



A Method of Isolation of Capparisterol from *Capparis decidua* and Antinephrolithiasis Activity

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ABSTRACT

A kidney stone is similar to formation of calculi in kidney. It forms when chemicals in the body aggregate together. A stone may stay in the kidney or travel through the urinary system. Very small stones may pass through the urinary system without causing much pain. Larger stones can block the flow of urine if they get stuck in the ureters or urethra.

The study was undertaken to investigate the anti nephrolithiasis activity of capparisterol. Capparisterol is administered by intra peritoneal route in 35 mg/kg and 70 mg/kg doses. In preventive regimen capparisterol 70 mg/kg (73.73% relief) show greater creatinine clearance then standard drug cystone 750 mg/kg and in curative regimen capparisterol 70 mg/kg (114.18% relief) has greater effect then cystone (97.45% relief). Test drug show dose dependent therapeutic efficacy. Therapeutic effect of test drug increase with increasing dose.

Keywords: Solanum xanthocarpum, Capparisterol, Nephrolithiasis, Kidney stone.

INTRODUCTION

Nephrolithiasis

A kidney stone is like a small rock that forms in the kidney. Stones form when certain chemicals in the body clump together. A stone can either stay in the kidney or travel through the urinary system.¹ Anyone can have a kidney stone, but it may be more likely if you:

- Are male

- Are Caucasian
- Are very overweight
- Have had kidney infections
- Have a family member with kidney stones
- Have had kidney stones before
- Eat a lot of animal protein (such as meat and eggs)
- Do not drink enough liquids

Other conditions and medicines can also put you at greater risk for kidney stones.

Very small stones might pass through the urinary system without causing much pain. Larger stones can block the flow of urine if they get stuck in the ureters or urethra. Kidney stones do not usually cause any symptoms until they start to pass. Some symptoms might include:

- Extreme pain in your back or side that will not go away
- Throwing up
- Blood in your urine
- Fever and chills

PLANT PROFILE

Capparis decidua

Collection and Identification of Plant Material

The chosen plant, *Capparis decidua* was identified and selected by the experts of Botany Department, Institute of biomedical and industrial research, Jaipur. For the present study various parts of the *Capparis decidua* i.e., fruits, flowers and barks were collected in and around Jaipur (India).

See fig. 1.

It is commonly known as karer, karel, kenr, karu etc is a densely branching shrub or small tree of the Thar Desert.

It is also found in the subtropical and tropical zones and other arid regions in southern Asia with a mass of slender, 4-5 meter high or occasionally a small tree with many green vines like apparently leafless branches, hanging in bundles. The bark turns into whitish- grey colour with age but most of the branches and twigs are a glossy dark green in colour. Small light brown spines occur in pairs on the twigs at each node. Leaves are very minute (2 mm long) with a very short life span on young shoots, so that the plant looks leafless most of the time. The new flush of leaves appears in November –

January. Flowers are pink in colour, red veined in small groups along the leafless shoots, in the axils of spines. Fruits are small many seeded ovoid or sub-globulous, slightly mucronate pink berry of the size and shape of a cherry, becomes blackish when dry.²

Phytoconstituent

The plant possesses a number of alkaloids, terpenoids, glycosides and some fatty acids. The different phytoconstituents of different plant parts are as follow- Capparine, Cappariline and capparinine The bark shows the presence of n-pentacosane, n-tricontanol and β -sitaosterol besides a water-soluble alkaloid, 1-stachydrine .

Besides these, six new phytoconstituents have been isolated and characterized from the root bark, which are capparisterol, Capparideciduasterol, Capparisditerpenol, in aliphatic hydroxyketone and capparisditerpenyl ester.

MATERIAL AND METHOD

Equipment

UV spectrophotometer (Systronic Double beam spectrophotometer 2203), IR Spectrophotometer, Flame photometer (Esico – Model 381) , Hot air oven, Refractometer, Extraction unit , Stage micrometer, Test tube, Beaker, Funnel, Slide, Spatula etc.

