



An Investigation on Anti-urolithiatic Activity of Apple Cider Vinegar in Experimental Rats

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ABSTRACT

The apple cider vinegar (ACV) was evaluated for its antiurolithiatic activity in experimentally induced urolithiatic rats. Urolithiasis was induced by administration of sodium oxalate (70mg/kg) intraperitoneally to adult male Wistar albino rats (150-200g) for seven days served as a preventive regimen and ethylene glycol (0.75% v/v) in drinking water for 28 days served as a curative regimen. Two doses ACV (0.51ml/kg) and ACV (1.02 ml/kg) were subjected for the evaluation of antiurolithiatic activity. Cystone (750mg/kg) was served as standard in both the models. It was observed that experimentally induced Urolithiasis in both the conventional model elevated calcium, oxalate, uric acid concentration in urine also serum creatinine and uric acid levels were elevated. Similarly, reduction in magnesium levels was seen. Treatment with ACV (0.51ml/kg) and ACV (1.02ml/kg) significantly restored all elevated Urine and serum biochemical parameters in both prophylactic and curative regimen. Kidney homogenate analysis and assessment of physical parameters confirmed the antiurolithiatic effects of ACV. Histopathological findings also showed improvement in kidney architecture after treatment with ACV. The present study concluded that apple cider vinegar was found to be effective against Urolithiasis.

Keywords: Antiurolithiatic, Apple cider vinegar, Ethylene glycol, Sodium oxalate.

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INTRODUCTION

The formation of stone in the urinary system, i.e., in the kidney, ureter and urinary bladder or in the urethra is called urolithiasis. 'Urolithiasis' = ouron (urine) and lithos (stone). Urolithiasis is one of the major diseases of the urinary tract and is a major source of morbidity. Stone formation is one of the painful urologic disorders that occur in approximately 12% of the global population and its re-occurrence rate in males is 70-81% and 47-60% in female¹. It is assessed that at least 10% of the population in industrialized part of the world are suffering with the problem of urinary stone formation. The rate of occurrence is three times higher in men than women, because of enhancing capacity of testosterone and inhibiting capacity of estrogen in stone formation². The problem of stone formation is considered as a medical challenge due to its multifactorial etiology and high rate of reoccurrence.

Urolithiasis is clinically characterised by calculus formation in any region in the urinary tract, leading to urolithiasis. Kidney stone formation is a complex process that results from succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules³. Symptoms include

dysuria, burning and painful micturition, pain in the pelvic and lumbo-sacral region and the presence of small stone crystals in the urine. Urine is supersaturated with common stone forming minerals, however the crystallization inhibiting capacity of urine does not allow urolithiasis in most of individual, whereas the natural inhibition capacity is on deficient in stone former⁴. Globally Calcium oxalate is considered as the main constituent in the renal calculi accounting for 80% of the stones, followed by struvite to an extent of 10-15%, uric acid 3-10% and cysteine 0.5-1%⁵.

Many countries such as Malaysia, Indonesia, India and China have a rich tradition of folk medicine from centuries and provided effective remedies to various ailments using plants and plant derived compounds. There is no such risk factor to use the plant medicine as compared with the allopathic drugs. The uses of complementary and traditional medicine have been increasing worldwide because of fewer side effects. Medicinal plants are used for various research purposes and has been reported that traditional systems have immune potential against various diseases⁶.

One of the natural substances known for hundreds of years and nowadays living its renaissance is apple cider vinegar (ACV) which has been helping people to live healthy life. Apple cider vinegar is a solution of acetic acid produced by fermenting apples. The name vinegar comes from a French word "vin aigre", meaning "sour wine". Vinegar can be made from almost any fermentable carbohydrate source, including dates, sorghum, apple, grapes where yeasts ferments sugar to alcohol and later converted to acetic acid by acetobacter bacteria. Acetic acid is a volatile organic acid that identifies the product as a vinegar whose



concentration ranges from 3-5% and is responsible for the tart flavour and pungent, biting odour of vinegars⁷.

