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Research article

Structural identification through GC mass spectrophotometer and determine anti lithiotic activity of *hibiscus rosa sinensis* by using ethylene glycol induced method

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ABSTRACT

The present study investigated the antiurolithiatic effect of standardized ethanolic extract of *Hibiscus rosa sinensis* by structural elucidation of the responsible compound for the activity as well as Ethylene glycol model induce calcium oxalate crystal in Albino rats. The chemical structural elucidation of *H. rosa sinensis* performed by phytochemical study and GC-Mass spectrophotometry. Calcium oxalate urolithiasis was induced in rats in rats by ethylene glycol -ammonium chloride feeding in drinking water. Antiurolithiatic activity of *H. rosa sinensis* was evaluated in 2 different doses (300,600 mg/kg) in curative and preventive regimen by estimating the histological changes in kidney and biochemical change in urine, serum. Sodium oxalate was used as reference standard drug. Ethylene glycol-ammonium chloride feeding caused an increase in urinary volume, oxalate, total protein, phosphate and uric acid levels along with a decrease in urinary excretion of calcium, magnesium and citrate. Test group -1 and Test group-2 animals treated with *H. rosa sinensis* decrease the amount of calcium, oxalate, citrate, which is responsible for induce calcium oxalate crystals in significant amount. Histological study revealed minimum damage and a smaller number of calcium oxalate deposits in the kidney of ethylene glycol treated rats. These results indicated that the hibiscus rosa sinensis reduced and prevented the growth of urinary stones. However, the sodium citrate is more effective than the *H rosa sinensis* (600 mg/kg more effective than hibiscus 300 mg/kg) in the treatment of urolithiasis.

Keywords: *Hibiscus Rosa Sinensis*, Kidney Stone, Sodium Citrate, Ethylene Glycol, Calcium Oxalate.

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INTRODUCTION

Nephrolithiasis has been a recognized human disease for millennia. It was found in entombed Egyptian mummies dating back 4000 BC. The earliest recognized discovery was reported in America in a teenage boy. Nephrolithiasis is defined as calcification that lies in the collecting system, bladder, ureter and calyceal system. Most of the cases of calculi are found in the pelvicalyceal system and can be passed into the ureter. Computed tomography is the leading technique which are used in the imaging of nephrolithiasis and nephrocalcinosis. The first paper on intravenous urography was published in 1932, which described the features of a delayed nephogram and dilated collecting system in a patient with renal colic. This technique was considered to be the most reliable method in the assessment of renal calculi until the advent of helical CT in the early 1990s [1]. Kidney stones is the disease which found or affect up to 5% of the total population in the different regions of the world in living organism and are strongly associated with race or ethnicity and region of residence. Seasonal variations are important factors for urinary stone found mostly in the winter season because the consumption of the water decreases in these days. Since water Intake during the winter season commonly low therefore chance of stone forming increase. The age is prime factor for stone forming in human's body. Stones commonly found in men in the age of 30 years to 40 years while in women stones found in the age between 35 to 55 years. In most of the cases kidney stones repeatedly produced if once formed in the body. The probability of reoccurrence of kidney stone in the human body is approx 50% [2].

Medical treatment like lithotripsy is extensively used for the removal of calculi. However, continuous exposure to shock waves may cause acute kidney injury, infection and decrease in the kidney functions, and an increase in stone relapse. Drugs used in the treatment has showed some feasibility, but not without side effects.

Allopathic drugs like NSAIDS, stone expulsion (vasodilator), uric acid, calcium channel blockers, alkali citrate have side effects like risk of renal injuries, heart failure, reduced renal blood flow, inhibition of platelet aggregation, orthostatic hypotension [3].Medicinal plant remains an important alternative source of new drugs. In the Indian system of medicine, a number of plants have been claimed to be efficient to care and correct urinary stone. In the

present study we use *H. rosa-sinensis* plant that have analgesic, antispermatic activity however, so far, no scientific study has been reported regarding anti-urolithiasis potential of plant. In this study, we identified and evaluated the chemical responsible for antilithiatic property by using ethylene glycol and sodium oxalate model.

