International Journal of Advance and Applied Research (IJAAR)
Peer Reviewed Bi-Monthly
ISSN – 2347-7075
Impact Factor –7.328
Vol.9 Issue. 3 Jan-Feb-2022

AN EXPLORARTION FOR PROTECTIVE EFFECT OF RHIZOME EXTRACT OF IRIS PSEUDACORUS L.AND SEED EXTRACT OF DOLICHOS BIFLORUS L. IN SODIUM OXALATEINDUCED UROLITHIASIS IN RAT MODEL

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Abstract

The aim of our presented study is to assess the effect of Rhizome extract of Iris pseudacorus L. and seed extract of Dolicos biflorus L. as preventive agent in experimentally induced urolithiasis model in rats. Rats were administered Sodium Oxalate (70 mg/kg, i. p.) in drinking water for 28 days *in drinking water*. In addition to this, Saponin extract of Iris pseudacorusand Dolicosbiflorusof low dose and high dose were administered along with Sodium Oxalate on 14-28th day. After the experimental period, blood samples were collected by cardiac puncture to analyse for Creatinine, Calcium, Blood Urea Nitrogen (BUN), Phosphorus, Uric acid, Alkaline Phosphate, Potassium, and Alanine Amino Transferasesfollowed by various antioxidants and kidney histopathology. The ethylene glycol feeding resulted in an increased level of all parameters evaluated compared to normal rats. All these conditions were reversed with plant extract treatment. Histopathological analysis also showed that rats treated with Sodium Oxalate had large deposits of calcium oxalate crystals, and that deposits were reduced in rats treated with plant extract. Results were also compared with the marketed product cystone as a standard. These data suggest that Iris pseudacorusand DolicosbiflorusSaponin extracts has a protective activity against urolithiasis.

Keywords: Calcium oxalate, Kidney stone, Antiurolithiatic, Iris pseudacorus, Dolicosbiflorus.

Rationale of the Study:

Urinary calculi are the third most common urinary system problem. Urinary tract stone disease affects almost 10% of the population of the industrialised world, according to estimates. In developed countries, kidney stones represent for 0.5 to 1.9 percent of clinical cases.[1] Urinary calculi can lead to urinary tract blockage, hydronephrosis,

infection, and bleeding.[2]To remove the calculi, surgical procedures, lithotripsy, and local calculus disruption with a high-power laser are commonly utilised. These operations, however, are costly, and recurrence is prevalent.[3]

Various therapies are being employed to try to prevent recurrence, including thiazide diuretics and alkali-citrate, but empirical evidence for their efficiency is lacking.[4]Traditional remedies, on the other hand, have supplied a substitute for many ailments as well as some additional information on disease pathogenesis.[5]

As a result, the hunt for new antilithiatic therapies derived from natural sources has become more important, as herbal medicines are less expensive and have less adverse effects.[6]

Iris pseudacorus(Iridaceae) and Dolicosbiflorus (Fabaceae) is reported to be used in Urinary complaints.[11]There is no work reported on the antiurolithiatic activity of Iris pseudacorusandDolicosbiflorus, hence the present investigation has been undertaken.

Objectioves of the Study:

1. Collection and Authentication of Iris pseudacorus L. and Dolichos biflorus Linn Plants
2. To Study the Taxonomical Characters of the Plants and Investigation of Morphological and Microscopical characters of the drug.
3. Extraction, and Preliminary Phytochemical(s) Study of Iris pseudacorus L. Rhizome and Dolichos biflorus Linn Seeds.
4. Isolation, Identification and Purification Phytochemical(s) of Iris pseudacorus L. Rhizome and Dolichos biflorus Linn Seeds.
5. Acute Toxicity Studies as per OCED guidelines 425.
6. To study Pharmacological Effects of Phytochemical(s) Extracted from Iris pseudacorus L and Dolichos biflorus Linn Plants for Anti-Urolithiatic activity.

Materials and Methods:

Collection of plant material

The Iris pseudacorus L. Rhizome was procured from Iran and Dolichosbiflorus Linn Seeds were procured from Bangalore, Karnataka. Dr.Geetanjali (HOD of Botany Department SreeSiddaganga College Tumkur University, India.) has identified and authenticated the sample (Reference No. 507/20-21).

