sup>th</sup> hour, the urine is quantified and expressed in ml.
- Urine pH:** The acidity of the urine samples collected is assessed using pH meter. The acidity is concentrated in the urine because of the deposition of Uric acid crystals.
- Urinary oxalate:** To assay urinary oxalate, from the collected urine sample small volume of urine is taken (0.1-1ml) and acidified with concentrated HNO<sub>3</sub> in order to solubilize the crystals & adjusted to pH 7. To the neutralized urine 2ml of saturated CaSO<sub>4</sub> and 14 ml of pure ethanol is added and left undisturbed in the room temperature overnight in order to precipitate oxalate, it is centrifuged at 450g for 10mins, the pellet is solubilized again with acidified water and titrated against KMnO<sub>4</sub>

- d. **Urine calcium:** Estimated as per o-cresolphthalein complexone method using commercially available kits procured from Biolab diagnostics Pvt. Ltd. Tarapur (India).
- e. **Urine magnesium:** Estimated as per Calmagite method by using commercially available kits procured from Biolab diagnostics Pvt. Ltd. Tarapur (India).

**Serum analysis:** The experimental is anesthetized and sacrificed by cervical decapitation, blood is collected, centrifuged to collect the serum and the following assays are performed.

- a. **Serum uric acid:** Colorimetric enzymatic method is used to estimate the serum uric acid as per the protocol in the standard kit of Biolab diagnostics Pvt. Ltd. Tarapur (India).
- b. **Serum creatinine:** Urease/salisylate method is used to estimate serum creatinine.

**Kidney homogenate analysis:** The kidney is dissected from all the experimental animals, cleaned to remove the outer tissues and rinsed in ice-cold physiological saline., it's made sure that all the kidneys are stored in a separate containers with proper naming including the left side and right side kidneys are mentioned carefully until further use , since we wanted to work on the estimation of biochemical marker we selected the left kidney , minced and 10 % homogenate is prepared using 0.02 mol/l of Tris Hcl buffer of pH 7.4.

**Estimation of biochemical markers:** The homogenate is used to assay the marker enzymes in serum, urine and tissue constituents using the commercially available marker kits like acid phosphatase, alkaline phosphate, Aspartate aminotransferase, Alanine aminotransferase and Lactate dehydrogenase.

**Statistical analysis:** the data obtained is statistically analyzed with oneway ANOVA and at  $p < 0.05$  it is considered statistically significant.

## RESULTS AND DISCUSSION:

Total 6 volumes of PAMSE eluents is collected through column chromatography. Diluted fractions is spectrophotometric ally scanned from 380-420nm and similar valued fraction pooled and subjected to TLC, ethyl acetate: methanol: water in the ratio of 1.65:1.25:10 is the most suitable system confirmed by us during this study The bands found in this method and its values indicate the presence of compounds such as polyphenols and flavanols. The quantitative assessment on PAMSE showed substantial incidence of metabolites such as Alkaloids, Glycosides, Saponins, Tannins, Diterpenes, Phenols & flavonoids thus reflecting its importance. The active fraction of PAMSE sample is further analyzed using UHPLC, the profiles matched with standard DBA & Rutin and their retention time is compared. The profile below shows peak at retention time of 4.566 min for PAMSE sample. With reference to the standards with retention time 5 min for DBA and 3.56 min for Rutin respectively, we consider the group of compounds belong to “Flvanoids glycosides”.

After the qualitative and quantitative analysis of the bioactive components in PAMSE, the objective of our study to confirm the antiurolithiatic potentials is deliberated as follows

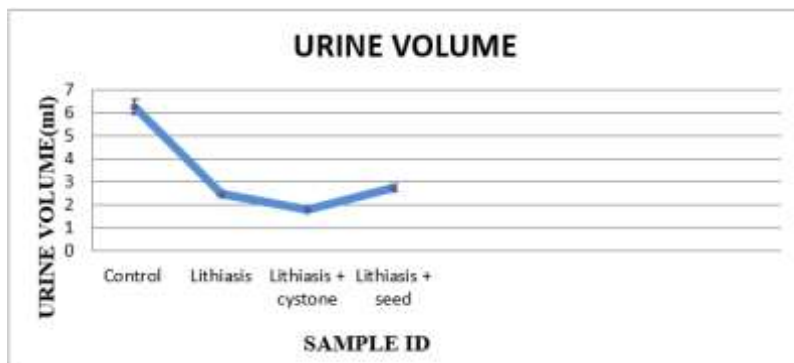
### a. Body Weight Analysis :