MATERIALS AND METHODS

The flowers of *Hibiscus Rosa-Sinensis were collected from Botanical garden of Sagar Institute of Research and Technology-Pharmacy* in the month of March 2020, and identified by Dr. Saba Naaz H.O.D Department of Botany, Safia Science College, A voucher specimen (File no. 194/Saif. /Sci./Collage/Bpl) was authenticated and has been deposited in the herbarium of the institute. Flowers of *hibiscus rosa sinensis* were shade dried and powdered to get coarse granules which were stored in air tight container in the dark.

For performing research work animal activity by animal models on the animals taken permission from the Institutional Animal ethical Committee, Sagar Institute of Research and Technology-Pharmacy the assigned Reference No. is SIRT-P/19/01/IAEC34.

Peperation of ethanolic extract of the flower of *Hibiscus rosa sinensis*

In present study, plant materials were extracted by using cold maceration method; the *hibiscus* flowers were collected, washed and rinsed properly. About 1kg of the powder was extracted with organic solvent ethanol and allowed standing for 4-5 days each. The extract was filtered using Whatman No.1 filter paper to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. Extract was transferred to beaker and evaporated in room temperature & excessive moisture was removed and extract was collected in air tight container.

Drugs and Chemicals

Sodium Citrate were used as standard drug for the anti-lithiotic respectively were purchased from the local market of Bhopal. All remaining chemicals used in the experiment were of the highest 99.5% pure, commercially grade available.

Apparatus and Instruments

Rotatory vacuum evaporator, GC-Mass Agilent 7890A, GC-MS Agilent 5975C, Heating mantle. Hot Plate, Magnetic stirrer, Glassware,

Phytochemical test

The freshly prepared ethanolic leaves extract was subject to qualitative test to identify the phytochemical compound present in it using the standard procedure [4].

GC-mass analysis

GC mass analysis carried out at Central Instrumentation facility of IISER Indian institute of Science Education and Research Bhopal. This technique is very important for the identification of various phytochemical of plants. The equipment used for GC-mass for detection of molecular weight and structure of chemical compound. For GC-mass detection an electron ionization system with ionizing energy of 70 ev was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate 1 ml/min and 1 µl of plant sample was employer (split ratio of 10:1) at the injection temperature 250˚ C ion source temperature of 280˚ C and total running time approx. 60 minutes.

Identification of the chemical was conducted using the GC mass database of National Institute Standard and Technology having>6200 patterns. The spectrum of the unknown component was composed with the spectrum of known component in the repository of NIST library. The retention of the molecular weight, molecular formula and composition percentage of the sample material were record [5].

Ethylene glycol

Male albino rats (aged 7-8 weeks) weighing 200-250 g will be housed under 12 h light/dark cycles at 23 + 2 C. The animals accessed to food and water and libitum. All animals' experiments were carried out in accordance with CPCSEA guidelines and Institutional Animal Ethical Clearance.

A total of 30 animals were divided into 5 groups for anti-inflammatory studies:

Group 1 Control (No drug)

Group 2 Standard (Sodium Oxalate)

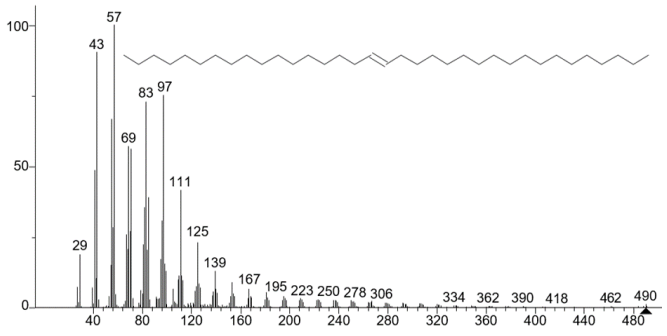
Group 3 Negative Control (Stone induced but drug not given

Group 4 Extract (hydroalcoholic extract of *Hibiscus rosa sinensis* 300 mg/kg)

Group 5 Extract (hydroalcoholic extract of *Hibiscus rosa sinensis* 600 mg/kg)