The influence of apple cider vinegar has been investigated for hundreds of years. It was in fact first used about 5000 years ago. In the year 400 B.C., Hippocrates, the father of modern medicine, prescribed the mixture of honey and apple cider vinegar for treatment of various diseases⁸. It has been particularly used during the American Civil War for disinfecting the wounds of soldiers. More valuable properties of apple cider vinegar and its ingredients, suggesting their therapeutic effects, have been recently discovered⁹. Apple cider vinegar which is made by fermentation of apple has been used as a folk medicine and its consumption as a home remedy is widely advertised in mass media. However scientific data base about its biological effects is scarce.

MATERIALS AND METHODS

Collection And Authentication of Fruit Material

The fresh fruits of Red delicious apple used for the present studies were collected from local market and authenticated by botanist.

Preparation of Apple Cider Vinegar¹⁰

Red delicious apples are used to produce natural apple cider vinegar with inclusion of maceration. The fresh fruits were washed and the cores and peels of apples are separated. It is important to pick up organic apples so that will get the full benefits of apple cider vinegar. Glass jar was selected and to this warm water, apple pieces and organic sugar was added and stirred well. Later covered the jar with a cotton cloth and put a rubber band around it and stored the glass jar in a cool and dry place. Allowed it to stand for 2 to 3 weeks so that fermentation takes place. During this period stirring of apple cider vinegar is done. This is very important step because it will help in fermentation process. On 27th day the peels are separated and filtered the liquid and tested for acidity of apple cider vinegar.

Estimation of Total Acidity % of Vinegar¹¹

Pipetted out 10ml of apple cider vinegar and transferred to conical flask. To this, 2 drops of phenolphthalein indicator was added. This is titrated against standardized solution of 0.1N NaOH. The NaOH solution was added drop by drop from the burette to the ACV sample until all the acetic acid in the sample was exactly consumed. At this point reaction is completed and no more NaOH is required and this is called the equivalent point of the titration i.e., change from colourless to very pale pink. The burette reading was noted and calculated the % TA of ACV using the formula.

% TA = (ml of NaOH) x (N of NaOH) x (60.05)/10 x Sample Weight.

Preliminary Qualitative Phytochemical Analysis^{12,13}

The preliminary phytochemical analysis of the apple cider vinegar was performed for the detection of the active constituents present in it.

Experimental Animals

Healthy Wistar albino rats (150–200g) of either sex were used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature $22^{\circ} \pm 2^{\circ}\text{C}$, relative humidity 60±5% and 12-hour light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (Approval no. SCP/IAEC/F150/P108/2017). The animals were acclimatized for one week before use.

Evaluation of Antiuro lithiatic Activity

Dose selection of ACV¹⁴

Two doses were selected and was administered post orally at a constant volume of 0.51 ml/kg (low dose) and 1.02ml/kg (high dose) for each animal.

An Investigation on Antiuro lithiatic Activity of Apple Cider Vinegar in an Experimental Rats

Sodium oxalate induced urolithiasis¹⁵ (prophylactic)

Animals were divided into five groups containing six animals in each group. Group I served as a normal control and received regular rat food and drinking water *ad libitum*. Group II served as a urolithiatic control and received regular rat food and sodium oxalate (70mg/kg of body weight) was given intraperitoneally from 1st to 7th day. Sodium oxalate (70mg/Kg of body weight) was given intraperitoneally to groups II-V from 1st day to 7th day for induction of renal calculi. Group III received standard drug Cystone (750mg/kg) from 1st day till 7th day. Group IV received ACV (0.51 ml/kg) and Group V received ACV (1.02ml/kg) from 1st to 7th day. Treatment with ACV and standard drug was given once daily through oral route.

Ethylene Glycol Induced Urolithiatic Model^{16,17} (Curative)

Animals were divided into five groups containing six animals in each group. Group I served as a normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75% v/v) in drinking water was fed to groups II-V for induction of renal calculi from 1st day till 28th day. Group II served as a urolithiatic control. Group III received standard drug Cystone (750mg/kg of body weight) from 15th day till 28th day. Group IV received ACV (0.51 ml/kg) and Group V received ACV (1.02ml/kg) from 15th to 28th day. Treatment with ACV and standard drug was given once daily through oral route.



Assessment of antiurolithiatic activity

Urine analysis¹⁸

Urine samples (24 hour) were collected on 7th and 28th day by keeping the animals in individual metabolic polypropylene cages for the analysis of prophylactic and curative parameters respectively. Animals had free access to drinking water during urine collection period. Urine was used for the estimation of calcium, oxalate, magnesium, creatinine using commercially available kits.