Titlle of the group	Average weight on 1 <sup>st</sup> day(in g)	Average weight on 14 <sup>th</sup> day(in g)	Average weight on 28 <sup>th</sup> day(in g)
Control (Group 1)	142	180.16	193.5
Induction control - Lithiasis (Group 2)	144	176.66	196
PAMSE (Group 3)	155	177	190
Cystone (Group 4)	142.66	174	181.66

**Table 2:** Weight of the Animals

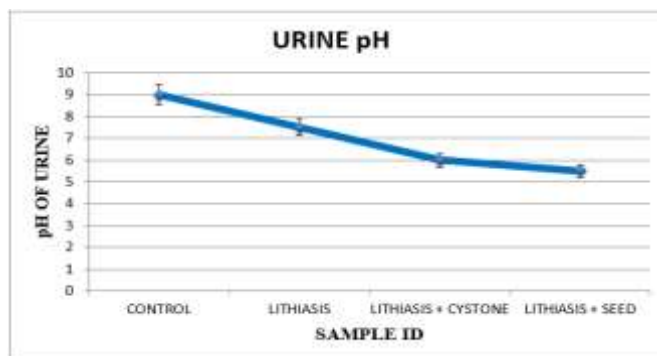
From the above table it is evident that the PAMSE extract improved health condition by stimulating cellular antioxidant difference.

### a. Analysis of Urine volume:



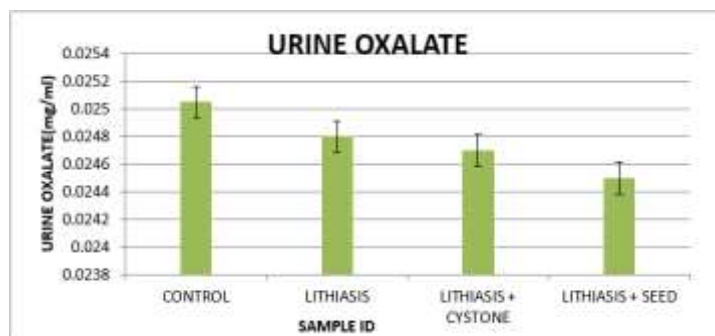
**Fig 1:** Urine Volume

b. Analysis of urine pH:



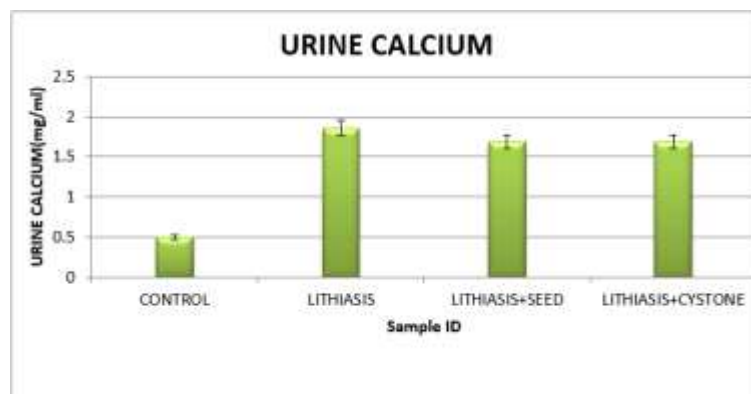
**Fig 2:** Urine pH

c. Analysis of urine oxalate:



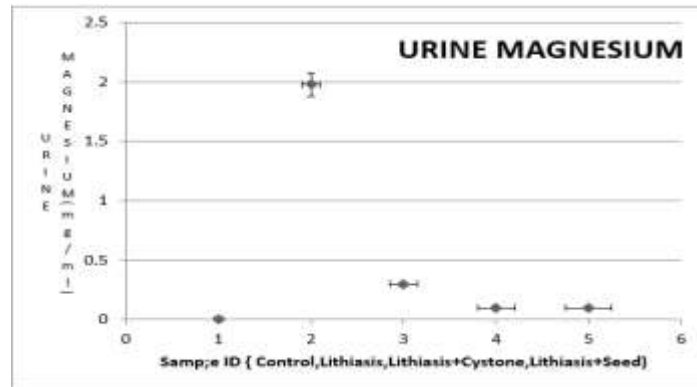
**Fig 3:** Analysis of Urinary Oxalate

d. Analysis of urine calcium:



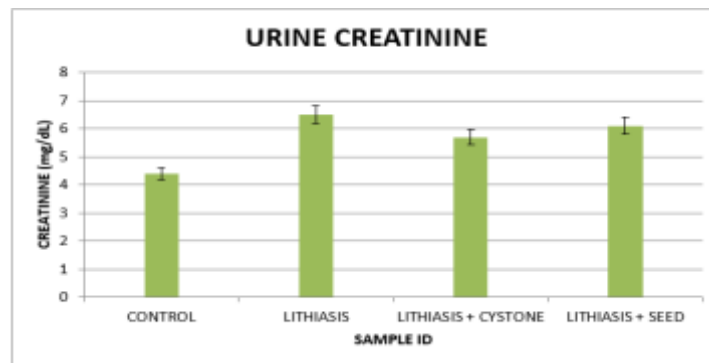
**Fig 4:** Analysis of Urine Calcium

e. Analysis of urine magnesium:



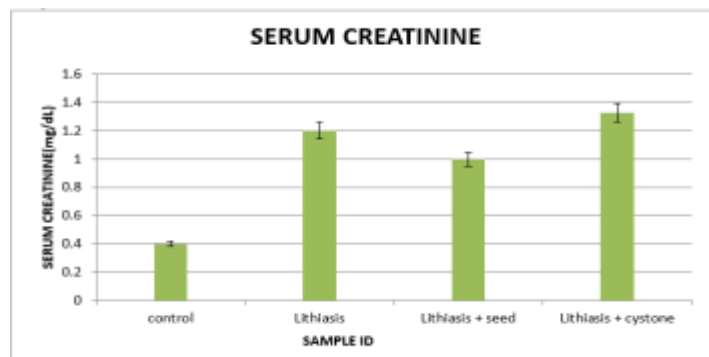
**Fig 5:** Analysis of Urine Magnesium

f. Analysis of urine creatinine:



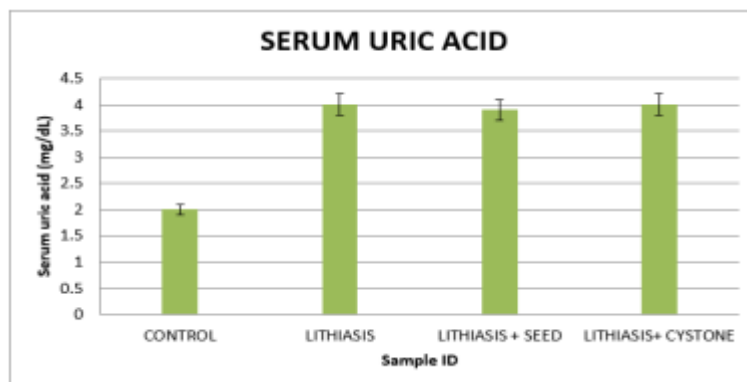
**Fig 6:** Analysis of Urine Creatinine

g. Analysis of serum creatinine:



**Fig 7:** Analysis of Serum Creatinine

h. Analysis of serum uric acid:



**Fig 8:** Analysis of Serum Uric Acid

i. Analysis of ACP-acid phosphatase:

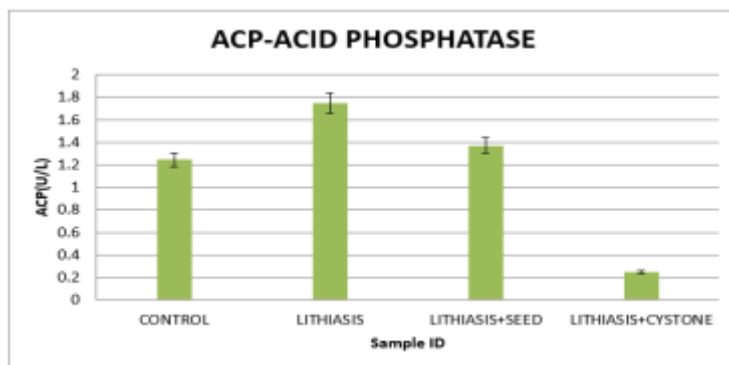


Fig 9: Analysis of Acid Phosphatase

j. Analysis of LDH:

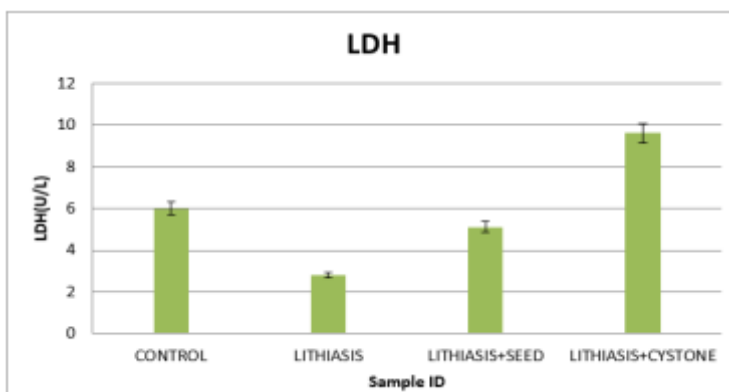


Fig 10: Analysis of LDH

k. Analysis of alkaline phosphatase (ALP):