Chemical and Drugs

Ethylene glycol, Ammonium chloride, Cystone, Sodium azide. Diethyl ether, Formaldehyde, Hydrochloric acid, Nitric acid, Ethanol, Methanol, Ether, chloroform, and other common laboratory reagents (Rankem & CDH Pvt. Ltd. New Delhi).

Experimental Animals

Healthy male rats of wistar strain weighing between 150 and 175 g of

equivalent age groups were obtained from central animal house of Institute of Biomedical and Industrial Research, Jaipur. Rats were acclimatized for one month in propylene cages under hygienic conditions and provided with standard animal feed and water ad libitum. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals and were approved by the Institutional animal ethical committee (ibir/iaec/02).

Isolation and identification method of Capparisterol from *Capparis deciduas*

The coarsely powdered fruit of *Capparis decidua* (2 kg) was subjected to hot extraction process with ethanol (95%) for seventy two hours. The combined extracts were concentrated in rotary vacuum evaporator. A dark brown extract (120 g) obtained was chromatographed over silica gel (100-120 mesh) in a column using various solvents in order of increasing polarity. Elution of the column with petroleum ether: chloroform (1:1) gave 180 mg yellowish crystal of compound, which were recrystallized from pure chloroform. Its melting point was observed at 301^oC.

Toxicity studies according to OECD Guideline 420

The toxic dose was determined on the basis of a pre-studied experiment which was carried out on the rats. Four doses that were less than the lethal dose (LD50) (700 mg/kg, 1400 mg/kg, and 2100 mg/kg and 8000 mg/ kg as lower and higher dose, respectively) were taken as effective dose in the current study.

Four doses for *Capparisterol* to achieve lethal dose (LD50) were taken as effective dose in the current study.

Groups are divided as follow:

- Group A: 700 mg/kg; 10 albino Wistar rats
- Group B: 1400 mg/kg; 10 albino Wistar rats
- Group C: 2100 mg/kg; 10 albino Wistar rats

EXPERIMENTAL MODEL

Anti Nephrolithiasis activity model

Dosage

Capparisterol was suspended in distilled water and was administered (i.p) at doses of 35 and 70 mg/kg body weight based on preliminary experimentation.

Experimental procedure

Ethylene glycol induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats following procedures as under.

Prophylactic regimen (PR)

Animals were divided into five groups containing six animal in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water ad libitum. All remaining groups (Group II-V) received calculi inducing treatment, comprised of ethylene glycol (EG, 0.4% v/v) with ammonium chloride (NH₄Cl, 1% w/v) in drinking water *ad libitum* for 15 days to accelerate lithiasis. Group III - administered cystone (750 mg/kg body wt.)
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Group IV – Capparisterol (35 mg/kg)
Group V- Capparisterol (70 mg/kg),
Group III – V were administered above mentioned doses from day 1 to day 15 of calculi induction. Capparisterol and standard drug were suspended in distilled water and given intraperitoneally once daily.³

Curative regimen

Animals were divided into five groups containing six animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water *ad libitum*. All remaining groups (Group II- V) received calculi inducing treatment, comprised of ethylene glycol (EG, 0.4% v/v) with ammonium

chloride (NH₄Cl, 1% w/v) in drinking water *ad libitum* for fifteen days to accelerate lithiasis, followed by only EG (0.4% v/v) for next thirteen days.

Group III - administered cystone (750 mg/kg body wt.)

Group IV – Capparisterol (35 mg/kg)

Group V- Capparisterol (70 mg/kg),

Group III – V were administered above mentioned doses from day sixteen to day twenty eight of calculi induction respectively. Capparisterol and standard drug were suspended in distilled water and given intraperitoneally once daily.

After the treatment, the rats were placed in metabolic cages and urine was collected in a glass bottle having 20 µl of 20 % sodium azide as a preservative for twenty four hour.

The urine was frozen at - 20⁰C and used for determination of alkaline phosphate (ALP) and lactate dehydrngene (LDH) and creatinine content. The rats were anaesthetized with diethyl ether and sacrificed by decapitation after twenty four hour of above treatment. Before sacrificing, the blood was taken from orbital sinus into centrifuge without anticoagulant and allowed to clot at room temperature to collect serum. Urine from urinary bladder was directly obtained by puncturing with a needle (5/8 in.) attached to a 1ml tuberculin syringe. After dissection both kidneys were removed and transverse section from both the kidneys was fixed for histological analysis.

