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THE STUDY ON UROLITHIATIC ACTIVITY OF *CISSUS QUADRANGULARIS* PLANT IN EXPERIMENTAL ANIMAL MODEL

MD Faheemuddin[1*], Mohammed Arshad[2], P. Sai Priya[3], Putta Pravalika[4], Shruthi Verma[5], G. Shravani[6] and Suryam Gugulothu[7]

[1]Assistant Professor, Department of Pharmacology Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India-502313.
[2,3,4,5]Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India-502313.
[6]Assistant Professor, Department of Pharmaceutical Chemistry, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India-502313.
[7]Assistant Professor, Department of Pharmaceutics, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India-502313.

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MD Faheemuddin
Assistant Professor, Department of Pharmacology Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak, Telangana, India-502313.

ABSTRACT

The present study was undertaken to evaluate the Urolithiatic Activity of the medicinal plant Cissus quadrangularis. The study was carried out for 14 days in which the aqueous extract of Cissus quadrangularis was used at a dose at which it is used to cure bone fracture treatment in ayurvedic practices 7. Four groups consisting of three Wister rats in each group weighing 120 to 150 grams were taken. Normal group (normal water), control group (Ethylene Glycol (EG) induced urolithiasis) Test group-I (500mg/kg) Test groupII (750mg/kg) after 14 days the animals weight analysis, urine analysis, blood analysis and histopathological studies were carried out. The results shown the significant development of calcium oxalate crystals in control, TestI & Test-II groups. From the study it is concluded that the dose used for the bone fracture treatment leads to urolithiasis, so the dose adjustment is required. Further study is required to prove exact mechanism of calcium oxalate crystals.

KEYWORDS: The results shown the significant development of calcium oxalate crystals in control, TestI & Test-II groups.

INTRODUCTION

Kidney stones, medically termed as nephrolithiasis, are hard deposits formed in the kidneys from minerals and salts found in urine. They can vary in size, from as small as a grain of sand to as large as a golf ball, and can cause excruciating pain when they pass through the urinary tract. There are different types of kidney stones, including calcium stones (the most common type), uric acid stones, struvite stones, and cystine stones. The type of stone can influence treatment options and prevention strategies. Kidney stones are prevalent in India, impacting individuals of varying ages and genders across the country. Below are demographic specifics regarding the occurrence of kidney stones in India: Age: While kidney stones can develop in individuals of any age, they tend to be more prevalent among people between the ages of 30 and 60 years. Gender: Research indicates that kidney stones occur more frequently in men compared to women. Studies have demonstrated that men are three times a likely to develop kidney stones as women. Geographical Location: The occurrence of kidney stones in India differs depending on the geographical location. Research indicates that individuals residing in hot and arid regions of the country, such as Rajasthan and Gujarat, have a higher likelihood of developing kidney stones. Dietary Habits: Diet significantly influences the formation of kidney stones. In India, the elevated consumption of animal protein, salt, and foods rich in oxalate, such as spinach and nuts, are contributing factors to this condition. Genetics: There is evidence to suggest that genetics might contribute to the occurrence of kidney stones. Research indicates that certain families exhibit a greater prevalence of this condition. Kidney stones form when there is an imbalance in the substances that make up 14 urine, such as calcium, oxalate, and uric acid. When these substances become highly concentrated, they can crystallize and form stones.

MATERIALS AND METHODS

Cissus quadrangularis (Horjora, Nalleru) Cissus quadrangularis, also known as veldt grape, adamant creeper, or devil's backbone, is a plant that belongs to the grape family (Vitaceae).

Cissus quadrangularis is a perennial herb with medicinal properties distributed throughout the tropical world. It is one of the most frequently used medicinal plants in India. This plant is studied for its phytochemical

constitution, pharmacological activities and toxicological evaluation. It is used for bone healing. Ayurveda prescribe this plant for several medicinal ailments. The anabolic steroid from the Cissus quadrangularis plant showed a marked influence in the rate of fracture healing by early generation of all connective tissue. Cissusquadrangularis contains vitamins and steroids, which are found to have specific effect on bone fracture healing. The stem parts of plant contain A and βamyrins, β-sitosterol, ketosetosterol, phenols, tannins, vitamin, carotene, Calcium oxalate, 31 methyl tritriacontanoic acid, taraxeryl acetate, taraxeroliso-pentadecanoic acid, Calcium ions and phosphorus.