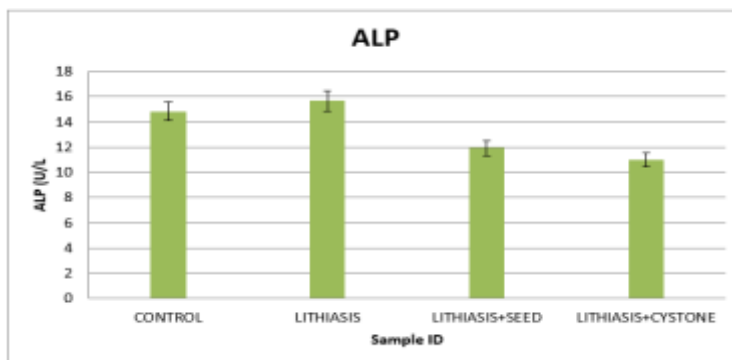


Fig 11: Analysis of Alkaline Phosphatase

l. Analysis of aspartate amino transferase (AST):

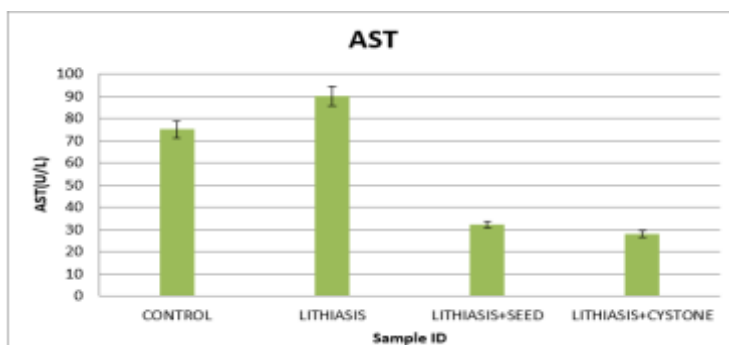
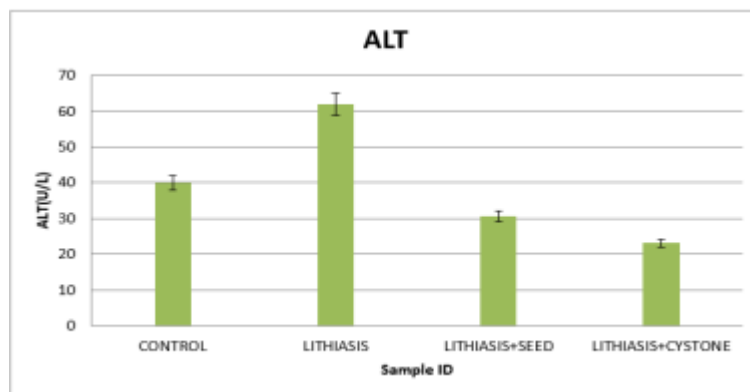


Fig 12: Analysis of Aspartate Amino Transferase

#### m. Analysis of alanine amino transferase (ALT):



**Fig 13:** Analysis Of Alanine Amino Transferase

#### DISCUSSION:

We are aware of the fact that the kidney stones affect the mankind, if untreated it poses serious issues one of them being the renal failure, there are treatments available but they seem like to be no relief and there is always a search for single effective drug.

The key mineral in the urolithiasis patients as per the epidemiological data available is the Calcium oxalate (CaOx), these kidney stones over the course of time shows the symptoms the first being renal colic, blocking the flow of urine in the urinary tract causing the irritation and severe pain it may be accompanied by bloody urine if the stones are too large.

For Urolithiasis unfortunately surgery is considered to be the best option when alternate treatments fail. The safe option beheld for since ancient times are natural drugs. Based on the previous results reported by Smitha et al, on *in vitro* antiurolithic activities of *Persea Americana*, it's seen that seed extracts have potent ability in inhibiting the crystallization of the calcium oxalate comparable to the standard Cystone, and this effect is dose dependent suggesting antiurolithic activity. So far no scientific evaluation has been done on seed extract *in vivo*, which motivated us to carry out the study using Ethylene glycol and ammonium chloride induced hyperoxaluria model.