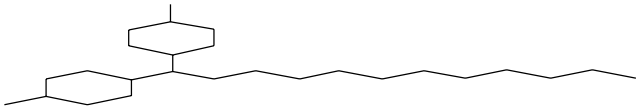
GC-mass interpretation of *Hibiscus rosa* sinensis 17-Pentatriacontene, molecular formula: $C_{35}H_{70}$

Figure 1: GC-mass interpretation of *Hibiscus rosa*



Administration of Ethylene Glycol in drinking water has been shown to result in consistent induction of hyperoxaluria, crystalluria and calcium oxalate nephrolithiasis. Delivering solely 0.75% EG male rate eventually yielded persistent crystalluria at 12 days and renal crystal deposits at 3 weeks. To enhance the development of crystal

deposition. Ethylene glycol often has been combined with other agents such as ammonium chloride (AC) to reduce urinary pH as well as vitamin D or calcium chloride to result in subsequent hypocalcemia. This lithogenic combination of ethylene glycol and



ammonium chloride is determined to be health-with rats having lower weights worsening renal function, and increased free radicals and metabolic acidosis take place as a result of EG rats [6-7].

Figure 2: 17-Pentatriacontene, Molecular weight 480

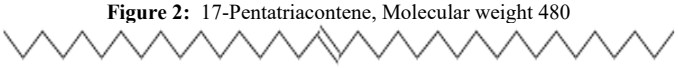


Table 1: 10 largest peaks: NIST: 233160

57999	43904	97751	83727	55 666
69569	71560	41 487	111414	85 391

Figure 3: Cyclohexane, molecular weight : 362

Chemical Name; Cyclohexane, Molecular formula:

Table 2: 10 largest peaks: NIST 23514 reference

55999	97543	41393	43333	69 268
83 261	111239	81212	96200	57 187

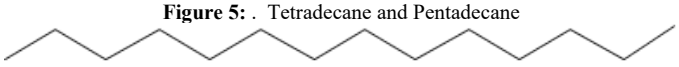
Chemical Name: Tetradecane, Molecular Formula: $C_{14}H_{30}$

Figure 4: Tetradecane, molecular weight: 198

Table 3 :10 largest peaks: NIST: 229858 reference

57999	43740	71643	85423	41 261
29163	55143	56143	70117	42 86

Figure 5: . Tetradecane and Pentadecane



After the GC-mass study different chemical compound identified from the hibiscus rosa sinensis compound which is mentioned in the table no 1. Tetradecane and Pentadecane are two compound which have structural similarity with sodium citrate responsible for anti-lithiotic activity.

Table 4: Chemical compound identified by GC-Mass method with identified research activity based on previous research work and literature review**.**

Chemical Name	Molecular formula	M.W.	%	Identified Research activity
Octatriacontyl pentafluoro propionate	$C_{41}H_{77}F_5O_2$	696	4.37	Antiviral drug used in the treatment of covid virus.
Hexatriacontyl pentafluoro propionate	$C_{39}H_{73}F_5O_2$	668	2.74	Insecticidal activity
Tetratriacontyl heptafluorobutyrate	$C_{38}H_{69}F_7O_2$	690	2.63	Antimicrobial property
Nonahexacontanoic acid	$C_{69}H_{138}O_2$	998	2.63	Fatty acid esters
17-Pentatriacontene	$C_{35}H_{70}$	490	34	Hydrocarbon compound
Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	914	10.2	Activity not reported
Cyclohexane, 1,1'-dodecylidenebis [4-methyl-	$C_{26}H_{50}$	362	3	Antibacterial, antifungal, analgesic, Antilithiatic [9].
Tetratriacontyl heptafluorobutyrate	$C_{38}H_{69}F_7O_2$	690	1.54	Anti-microbial property
tert-Hexadecanethiol	$C_{16}H_{34}S$	258	1.54	Diabetes, inflammation, cancer
Tetradecane	$C_{14}H_{30}$	196	2.44	Alkane Hydrocarbon, antimicrobial activity, Antilithiatic activity [8].
Pentadecane	$C_{15}H_{32}$	212	20.3	Alkane Hydrocarbon
Dodecane	$C_{12}H_{26}$	170		Alkane Hydrocarbon [10].
Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$		12.0	Alkane Hydrocarbon, solvent, diluent [10-11].
Hexadecane	$C_{16}H_{34}$	226	5.4	Alkane Hydrocarbon, antibacterial, and anti-fungal, Anti-oxidant, [10].
Dodecane, 2,7,10-trimethyl-	$C_{15}H_{32}$	212	5.84	Alkane Hydrocarbon, Solvent, Diluent
17-Pentatriacontene	$C_{35}H_{70}$	490	5.23	Anti-inflammatory anticancer, antibacterial, Antilithiatic activity, [10].
1-Hexacosene	$C_{26}H_{52}$	364	3.43	Anti-inflammatory property
Hexadecane, 1,1-bis(dodecyl oxy)-	$C_{40}H_{82}O_2$	594	2.56	Anti-oxidant , nematicides hypocholesterolemia,
tert-Hexadecanethiol	$C_{16}H_{34}S$	258	5.61	Enzyme activator
1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	3.40	Anti-microbial property
Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	914	3.27	Antioxidant, Antitumor, hyperlipidaemic,