Extraction of the plant material and sample preparation

The Rhizome of Iris pseudacorus L. is sliced into small parts and dried under shades for 7 days at room temperature. The dried rhizome of Iris pseudacorus and seed

of Dolichosbiflorus were powdered, then the sieved (10/40). The powder was used for preparation of methanol extraction. The 1000 ml methanol reflux condenser extracted every 100 g powder for 3 periods of 7 hours till it gets half. After completion of extraction, the extract was filtered by using Whattman No.1 paper and evaporated to get dryness at room temperature. Methanolic extract was subjected to preliminary phytochemical screening.[12-15]

Isolation and purification of Saponin from Methanolic extraction of Iris pseudacorus L. Rhizome and Dolichosbiflorus Linn Seeds

Extraction of Saponin was done by TLC fractionation method. 5gms of methanolic extract was subjected to saponification in 50ml of 20% ethanol. Followed by filtrations and residues was once again extracted with 20%/50ml ethanol and filtered. Both the filtrates combined together and heated to residue the volume to 40ml at 900C. Fractioned with 40ml of diethyl ether in separating funnel (Repeated twice) and ether layer was recovered. Aqueous layer was fractionated with 60ml of n-Butanol in separating funnel (Repeated twice) and aqueous layer recovered.

N-Butanol layer was washed with 5% NaCl solution, dried and weighed. Finally, 10 ml methanol was added and methanol layer and white powder separated. White powder assumed that highly purified. Solubility and chemical tests were conducted to confirm the presence of Saponin. And this sample (Saponin) is used for all further experimentations.

Experimental animals:

Wistar rats (Both sex, 5-6 weeks old) weighing 150-200 gm and Albino mice (Male, 5-6 weeks old) 20-25 gm have been used for the current study. All the work carried out on the animals was in accordance with the CPCSEA guidelines and the Research protocols have been approved by the IAEC, KCP, and the Sl. No. was KCP/IAEC/08/20-21/16/13-03-21.

Drugs and Chemicals:

All the chemicals used for the study was procured from Himedia, Mumbai and Merck, India. Equipment's were used was purchased from Analytical Technologies limited, India and Thermo Scientific, USA. Acute toxicity test on the pure active Saponin*Iris pseudacorus L. Rhizome* and *Dolichosbiflorus Linn* Seedsas per OECD guidelines No. 425; *Albino mice* (Female, 5-6 weeks old) with a weight of 20-25 gm were fasted overnight and limit and test is carried out with an initial dose of 175mg/kg/b.w. The following order is followed: 175, 550, 1750 and 5000 mg/kg /b.w. All the Animals

have been observed during the time being especially first 30 minutes to 24 hours. The special attention is required during the first 4 hours and then every day up to 14 days.

Model : Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rat Model[16]

Experimental Methods

42 Wistar rats age 5 to 6 weeks weighing (150-200g) have been divided in to following groups, with 6 animals in each group (n=6), in the following manner:

Group 1	Normal control	Vehicle for 28 days.
Group 2	Disease control	Sodium Oxalate (70 mg/kg, i. p.) in drinking water for 28 days.
Group 3	Standard group	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Cystone (750 mg/kg, p.o.) on 14th -28th day.
Group 4	Test group 1	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Saponin of Iris pseudacorus at low dose (X mg/kg, p.o.) on 14-28th day.
Group 5	Test group 2	Sodium Oxalate (70 mg/kg, i. p., 28 days) in drinking water for 28 days + Saponin of Iris pseudacorus at high dose (Y mg/kg, p.o.) on 14-28th day.
Group 6	Test group 3	Sodium Oxalate (70 mg/kg, i. p., 28 days) + Saponin of Dolichosbiflorus at low dose (X mg/kg, p.o.) on 14-28th day.
Group 7	Test group 4	Sodium Oxalate (70 mg/kg, i. p., 28 days) in drinking water for 28 days + Saponin of Dolichosbiflorus at high dose (Y mg/kg, p.o.) on 14-28th day.

Parameters to Be Evaluated:

Biochemical Parameters:

Collection of Blood Samples

After the experimental period, blood samples were collected by cardiac puncture under mild pentobarbital anesthesia. Collected blood samples were allowed to clot for 10 mins at room temperature and Serum was separated by centrifugation at 10000×g for 10 minutes and analysed for Creatinine, Calcium, Blood Urea Nitrogen (BUN), Phosphorus, Uric acid, Alkaline Phosphate, Potassium, and Alanine Amino Transferases.

Histopathology Studies & Kidney Homogenate Analysis

At the end of the experiment, on day 28th the rats were sacrificed by high dose of pentobarbital and kidneys excised, isolated kidneys have been cleaned off extraneous tissue and rinsed in ice cold physiological saline. After paraffin infiltration the Tissue pieces were sectioned at 5µm and stained with haematoxylin and eosin for Histopathological examination.[17-19]