Serum Analysis¹⁹

On 7th day prophylactic rats and on 28th day curative rats were anaesthetised using ether and blood was withdrawn by retro-orbital puncture using micro capillary tubes. Serum was separated by centrifugation at 7000 rpm for 15 minutes and used for the estimation of creatinine and uric acid using commercially available kits.

Kidney Homogenate Analysis²⁰

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off some extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in hot air oven. A sample of 100 mg of dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenised. The homogenate was centrifuged at 2000 rpm for 10 min and supernatant was separated. The calcium, oxalate and lactate dehydrogenase content in kidney homogenate was determined using commercially available kits.

Physical Parameters

Measurement of Urine volume and kidney weight Urine samples (24hour) were collected on 7th day in prophylactic rats and on 28th day in curative rats by keeping the animals in individual metabolic propylene cages. Animals had free access to drinking water during urine collection period. The volume of urine from each group of animals were measured. Rats were sacrificed with ether anaesthesia at the end of experiment and kidneys were isolated, kidneys were cleaned off extraneous tissue and weighed individually.

Histopathological studies²¹

Rats were sacrificed with ether anaesthesia. Their kidneys were isolated and subjected to histopathological studies. The kidneys were cleaned off extraneous tissue and transferred to 10% neutralised formalin solution. Sections of the kidney was fixed in paraffin, stained with haematoxylin and eosin and observed for histopathological studies.

Statistical Analysis

All data were expressed as Mean \pm SEM. The statistical significance between groups were compared using one way ANOVA, followed by Dunnett's (multiple comparison test) and student t test.

RESULTS

Estimation of Total Acidity % of Apple Cider Vinegar

Table 1: Estimation of total acidity percentage of Apple cider vinegar

Content in conical flask	Burette reading		Volume of NaOH (ml)	End point
	Initial	Final		
10ml of ACV+2 drops of phenolphthalein	0	0.6	0.63	Pale pink
	0	0.7		
	0	0.6		

$$\% \text{ Of total acidity} = (\text{ml of NaOH}) \times (\text{N of NaOH} \times 960.05) / 10 \times \text{Sample weight.} = 0.63 \times 0.1 \times 960.5 / 10 \times 10$$

$$\% \text{ Of total acidity} = 3.81$$

Preliminary phytochemical screening of Apple cider vinegar

Preliminary phytochemical investigation of Apple cider vinegar has been performed and active constituents like flavonoids, triterpenoids and carbohydrates are found to be present.

Evaluation of Antiurolithiatic Activity of ACV in Sodium oxalate induced Urolithiasis (Prophylactic model)

In the present study, urolithiasis was successfully induced by administration of sodium oxalate (70 mg/kg) intra-peritoneally for 7 days and on 7th day urine was collected and analysed for various urinary and serum biochemical parameters.



Table 2: The effect of ACV on urine and serum analysis against sodium oxalate induced urolithiatic in rats.

Groups	Urine (mg/dl)				Serum(mg/dl)	
	Oxalate	Calcium	Magnesium	Uric acid	Creatinine	Uric acid
Normal Control	1.32 ± 0.14	1.282 ± 0.03	2.89 ± 0.14	1.31 ± 0.14	0.81 ± 0.03	0.68 ± 0.22
Urolithiatic control	6.78 ± 0.36***	5.55 ± 0.07***	1.43 ± 0.66***	5.43 ± 1.02***	2.52 ± 0.09***	2.1 ± 0.11***
Standard	1.64 ± 0.44**	1.57 ± 0.070**	2.70 ± 1.05***	1.98 ± 1.02***	0.93 ± 0.08***	0.75 ± 0.03
ACV (0.51ml/kg)	4.23 ± 0.15*	3.46 ± 0.06*	2.11 ± 1.06**	3.01 ± 0.70*	1.76 ± 0.02*	1.39 ± 0.51*
ACV (1.02ml/kg)	3.22 ± 0.25**	2.745 ± 0.05**	2.48 ± 1.43**	2.78 ± 0.88***	1.22 ± 0.06**	1.06 ± 0.08***

All values are expressed as mean ± SEM, n= 6 One-way analysis variances (ANOVA followed by Dunnett's multiple comparison test *P<0.05 **P<0.01 ***P<0.001 compared with urolithiatic control. a -p<0.001, values are significantly different from normal control group.