Effect of ethanolic extract of Hibiscus rosa sinensis on urine volume, calcium ion, oxalate, total protein, phosphate and uric acid level in ethylene glycol induced urolithiasis [12-13].

Table 5: Effect of hibiscus rosa sinensis flower extract on urinary parameter in urolithiasis-induced rats

Days	Group I	Group II	Group III	Group IV	Group V
Urine Volume (ml/24h)	Vehicle Control	Negative Control	Standard 720 mg/kg	Hibiscus 300 mg/kg	Hibiscus 600mg/kg
0	8.12 ± 0.36	7.85 ± 55	9.10 ± 0.32	8.00 ± 0.55	8.50 ± 0.46
14	8.01 ± 0.25	7.70 ± 1.39	10.20 ± 1.35	7.90 ± 1.02	8.70 ± 1.02
28	7.58 ± 0.27	7.10 ± 1.41	12.12 ± 1.22	10.10 ± 0.69	10.50 ± 0.86
Calcium					
0	0.60 ± 0.02	0.63 ± 0.03	0.55 ± 0.03	0.60 ± 0.03	0.60 ± 0.02
14	0.58 ± 0.03	1.65 ± 0.02	0.48 ± 0.02	1.00 ± 0.03	0.70 ± 0.02
28	0.60 ± 0.02	2.55 ± 0.02	0.40 ± 0.01	1.60 ± 0.01	0.60 ± 0.01
Magnesium					
0	2.37 ± 0.08	2.66 ± 0.03	2.68 ± 0.06	2.75 ± 0.11	2.67 ± 0.10
14	2.71 ± 0.09	6.60 ± 0.01	2.62 ± 0.10	3.75 ± 0.13	3.48 ± 0.11
28	2.67 ± 0.11	7.15 ± 0.02	2.56 ± 0.09	4.17 ± 0.10	3.33 ± 0.11
Citrate					
0	5.57 ± 0.22	5.80 ± 0.11	5.75 ± 0.24	5.77 ± 0.15	5.80 ± 0.25
14	5.90 ± 0.37	10.90 ± 0.17	4.69 ± 0.37	5.88 ± 0.21	4.77 ± 018
28	5.83 ± 0.35	12.15 ± 0.04	5.30 ± 0.21	6.87 ± 0.22	6.50 ± 0.15
Oxalate					
0	5.14 ± 0.04	5.44 ± 0.03	5.25 ± 0.26	6..35 ± 0.22	6.80 ± 0.21
14	5.23 ± 0.06	8.10 ± 0.29	5.45 ± 0.28	8.45 ± 0.23	8.00 ± 0.22
28	5.21 ± 0.04	10.12 ± 0.36	5.75 ± 0.29	6.62 ± 0.25	6.20 ± 0.22
Total Protein					
0	5.86 ± 0.03	7.85 ± 0.27	5.90 ± 0.36	7.15 ± 0.40	6.85 ± 0.35
14	5.71 ± 0.02	11.05 ± 0.64	6.12 ± 0.39	9.45 ± 0.38	9.26 ± 0.39
28	5.97 ± 0.02	12.70 ± 0.73	6.49 ± 0.39	9.00 ± 0.45	7.35 ± 0.52
Phosphate					
0	5.56 ± 0.11	5.81 ± 0.22	5.66 ± 0.19	5.80 ± 0.20	5.80 ± 0.21
14	5.57 ± 0.17	6.69 ± 025	5.70 ± 0.23	6.50 ± 0.24	6.24 ± 0.22
28	5.84 ± 0.04	7.54 ± 0.27	5.72 ± 0.21	6.60 ± 0.25	6.25 ± 0.30
Uric Acid					
0	1.40 ± 0.21	1.57 ± 0.23	1.45 ± 0.06	1.49 ± 0.12	1.48 ± 0.06
14	1.36 ± 0.32	2.00 ± 0.33	1.54 ± 0.16	1.83 ± 012	1.82 ± 0.23
28	1.38 ± 0.23	2.49 ± 0.37	1.66 ± 0.50	1.84 ± 0.12	1.87 ± 0.15