The stems of Cissus quadrangularis was collected from Medicinal Garden, VIPER, Narsapur and Urban Park, Narsapur. The collected stems were authentically identified by Dr. MadhavShetti, Botanist, Sri Venkateswara University, Tirupati.

Lakshadi Guggul: widely used in the Ayurvedic treatmentfor of bone related diseases and fracture healing. Zeotone softgel capsule.

Panchajeeraka Gudam: An effective Ayurvedic medicinefor post-natal care, useful in digestive and respiratory diseases. It is in herbal jam form.

Preparation of extract

The collected stems of Cissus quadrangularis were dried by using Tray Dryer. The dried stems were pulverized through a mechanical grinder. The powdered material was subjected to successive Maceration extraction by using water as a solvent. The extract was stored in a refrigerator**.**

Phytochemical Studies

The extracts of Cissus quadrangularis was subjected to phytochemical studies to get the active chemical compounds present in it. Extracts were sticky, green color, and stored at 4 °C until use. Phytochemical studies were performed according to the official methods prescribed for medicinal compounds using appropriate chemical reagents**.**

Experimental Design

The experimental rats were divided into four groups, each group comprising of three animals. Group I animals were administered with water and considered as a control for 2 weeks. 0.75% Ethylene glycol was administered to group II animals and had been treated as a Toxic Control. Extract of Cissus quadrangularis were given thrice daily to group III (500mg/kg) & VI (750mg/kg) containing animals. Administration of test and the toxic control drug was done by oral route. Experimental animals were examined by biochemical & histopathological studies. Table-2.

Table 2: Experimental Design.

Treatment	Drugs	Dose
Group 1	Vehicle Control	Normal Water Ad Libitum
Group 2	0.75% Ethylene Glycol	Water + Ethylene Glycol
Group 3	Extract PO	500mg/kg p.o
Group 4	Extract PO	750mg/kg p.o.,

Collection of Serum

Blood was collected from experimental rats by venipuncture using a 20-gauge needle. Bloodwascentrifuged for 5minat 2000 rpm to separate serum. The extracted serumwas transferred intothe plastic vialand storedat –80°C untilfurther biochemicalparameters estimation such as calcium, creatinine, uric acid, urea, and blood urea nitrogen (BUN).

Ethics statement

All experimental procedure and studies performed under the guidelines of scientific experiments on animal sasapprovedbytheInstitutionalanimalethicscommittee(IAEC) and care of laboratory animals taken for the entire study as per the guidelines of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Euthanasiaandanaesthesia

Toperformthehistopathologicalstudy,animalsweresacrificedbycervicaldislocationas it causes extensive damage to the brainstemresulting in immediate unconsciousness and death. Experimental rats were sedated with Chloroform before dislocation**.**

Histopathology

Attheendoftheexperiment, the rats were sacrificedbycervicaldecapitation. After sacrifice, kidney of each group was rapidly dissected out and washed immediately with saline and fixedin 10% phosphate-bufferedformalin. Paraffin-embeddedspecimenswere cutinto 5µm-thicksectionsandstainedwith haematoxylin and eosin. Thesectionswere examined under the light microscope for the presence of histopathological changes and photomicrographs were taken.

Collected stems

Dried stems

Grinded powder

Extraction

Materials and Methods

Dosing

Microscopy

Collected Blood & Organ Isolation

Collected Urine

Metabolic cage

RESULTS

Phytochemical analysis of water extract of *Cissus quadrangularis*

Theaqueousextractof*Cissusquadrangularis*issubjectedto phytochemicalscreening methods and it was shown the presence of Alkaloids, Glycosides, Flavonoids, Tannins and amino acids.