Table 3: Effect of ACV on urine volume and kidney weight against Sodium oxalate induced urolithiasis in rats.

Groups	Normal	Urolithiatic control	Standard	ACV (0.51ml/kg)	ACV (1.02ml/kg)
Volume of urine in (ml)	17.72±0.73	9.14±0.42***	15.43±0.04**	12.5±0.12*	13.9±0.04**
Kidney weight	0.70 ± 0.55	2.04±0.05***	0.95 ± 0.02**	1.64±0.08*	1.30±0.14**

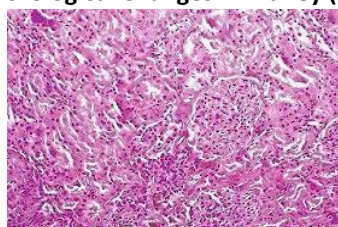
All values are expressed as mean ± SEM n=6, One way analysis variance (ANOVA) followed by Dunnett's multiple comparison test, when compared with normal group, and *P<0.05 **P<0.01 ***P<0.001 compared with urolithiatic control group. A p<0.001, values are significantly different from normal control group.

Table 4: Effect of ACV on kidney homogenate analysis against sodium oxalate induced Urolithiasis in rats.

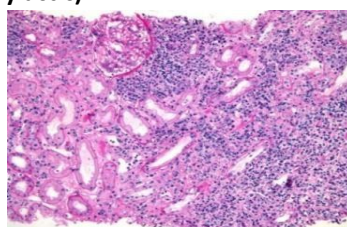
Groups & Drug Treatment	(mg/dl)		(U/g)
	Oxalate	Calcium	LDH
Group I Normal Control	1.98±0.17	7.43±0.35	1.08±0.44
Group II Urolithiatic control Ethylene Glycol (0.75%)	10.23±0.19***	12.1±0.84***	4.08±0.46***
Group III Reference control Cystone (750 mg/kg)	5.42±0.04***	8.91±.055***	1.84±0.35***
Group IV ACV (0.51ml/kg)	7.12±0.31*	10.32±0.43**	2.75±0.46*
Group V ACV (1.02ml/kg)	6.44±0.55**	9.13±0.51**	2.09±0.23**

All values are expressed as mean ± SEM, n= 6 One-way analysis variances (ANOVA) followed by Dunnett's multiple comparison test *P<0.05 **P<0.01 ***P<0.001 compared with urolithiatic control. a -p<0.001, values are significantly different from normal control group.

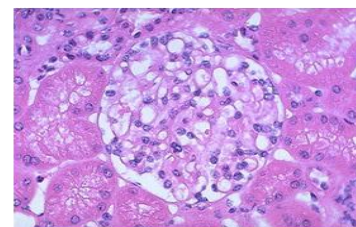
Histopathological Changes in Kidney (Prophylactic)



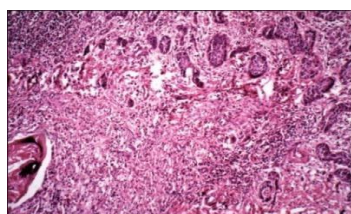
(a) Normal



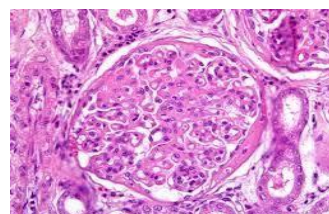
(b) Urolithiatic control



(c) Standard



(d) ACV (0.51ml/kg)



(e) ACV (1.02ml/kg)

Figure 1: Histopathological images of rat kidney sections of different group

(a) Control group showed normal renal architecture without any crystal deposition vial lining; (b) In urolithiatic control group a large sized crystals of Calcium oxalate were observed along with glomerular atrophy and damaged tubular cells (c) Standard group rats showed significant protection with mild lymphocytic infiltration with no evidence of thickening of synovial lining and angiogenesis (d) ACV low dose (0.51ml/kg) and (e) ACV high dose (1.02ml/kg) treated group also significantly restored the normal architecture. No CaOx crystals were observed, the cellular organizations of proximal and distal convoluted tubules were restored.