Effect of ethanolic extract of Hibiscus rosa sinensis on blood serum and kidney histology, In the serum test count creatine, uric acid, Blood urea nitrogen (see table 2). In the kidney histology (see table 3) calcium, oxalate, phosphate, lipid peroxidation, CaOx deposit, damage index level in Ethylene Glycol induced urolithiasis [11].

Table 6: Effect of hydro alcoholic extract of hibiscus rosa sinensis on serum and kidney parameter

Parameter	Group I	Group II	Group III	Group IV	Group V
Serum	Vehicle Control	Negative Control	Standard 720 mg/kg (sodium Oxalate)	Hibiscus 300 mg/kg	Hibiscus 600mg/kg
Creatine	0.66 ± 0.05	1.50 ± 0.08	0.81 ± 0.04	1.31 ± 0.05	0.98 ± 0.05
Uric acid	1.47 ± 0.04	4.57 ± 0.18	2.24 ± 0.06	4.00 ± 0.08	2.95 ± 0.06
BUN	35.25 ± 0.08	49.22 ± 0.25	38.05 ± 0.65	45.05 ± 0.77	41.15 ± 0.83
Kidney					
Calcium	0.16 ± 0.02	0.33 ± 0.03	0.13 ± 0.01	0.35 ± 0.03	0.31 ± 0.02
Oxalate	1.36 ± 0.10	6.00 ± 0.18	1.55 ± 0.10	3.98 ± 0.35	2.80 ± 0.21
Phosphate	2.36 ± 0.10	3.96 ± 0.31	2.50 ± 0.20	3.10 ± 0.29	2.80 ± 0.19
Lipid Peroxidation	30.35 ± 0.84	95.00 ± 3.88	45.00 ± 3.25	59.04 ± 4.10	56.32 ± 3.36
CaOx deposit	0.00 ± 0.00	85.55 ± 2.16	5.01 ± 0.44	20.0.8 ± 1.22	15.12 ± 1.65
Damage index	0.00 ± 0.00	3.02 ± 0.09	0.32 ± 0.06	2.25 ± 0.05	1.56 ± 0.04