In this study the weight of the male winstar rats is taken on the first 14<sup>th</sup> and 28<sup>th</sup> day respectively and it's a clear indication there is no change in the weight of the experimental animals with respect to control, lithiasis and cystone group. Based on the measurement of the urine volume in comparison to the cystone the standard drug the PAMSE has increased the urine volume, this is an indication that there is no obstruction for the urine to flow in the urinary tract. From the urine biochemistry done with respect to urine oxalate, urine phosphate, urine calcium, the level of the ions in the urine samples of the experimental animals treated with PAMSE is significantly lower. However, the level of magnesium is significantly increased in PAMSE treated rats the reason being the oxalate absorption & excretion consequently preventing supersaturation, reducing the risk of calcium oxalate stone formation, but magnesium level is decreased in lithiasis models. The pH of the urine in PAMSE treated animals is slightly alkaline

The increase in the urinary oxalate in ethylene glycol Urolithiasis and decreased in PAMSE treated rats is due to the inhibition of formation of oxalate by the constituents present in the extract further it can also be because of inhibition of activity of oxalate oxidase enzyme which is responsible for the stone formation.

The microscopic examination of urine reveals the concentration of various ions are changed radically after treatment with PAMSE, creating an environment favorable for the reduction of crystal formation.

The kidney damage is minimized, serum uric acid & creatinine is decreased in PAMSE treated experimental animals, it's known that nitrogenous substances such as creatinine cause kidney damage and is observed in lithiasis rats as there is significant increase in serum creatinine, serum uric acid.

With respect to the assays conducted on biochemical markers from the kidney homogenate there is significant increase in the biochemical parameters such as ACP, ALP, AST, ALT levels and decrease in LDH level in induction control group compared to normal control group, these alterations were reverted in PAMPSE treated animals indicating significant antiurolithic activity. Data is considered as statistically significant at  $p < 0.05$ .

#### CONCLUSION:

We the mankind is enormously blessed with the natural immune system that provides the protection against life threatening diseases, however at some instances the diet also plays a significant role in the protection from endogenous toxins, in this century we are also trending towards the herbal therapies as they contain enormous amount of

phytoconstituents among this are the greatest group called Flavanoids , they are rich sources of antioxidants and have different classes with different properties like anticancer, antimicrobial ,anti-diabetic, antimalarial, neuroprotective, cardio-protective, anti-inflammatory. Our PAMSE contains the Flavanoids glycosides and have exhibited significant antiurolithiatic activity. The possible mechanism related to antilithiatic effect by Flavanoids still stand elusive, our future plan lies in this perspective.

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**CONFLICT OF INTREST:** The authors declare no conflict of Interest.

## REFERENCE:

1. Mark L. Dreher<sup>1</sup> and Adrienne J. Davenport<sup>2</sup>, Hass Avocado Composition and Potential Health Effects, *Crit Rev Food Sci Nutr.* 2013 May; 53(7): 738–750.
2. P A Bergh<sup>1</sup>, D Navot, The impact of embryonic development and endometrial maturity on the timing of implantation, *Fertil Steril.* 1992 Sep; 58(3):537-42.
3. Bijarnia RK, Kaur T, Aggarwal K, Singla SK and Tandon C. Modulatory effects of N-acetylcysteine on hyperoxaluric manifestations in rat kidney. *Food Chem Toxicol.* 2008; 46: 2274–2278.
4. Fan J, Shen SJ. The role of Tamm Horsfall mucoprotein in calcium oxalate crystalluria. N-acetyl cysteine- A new therapy for calcium oxalate urolithiasis. *Br J Urol* 1994; 74: 288-293.
5. Divakar K, Pawar AT, Chandrasekhar SB, Dighe SB and Divakar G. Protective effect of the hydro-alcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats. *Food Chem Toxicol.* 2010; 48:1013-8.
6. Khan SR, Shevock PN, Hackett RL. Urinary enzymes and calcium oxalate urolithiasis. *J Urol.* 1989; 142: 846- 849.
7. Yamaguchi S, Wiessner JH, Hasegawa AT, Hung LY, Mandel GS and Mandel NS. Study of a rat model for calcium oxalate crystal formation without severe renal damage in selected conditions. *Int J Urol.* 2005; 12: 290–298.
8. Yoganarasimhan SN. Medicinal Plants of India, Tamil Nadu. Srinivasan V, Kosal N Ram. Cyber Media, Bangalore. 2000.