Evaluation of Antiurolithiatic Activity of ACV in Ethylene glycol induced Urolithiasis (Curative model)

Urine samples were collected on 15th day from overnight fasted animals by keeping in individual metabolic cages. Animal had free access to drinking water during the urine collection period. On 15th day analysis of urine was done for the confirmation of urinary stone formation. The test reports confirm the presence of calcium oxalate crystals and triple phosphate in urolithiatic control animal.

Table 5: Effect of ACV on urine and serum analysis against sodium oxalate induced urolithiasis in rats.

Groups	Urine (mg/dl)				Serum(mg/dl)	
	Oxalate	Calcium	Magnesium	Uric acid	Creatinine	Uric acid
Normal Control	0.5 ± 0.14	2.83±0.15	2.66±0.14	1.23 ±0.14	0.46±0.14	1.18±0.21
Urolithiatic control	2.21 ± 0.36***	7.58±0.14***	1.48±0.76***	4.39 ±1.02***	1.56±0.14***	3.10±0.34***
Standard	0.58 ±0.44***	3.5 ±0.08***	2.54±1.06***	1.48 ±1.02***	0.52±0.14***	1.56± 0.08***
ACV (0.51ml/kg)	0.81 ± 0.15*	5.32 ± 1.28*	1.75±1.06*	3.19±0.70**	0.97±0.14*	2.43±0.53**
ACV (1.02ml/kg)	0.72±0.05**	4.53± 1.21***	1.98±1.43**	2.12±0.88**	0.68±0.63**	2.16±0.12**

All values are expressed as mean± SEM, n= 6 One-way analysis variances (ANOVA followed by Dunnett's multiple comparison test *P<0.05 **P<0.01 ***P<0.001 compared with urolithiatic control. a -p<0.001, values are significantly different from normal control group.

Table 6: Effect of ACV on urine volume and kidney weight against Ethylene glycol induced urolithiasis in rats.

Groups	Normal	Control	Standard	ACV (0.51ml/kg)	ACV (1.02ml/kg)
Volume of urine in (ml)	18.54±0.03	11.14±0.02***	16.11± 0.04***	13.95±0.02*	14.74±0.04**
Kidney weight	0.78±0.04	2.13±0.05***	0.83±0.02***	1.54 ±0.06**	1.21±0.01**

All values are expressed as mean± SEM n=6, One way analysis variance (ANOVA followed by Dunnett's multiple comparison test *P<0.05 **P<0.01 ***P<0.001 Compared with urolithiatic control group. a -p<0.001, values are significantly different from normal control group.

Table 7: Effect of ACV on kidney homogenate analysis

Groups	Oxalate (mg/dl)	Calcium (mg/dl)	LDH (U/g)
Normal Control	2.6±0.18	4.43±0.35	1.66±0.44
Urolithiatic control	7.23±0.36***	12.1±0.84***	5.08±0.46**
Standard	3.1±0.24***	4.98±.052***	2.84±0.35***
ACV (0.51ml/kg)	5.11±0.41**	8.32±0.43*	4.75±0.46*
ACV (1.02ml/kg)	4.24±0.25**	6.53±0.51**	3.98±0.23**

All values are expressed as mean± SEM n=6, One way analysis variance (ANOVA followed by Dunnett's multiple comparison test *P<0.05 **P<0.01 ***P<0.001 Compared with urolithiatic control group. a -p<0.001, values are significantly different from normal control group.