Figure 6: Light microscope image of kidney section from animals

Figure 6 (A) Vehicle control

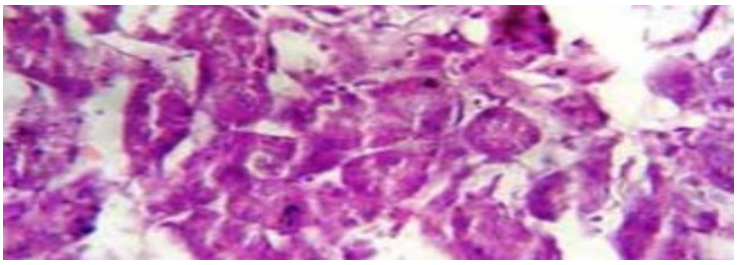


Figure 6 (B) Negative Control

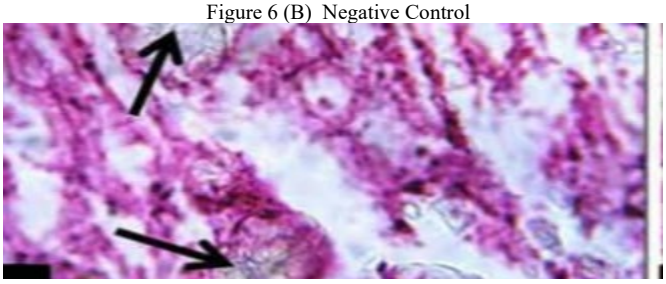


Figure 6 (C) Standard drug sodium citrate

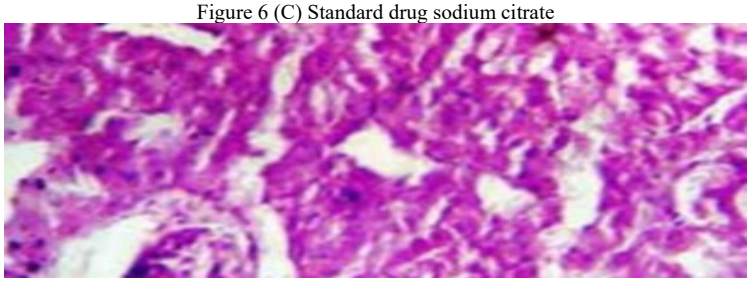


Figure 6 (D) Hibiscus rosa sinensis 300 mg/kg

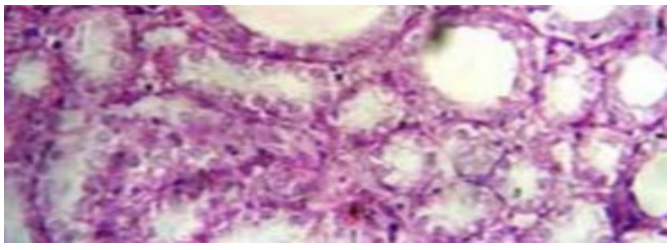
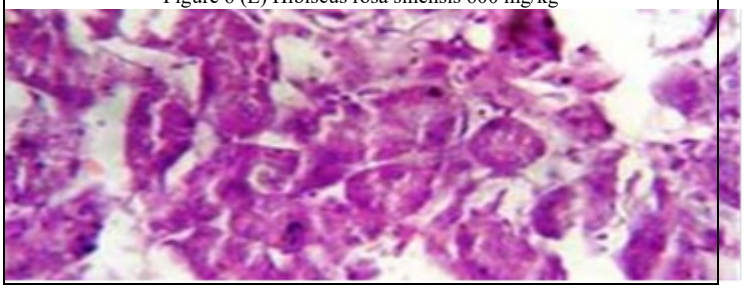


Figure 6 (E) Hibiscus rosa sinensis 600 mg/kg



The evidence presented earlier showed increased phosphate excretion in calcium induced lithiasis animals. In the lithiotic negative control group phosphate level increased in the urine sample with oxalate ion which is suitable for stone formation by forming calcium phosphate crystals, which induce calcium oxalate deposition. The sodium citrate treatment shows to decrease the rate of urinary phosphate excreation and thereby reduce the risk of formation.

Magnesium presence reflect the calcium oxalate as reported in some earlier finding and study results. Magnesium is the ion responsible for inhibition of crystallization. It is seen in some studies that magnesium level return to normal on drug therapy in renal stone patients. If high magnesium is reported to form a complex with oxalate and reduce the saturation of calcium oxalate and as a consequence reduce the growth and nucleation rate of calcium oxalate crystals.

The increase in the amount of uric acid in urine indicated the calcium induce in the kidney of rats. Uric acid interferes the solubility of calcium oxalate in urea medium. The rise of amount of uric acid crystals in rise calcium oxalate and modulation the crystallization also suggests its primarily role in stone formation. Treatment of *H. rosa sinensis* reduce the excreation of uric acid and thereby reduce the risk of stone formation.

Similarly it is seen in previous study increased urinary protein excretion was observed in calcium induced animals. Super saturation of urinary collides result in perception of crystal initiation particle treatment of *Hibiscus rosa sinesis* show significant decrease in urine protein and it indicates the calcium oxalate formation in the urine.