Biochemical assays in urine and serum

Serum urea level was estimated by diacetylmonoxime method. The creatinine in both serum and urine was estimated by the method of Bonsnes and Tauskey. Creatine clearance was calculated. Urinary LDH was measured by decrease in absorbance at 340 nm resulting from the oxidation of NADH. The activity of ALP was determined by

measuring the conversion of p-nitrophenyl phosphate to p-nitrophenol at 405 nm.

Histopathological studies

Transverse sections of kidney tissue were fixed in formaldehyde (10%). The tissue were then dehydrated and embedded in paraffin wax. The paraffin sections (8µ) were then cut and stained in H & E staining and viewed under light microscope.⁴⁻⁹

Statistical analysis

The results are expressed as mean ± SE. Comparison between the treatment groups and control were performed by analysis of variance (ANOVA) followed by Dunnet's multiple test. In all tests the criterion for statistical significance was P < 0.05.

Observation

Toxicity Study of aqueous extract of Capparid decidua extract

Group A: No behavioral change and mortality rate observed.

Group B: 30 % mortality rate observed.

Group C: 50 % mortality rate observed.

See fig. 2, 3 & 4.

RESULT

Capparisterol found no behavioural change and mortality at dose 700 while at 1400, 2100 mg/kg 0%, 30%, 50% respectively mortality was observed.

According to Table 1 and Table 2, test drug at both doses shows nephrolithiatic activity against ethylene glycol induced nephrolithiasis in albino wistar rat with comparison to standard drug cystone.

In preventive and curative regimen test sample show significant changes with comparison of hyperoxaluria group in Urinary LDL (Units/ml/mg prt), Urinary ALP (IU/L), Serum Urea (mg/dl) and Serum Creatinine (mg/dl).

In preventive regimen capparisterol 70 mg/kg (5.49 ml/min, 73.73% relief) show greater creatinine clearance then standard drug cystone (5.44 ml/min, 72.15% relief) and in curative regimen capparisterol 70 mg/kg (5.89 ml/min, 114.28% relief) has greater effect then cystone (5.43 ml/min, 97.45% relief).

DISCUSSION AND CONCLUSION

A kidney stone is like a small rock that forms in the kidney. Stones form when certain chemicals in the body clump together. A stone can either stay in the kidney or travel through the urinary system. Very small stones might pass through the urinary system without causing much pain. Larger stones can block the flow of urine if they get stuck in the ureters or urethra. Kidney stones do not usually cause any symptoms until they start to pass. The study was undertaken to investigate the anti nephrolithiasis activity of *Capparis decidua*. In preventive and curative regimen test sample show significant changes with comparison of hyperoxaluria group in Urinary LDL (Units/ml/mg prt), Urinary ALP (IU/L), Serum Urea (mg/dl) and Serum Creatinine (mg/dl).

In preventive regimen capparisterol 80 mg/kg (73.73% relief) show greater creatinine clearance then standard drug Cystone 750 mg/kg and in curative regimen capparisterol 70 mg/kg (114.18% relief) has greater effect then cystone (97.45% relief). Test drug show dose dependent therapeutic efficacy. Therapeutic effect of test drug increase with increasing dose.

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Table 1. Anti nephrolithiasis activity of capparisterol (Preventive regimen)

PR	Urinary LDL (Units/ml/mg prt)	Urinary ALP (IU/L)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Crcl (ml/min)
Control	12.50± 0.9193****	44.60± 0.7924****	197.99± 4.3668****	0.58± 0.0794****	6.17± 1.1411****
Hyperoxaluria	39.62± 2.8891	115.63± 2.8209	350.33± 37.9658	0.91± 0.1146	3.16± 0.5859
Cystone 750 mg/kg	17.22± 0.4898****	54.4± 2.5812****	236.10± 4.2220****	0.55± 0.0735****	5.44± 0.7897***
Capparisterol 35 mg/kg	24.52± 2.1963****	93.81± 2.5069****	308.73± 7.4032***	0.53± 0.0711****	5.25± 0.9869**
Capparisterol 70 mg/kg	18.07± 1.5070****	66.24± 3.8556****	252.29± 12.4490****	0.55± 0.0779****	5.49± 0.8825****