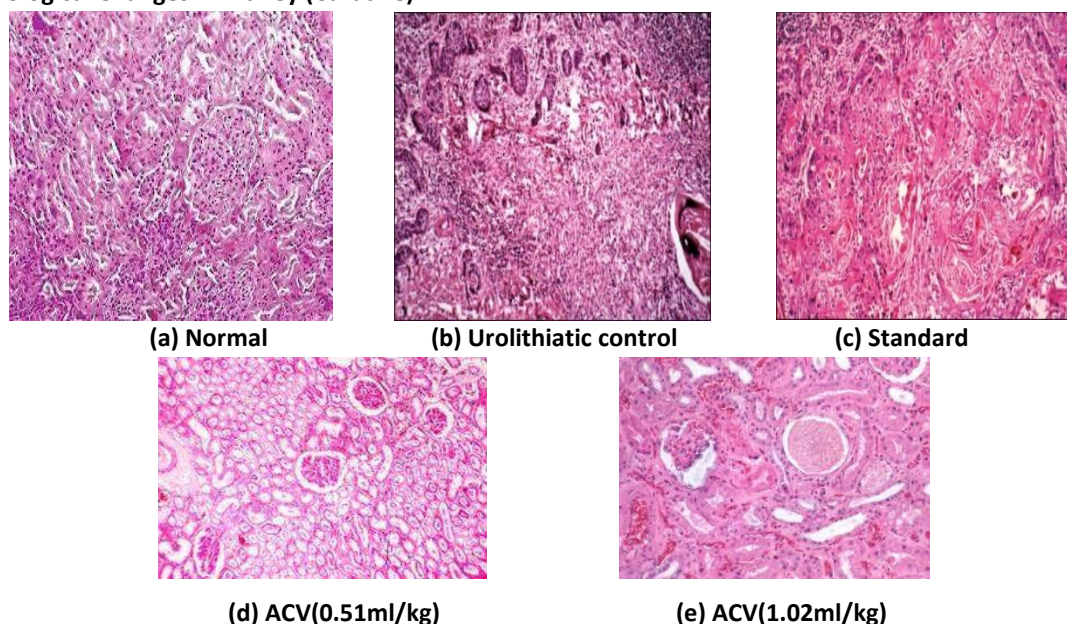
Histopathological Changes In Kidney (Curative)

Figure 2: Histopathological studies of Kidney in different Groups

(a)Control group showed normal renal architecture without any crystal deposition vial lining; (b) In urolithiatic control group a large sized crystals of Calcium oxalate were observed along with glomerular atrophy, damaged tubular cells and dearranged tubular organization. (c) Standard group rats showed significant protection with mild lymphocytic infiltration with no evidence of thickening of synovial lining and angiogenesis (d) ACV low dose (0.51ml/kg) and (e)ACV high dose (1.02ml/kg) treated group significantly restored the normal architecture. No CaOx crystals were observed, the cellular organizations of proximal and distal convoluted tubules were restored.

DISCUSSION

Currently available drug regimens for management of kidney stone have certain drawbacks. Therefore, there is a need for safer and more effective antiurolithiatic drugs. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, hydroxyl-L-proline and glycolic acid which leads to hyperoxaluria which is one of the major initiative factors for Urolithiasis²². In the present study, the chemical ethylene glycol (0.75%) and sodium oxalate (70mg/kg) is used to induce Urolithiasis in rats. The male rats are selected to induce Urolithiasis because the urinary system of male rats resembles that of humans²³. Also, earlier studies shown that the amount of stone deposition in female rats was significantly less²⁴.

Administration of ethylene glycol to the experimental animals for 28 days resulted in substantial excretion of oxalate and deposition of microcrystals in kidney. Ethylene glycol is non-toxic and gets eliminated by the

kidney. Ethylene glycol gets metabolized into glycolic acid, glyoxalic acid and oxalic acid. The formed oxalic acids were largely excreted in the urine as calcium oxalate and gets deposited in the renal tubule. Due to the deposition of calcium oxalate, there is an obstruction in urine flow in urinary system. Because of this the glomerular filtration rate (GFR) also decreases. Due to this the waste products particularly nitrogenous substances such as urea, creatinine, uric acid gets accumulated in the blood²⁵. Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that, 28 days administration of ethylene glycol to the male albino rats resulted into the formation of renal calculi mainly composed of calcium oxalate. The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Renal calcium oxalate deposition by ethylene glycol in rats is frequently used to mimic the urinary stone formation²⁶. Therefore, this model was used to evaluate the curative effect of Apple cider vinegar against Urolithiasis.

Oxalocalcic lithiasis was induced by an intraperitoneal injection of sodium oxalate (70mg/kg) for seven days. The mechanisms by which oxalate causes its deleterious effects to kidneys, liver and the haematological system have yet to be determined. In recent studies, however, some of toxic effects of oxalate have been attributed to oxalate induced oxidative stress. The oxalate induced-membrane peroxidation leads to membrane integrity loss renal cell damage and, finally, calcium oxalate crystal deposition. NaOx administration causes an increase in the severity of microscopic calcium oxalate crystals along with high crystal concentration in the kidney. Thus, this model was used to evaluate the preventive effect of apple cider vinegar against Urolithiasis.