Table 3.

S.No.	TestforPhytoconstituents	Waterextract
1.	Alkaloids, Dragendroff'stest Hager'stest	+
2.	Glycosides Zinc HCltest Alkalinetest	+
3.	Flavonoids ZincHCltest Alkalinetest	+
4.	Tannins Aq.FeCl3 Gelatintest	+
5.	Aminoacids Millon'stest	+

The oraladministrationof*Cissusquadrangularis*extractinratsup tothedose500mg/kg & 750mg/kg did not show any sign of toxicity and there was no mortality for 14 days.

Urinalysis

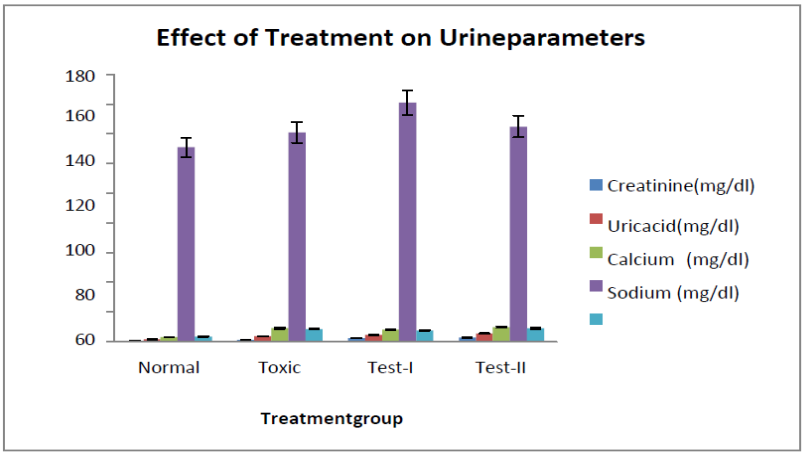
The qualitative urine analysisonday14 showed a significant inductionofcrystalurea in Toxic controlgroup (G2), whencompared to Normalcontrol(G1). Inadditionto normal urine crystals such as Uric acid, the calcium oxalate and phosphate crystals were significantly increased in ethylene glycol treated Toxic control group (G2). Treatment with test extracts showed an increased in the calcium oxalate and phosphate crystals. Among the extract treated groups, animal streated with Cissus *quadrangularis* at 500 and 750mg/kg, p.o., showed Urolithiatic activity.

Ethylene Glycol (0.75%v/v) Induced Urolithiasis Rat Model.

Table 4: Effect of oral administration of *Cissus quadrangularis* on urine parameters (Creatinine, Calcium, Potassium, Uric acid and ALP) after 14 days of treatment. the increase in the electrolyte and urine creatinine and uric acid in the urine ofthe treatment and toxic control group show that formation of Urolithiasis induction.

Groups	Creatinine (mg/dl)	Calcium (mg/dl)	Sodium (mg/dl)	Potassium (mmol/L)	Uricacid (mg/dl)
Normal	0.78±0.02$^{\#\#}$	3.24±0.32$^{\#}$	131±1.36$^{\#\#}$	3.62±0.11$^{\#\#\#}$	1.78±0.46$^{\#\#}$
ToxicControl	1.37±0.21*	9.14±0.34**	141±1.78**	8.74±0.32**	3.73±0.29**
Test-I	2.75±0.32**	8.39±0.84**	161±1.94***	7.81±0.75**	4.65±0.17***
Test-II	2.84±0.74***	9.97±0.75**	145±0.43**	9.25±0.75**	5.64±0.30*