Analysis of Tissue Antioxidant Enzyme

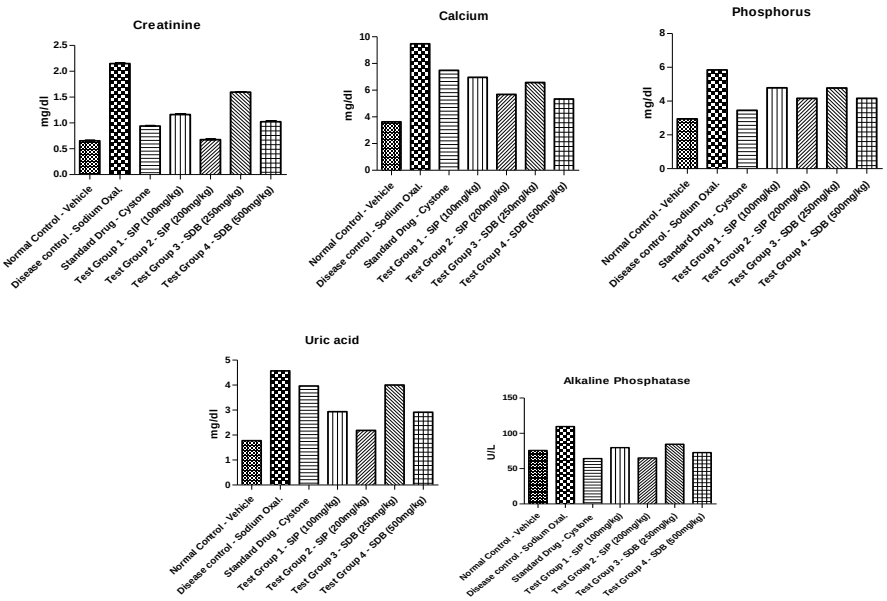
The remaining half portion of the right kidney was used for the estimation of various marker enzymes like MDA or LPO, GSH and LDH. 10% homogenate of the tissues were prepared in 0.1M Tris HCL buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 12000 × g for 30 minutes. The supernatant obtained after centrifugation were used for the estimation of various marker enzymes.[20, 21]

Statistical Analysis:

The data were presented as Mean ± S.E.M. from N = 6 rats in each group and analyzed using one way of Variance ANOVA followed by Tukey multiple comparison tests. P value <0.05 was considered statistically significant. Graph pad Prism 5.0 and Excel software were used for statistical analysis.

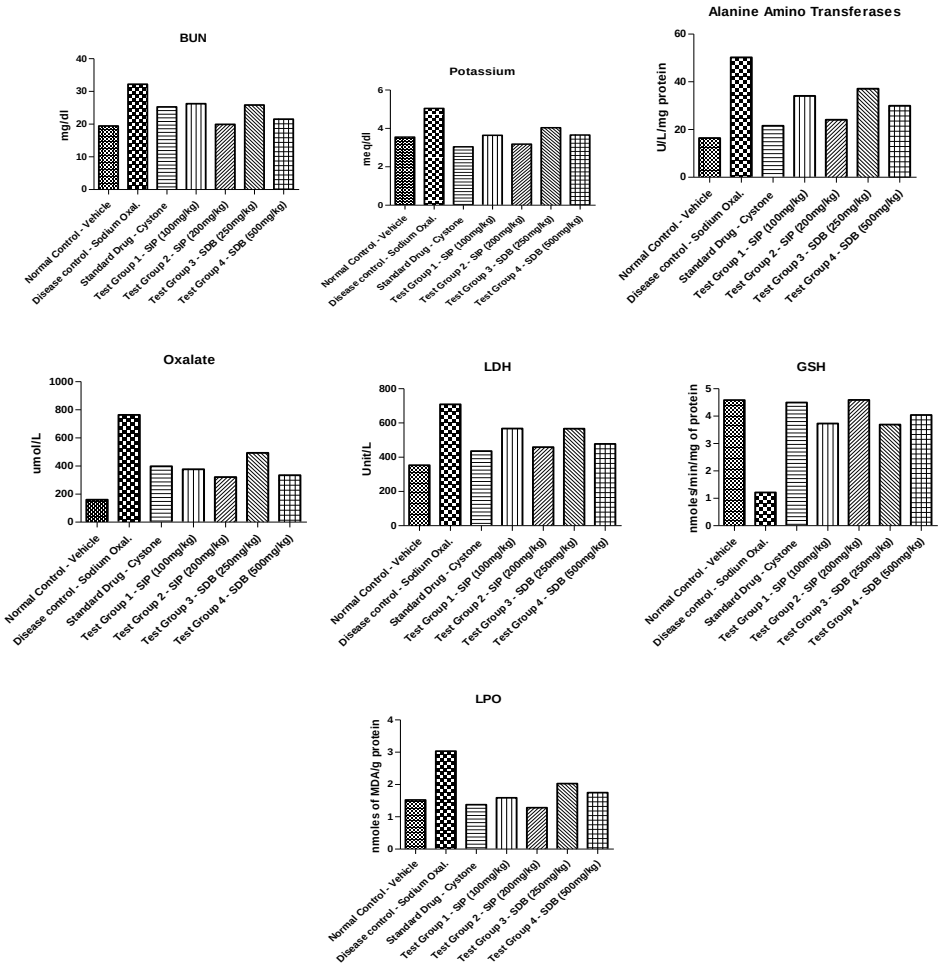
Results:

Figure: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on Creatinine,Ca, Phosphorus, Uric acid and ALP Analysis after 28 days of treatment



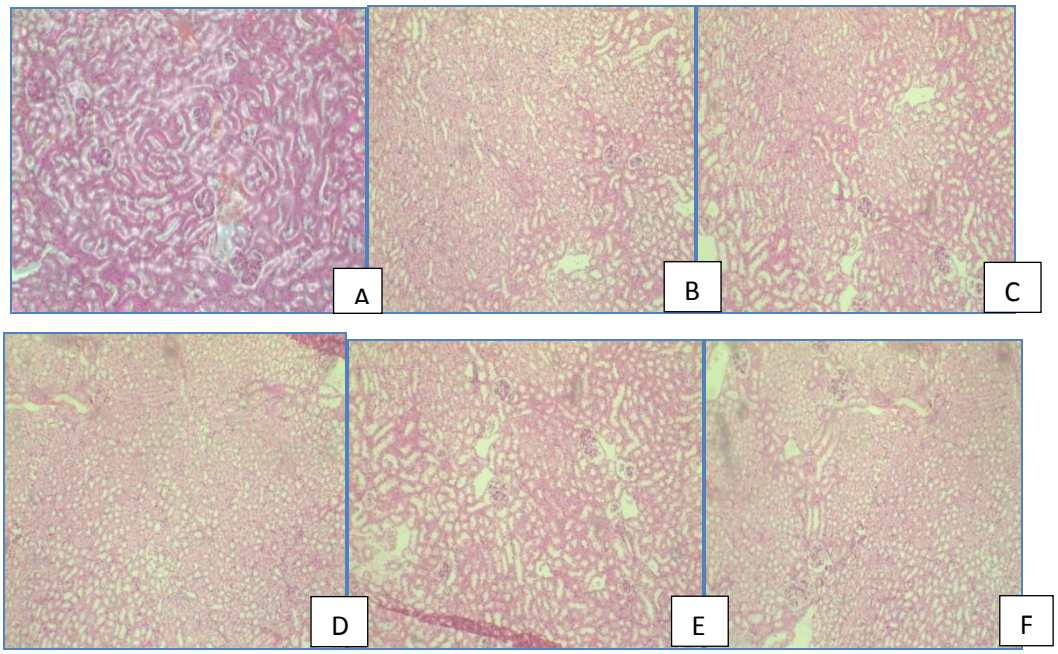
Values are expressed as Mean ± SEM, n = 6 in each group

Figure: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on BUN, Potasium, AAT, and Oxalate Analysis after 28 days of treatment**.**



Values are expressed as Mean ± SEM, n = 6 in each group

Figure: Effect of oral administration of SIP and SDB on Sodium Oxalate (70 mg/kg, i. p.) Induced Urolithiasis Rats on tissue histology (Kidney) after 28 days of treatment



1. Disease control -
2. Standard Drug – Cystone:
3. Test Group 1 - SIP (100mg/kg):
4. Test Group 2 - SIP (200mg/kg):
5. Test Group 3 - SDB (250mg/kg):
6. Test Group 4 - SDB (500mg/**kg):**

Conclusion:

Elevated creatinine, Serum ALP levels and presence of Uric acid crystals signifies impaired kidney function. As the kidneys become impaired for any reason, all these papameters in the blood will rise due to poor clearance of creatinine by the kidneys.

Calcium and phosphorous usually keep each other in check. With the progression of kidney disease, high phosphorus levels may lead to low serum calcium by depositing it onto the bones and other tissues. An excess BUN, potassium and serum enzyme (Alanine amino Transferases) indicates the decline in kidney function due to a disease or kidney damage which can be advanced stages of chronic kidney disease.Elevated lipid peroxides, LDH and decreased glutathione (GSH) indicates some form of tissue damage. An excess amount of oxalate can combine with calcium in the urine and cause kidney stones and crystals to form. Recurrent kidney stones and crystals can damage the kidney and lead to kidney failure.

In conclusion, the presented data indicate that administration ofSaponin Iris pseudacorus L. Rhizome and Dolichosbiflorus Linn Seedsto rats with Sodium Oxalate induced lithiasis reduced the growth of urinary stones by reversing all the abnormal parameters, thus supporting folk information regarding the antiurolithogenic activity of theplant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone constituents as detergent nature of saponines.

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