Regular increase of lipid peroxidation has been reported in the kidney of rats supplemented with a caliculi-producing diet. An elevated urinary oxalate concentration has been reported to induce lipid peroxidation and renal damage reacting with polyunsaturated fatty acids in the cell membrane.

Rise in blood urea nitrogen is another cause of kidney damage. In the negative control group animal showed rise in the blood urea level while the animal treated with *Hibiscus rosa* sinensis show significant decrease in blood urea level in test group animals.

It is showed in previous data increase calcium oxalate phosphate were observed in calcium induced animals. Treatment of Hibiscus rosa sinensis showed to present the rise in renal calcium, phosphate oxalate and phosphate level. Results of decrease in phosphate oxalate and protein and blood urea nitro hibiscious show significant antilithiatic activity.

Microscopic examination of kidney section of calcium induced animals showed the accumulation of calcium oxalate deposit inside the tubules which causes marked histological changes such as dilation of the proximal tubules along with inflammation. The hibiscus treatment decreases the number and size of calcium oxalate deposit in the different part of the Nephron.

Sodium citrate was the standard drug used in the study. Sodium citrate basically it is alkaline drug which change the pH acidic to basic level. Basic medium decreases the formation of calcium oxalate stone. Citrate is also work as anticoagulant so it decreases the calcium level by producing excreation of phosphate, oxalate, urea from the blood. Increase in the ph decrease the presence of uric acid in blood and urine

CONCLUSION

The present investigation support the use of Hibiscus rosa sinensis in folk medicine against urolithiasis . It is concluded that administration of ethanolic extract of hibiscus flower prevent the growth of urinary stones. The mechanism collectively supported by the activity of analgesic, hepatoprotective, antioxidant, hair growth activity.

REFERENCES

1. Phillips S, Graeth R, Tudor, 2011. Nephrocalcinosis and nephrolithiasis. Radiological imaging of kidney, Springer Verlag Berlin Heidelberg, 395-401.
2. Rohit kumar Bijauliya, Shashi Alok, Jain S K, et al, 2017. herbal and allopathic medicine for kidney, gallbladder and urinary stones, J Med. P'ceutical Allied Sci Volume 8 (5), 1935-1952.
3. Jadhav V M, Thorat R M, Kadam V J, Sathe N S, 2009. Traditional medicinal uses of Hibiscus rosa-sinensis. J. Pharm. Res, *2*(8), 1220-2.
4. Pawar A T, Vyawahare N S, 2015. Protective effect of standardized extract of Biophytum sensitivum against calcium oxalate urolithiasis in rats. Bulletin of Faculty of Pharmacy, Cairo University, *53*(2), 161-172.
5. Philip Rowe, Essential Statistics for the pharmaceutical science 2nd Edition 2015 published by Wiley Blackwell.
6. Pawar T Anil, Vyawahare S Niraj, 2017. Protective effect of standardized extract of Biophytum sensitivam against sodium oxalate-induced urolithiasis in rats, J. Traditional and Complementary Medicine, (7), 475-486.

7. Reddy V, NVL Swarchala, M Ganga Raju, 2021. GC-MS analysis, Bulletin of Environment, Pharmacology and Life Sciences Bull. Env. Pharmocol. Life Sci., 10 (3) 42-50.
8. Pauzi A N, Muhammad N, Sairi N H, 2015. The effect of different solvent extraction toward antilithiatic properties of Euphorbia hista and orthosiphon stamineus. IOP publishing 105-112.
9. De Ruiter J, hydrocarbon structure and chemistry. 2005. Alkanes, Principal of Drug action, Spring.
10. Yusoff D F, Raja Abd Rahman R N Z, Masomian M, et al, 2020. Newly isolated alkane hydroxylase and lipase producing Geobacillus and Anoxybacillus species involved in crude oil degradation. Catalysts, 10(8), 851.
11. Soundararajan, P, Mahesh R, Ramesh T, et al, 2006. Effect of Aerva lanata on calcium oxalate urolithiasis in rats. Indian journal of experimental Biology, 44 , 981-1008.

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