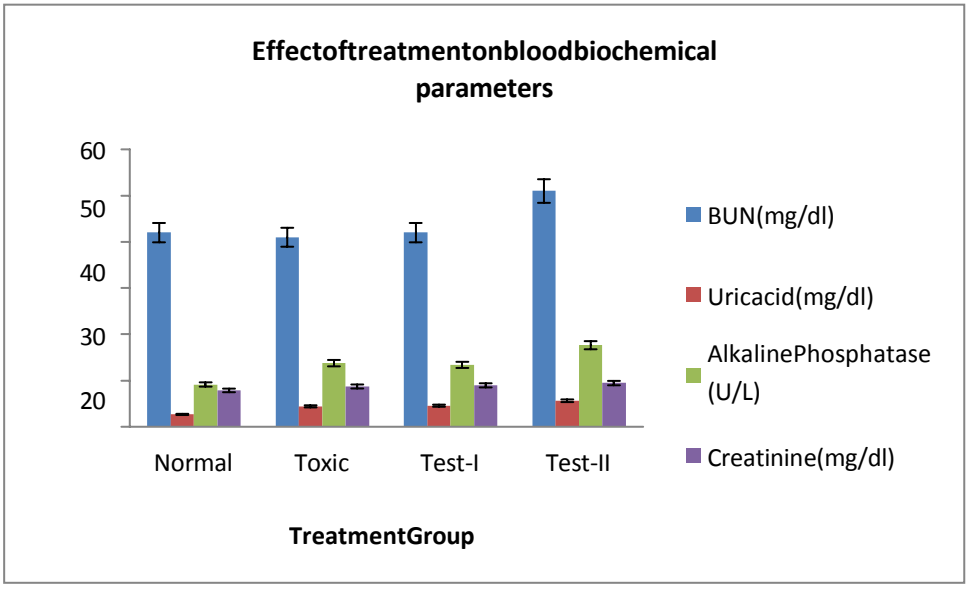
Al lthe valuesare calculated using ANOVA, Dunnett'stest, SEM(Standard ErrorMean) using Graph Pad Prism 5.0 version, ***P<0.001 as compared to ethylene glycol treated group.



Blood Analysis: Effect of oral administration of *Cissus quadrangularis* on Blood electrolytes (Sodium, Calcium, Potassium) after 14 days of treatment.

Groups	Calcium(mg/dl)	Sodium (mg/dl)	Potassium (mmol/L)
Normal	121.84±4.37$^{\#}$	111±2.44$^{\#\#}$	2.62±8.91$^{\#\#\#}$
ToxicControl	119.67±2.64**	153±3.88**	8.24±5.72**
Test-I	158.97±6.74**	174±3.94***	7.88±7.78**
Test-II	179.97±7.74**	197±5.43**	8.75±5.29**

Allt he valuesare calculated using ANOVA, Dunnett'stest, SEM(Standard ErrorMean) using Graph Pad Prism 5.0 version, ***P<0.001 as compared to ethylene glycol treated group.