In the present study urinary output was markedly decreased in lithiatic control group on 7th day and 28th day of prophylactic and curative regimen respectively. However, in standard and ACV treated groups the urinary volumes were increased when compared to lithiatic control group. It was also observed that experimentally induced calculogenesis caused an increase in kidney weight compared to normal group animals. Treatment with standard drug Cystone (750 mg/kg) prevents the accumulation, deposition and super saturation of calciurogenic chemicals in the kidney. Similarly significant decrease in kidney weight was seen in both ACV treated preventive and curative regimen.

Urolithiasis in both the conventional model was evidenced by significant elevation in the urine and serum biochemical parameters. Following the induction of Urolithiasis, the urinary volume and composition were found to be altered. Administration of Ethylene glycol (0.75%) and sodium oxalate (70mg/kg) facilitates the increased risk of urolithiasis by elevating urinary level of stone forming constituents' calcium, oxalate, uric acid and provides favourable environment for the nucleation and stone formation. In the present study, calcium, oxalate and uric acid excretion were progressively increased in calculi induced animals (Group II). It has been reported that oxalate plays an important role in stone formation and have about 15-fold greater effect than calcium²⁷. Elevated urinary calcium is favouring factor for nucleation of calcium oxalate from urine and further crystal growth. However, ACV at both low dose (0.51ml/kg) and high dose (1.02ml/kg) lowered the level of oxalate as well as facilitates the excretion of calcium, creatinine, urea and uric acid via urine, which is valuable in the prevention of calculi formation in both preventive and curative regimen.

In Urolithiasis glomerular filtration rate decreases due to the obstruction to the flow of urine by stones in urinary system. In calculi induced rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid which are markers of glomerular and tubular damage. Treatment with standard drug Cystone (750 mg/kg) and ACV low dose (0.51 ml/kg) and high dose (1.02ml/kg) showed significant decrease in in the elevated serum levels of creatinine and uric acid when compared to lithiatic control group.

Normal urine contains many inorganic and organic inhibitors of crystallization. Magnesium is one such well-known inhibitor. Magnesium has been found to decrease the growth and nucleation rates of calcium oxalate crystals. Urinary magnesium was significantly diminished in EG and sodium oxalate induced urolithiatic rats. Treatment with ACV restored magnesium levels in both preventive and curative regimen.

Experimentally induced Urolithiasis increased the activity of LDH, which is an oxalate synthesizing enzyme present in the liver and kidney. It is also considered as the marker enzyme of cellular injury. The increased LDH activity

indicated the increased oxalate synthesis as well as enhanced injury of renal epithelial cells. Both the conventional model depicted raised serum LDH level in hyperoxaluric rats (Group II). The ACV significantly reduced LDH level in kidney tissue homogenates in a dose dependent manner in both preventive and curative regimens.

Histopathological studies showed changes like damaged epithelial cells at the inner layer, dilation of tubules and presence of crystals in tubules of calculi induced (group II) rats. However, kidney section of treatment groups in both preventive and curative treatment with ACV(0.51ml/kg) and ACV(1.02ml/kg) shows improvement in dilation of tubules, tubule interstitial inflammatory infiltration and reduced crystal deposition This support the protective effect of ACV on curative and prophylactic Urolithiasis. Probable mechanism of action Apple cider vinegar may be due to its important phytoconstituents present such as flavonoids, terpenoids and polyphenols, which were confirmed in phytochemical study. Previous studies reported that flavonoids and polyphenolic compounds exert their curative effect in urolithiasis via reducing the spasmodic pain, inflammation, increasing the output of urine and antioxidant effect²⁸.

CONCLUSION

In conclusion, the results of the present study indicate that co-administration of apple cider vinegar (ACV) reduced and prevented the growth of urinary stones. The probable mechanism could be due to its extractive phytoconstituents possessing diuretic, antioxidant property, and decreasing the concentration of urinary stone-forming substances. The present study suggests the potential of Apple cider vinegar in the treatment of urolithiasis confirming their traditional use and modern claims of being a very good remedy for preventing urinary stone. However, the anti urolithiatic activity was found to be dose dependent in both prophylactic and curative model. The exact mechanism for the antiurolithiatic activity is still unclear. Thus, it's worth for further investigations for isolation of more bioactive molecules for treatment and using more experimental paradigms are required for further confirmation of Antiurolithiatic potential of Apple cider vinegar.

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