Mean± Standard Deviation

Table 2. Anti nephrolithiasis activity of capparisterol (Preventive regimen)

CR	Urinary LDL (Units/ml/mg prt)	Urinary ALP (IU/L)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Crcl (ml/min)
Control	12.24± 0.9879****	43.97± 1.9195****	202.51± 7.4992****	0.57± 0.0345****	6.40± 0.6883****
Hyperoxaluria	35.36± 1.7963	108.3± 5.3020	351.02± 6.2643	0.92± 0.0828	2.75± 0.4960
Cystone 750 mg/kg	15.32± 0.7029****	54.13± 1.2909****	237.20± 10.4138****	0.56± 0.0787****	5.43± 0.6703****
Capparisterol 35 mg/kg	21.29± 2.2303****	89.34± 2.1654****	297.2117± 4.7606****	0.70± 0.0956***	4.48± 0.3476***
Capparisterol 70 mg/kg	15.99± 0.855827****	56.38± 3.0206****	239.46± 4.3390****	0.61± 0.1056****	5.89± 0.7677****

Mean± Standard Deviation



Figure 1. Small tree of the Thar Desert.

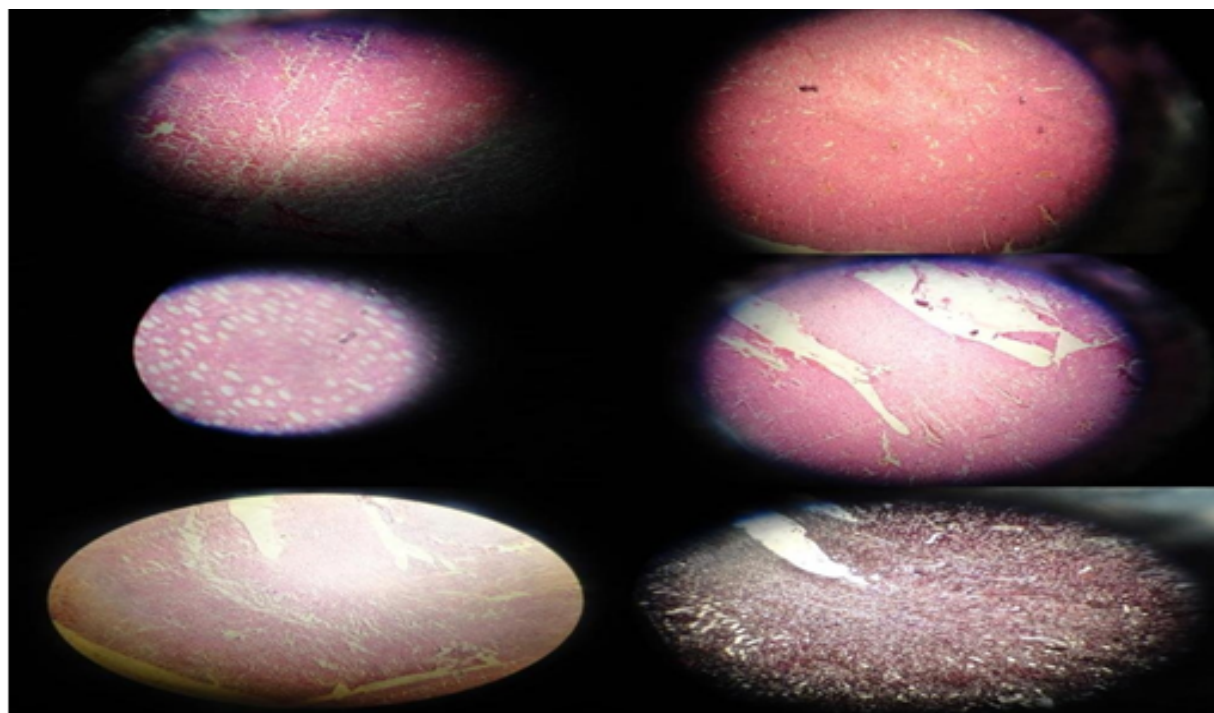


Figure 2. Histopathology slides for toxicity study of *Capparis*

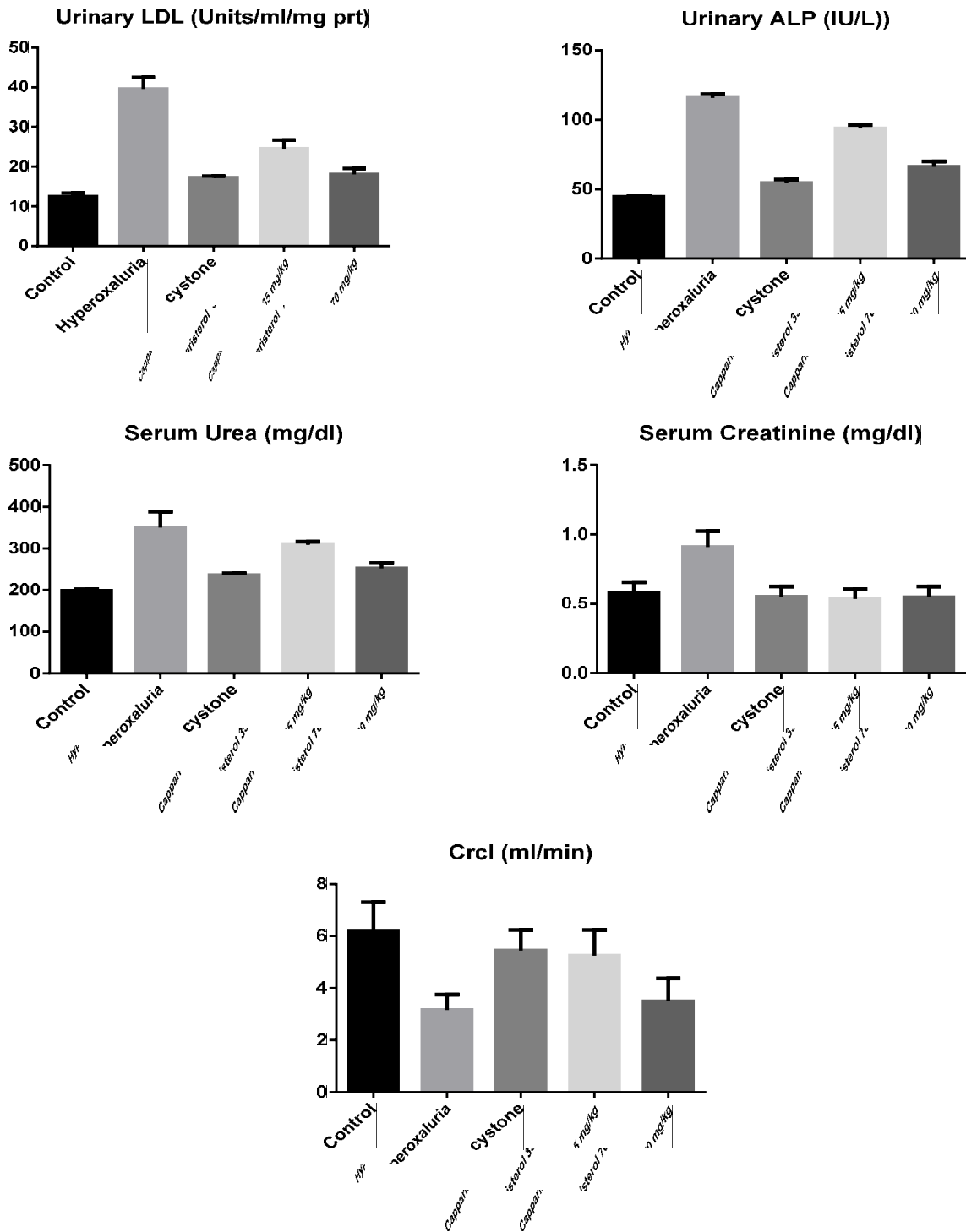


Figure 3