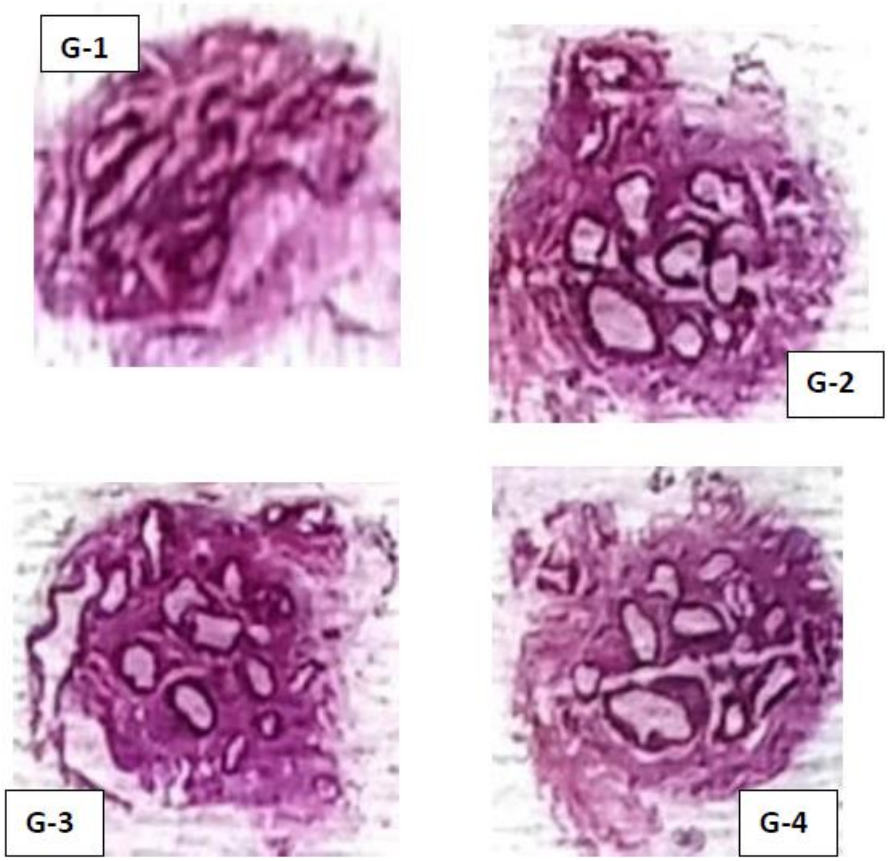


Histophatologicalstudies

Normal Group: The histology of normal group (G-1) hasnot show nanysigns of renal damage, thet issue is intactand the glomerul arapparatus and the renal tubules can beseen evident in the images.

Toxiccontrol Group: The histology of toxicgroup(G-2)hasshownrenaldamage, the tissue is intact and the glomerular apparatus and the renal tubules are not clear and the gaps in the arrangement of renal structure shows renal damage occurred due to calculli formation.

Test-I & Test-II Group: The histology of Test-I & Test-II group (G-3 & G-4 Respectively) has shown renal damage, the tissue is intact and the glomerular apparatus and the renaltubules are not clear and the gaps in the arrangement of renal structure shows renaldamage occurred due to calculli formation. The test-II group kidney images shows large gaps between the renal cellular arrangements, this suggest the renalcalculiformation due to treatment is dose dependent.



RESULTS AND DISCUSSION

The study was designed to evaluate the Urolithiatic effect of water extractof *Cissusquadrangularis*(CQ) in the management of bone healing of Wistar albino rats. *Cissus quadrangularis* has beenwidelyused becauseofits variouspharmacologicalproperties in humans with is provenefficacy and safety. It canbe used as supplement invarious types of fractures for quicker healing with early remodelling of fracturecallus. Further studies can be made to evaluate its efficacy in variousdisorders in different ethnic groups.

Cissus quadrangularis is traditionally used as a cure for the bone fractures. The study carried out using a toxic control treated with Ethylene Glycol (EG) induced urolithiasis was compared withthat of Test drugtreated animals against a normalgroup. The results showsthat *Cissusquadrangularisaqueous*extractthat isused forbone fractureinbythe traditional medical show significant formation of urolithiasisinratsatadoseof500mg/kg & 750mg/kg dose.

The urine and biochemical analysis of blood has supported the claim and the histopathological studies also has supported the results. From the results it can be concluded that significant development of calcium oxalate crystals in control, Test-I & Test-II groups. Thus, it can be suggested for a dose adjustment for its therapeutic use. Further study is required to prove exact mechanism of calcium oxalate crystals.

CONCLUSION

Cissus quadrangularis is traditionally used as a cure for the bone fractures. The study carriedout using a toxic control treated with Ethylene Glycol (EG) induced urolithiasis was compared withthat of Test drugtreated

animals against a normal group. The results shows that *Cissus quadrangularis aqueous* extract that is used for bone fracture in by the traditional medical show Urolithiasis activity in rats at a dose of 500mg/kg & 700mg/kg dose.

From the results it can be concluded that significant development of calcium oxalate crystalsincontrol, Test-I&Test-IIgroups. Thus, it can be suggested for a dose adjustment for its therapeutic use. Further study is required to prove exact mechanism of calcium oxalate crystals.

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