Research Article

Evaluation of Antiurolithiatic Activity of *Solidago virgaurea* Against Ethylene Glycol Induced Renal Calculi in Rats

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**ABSTRACT**

Research statistics says that 12% of world's population are being suffered with Urolithiasis, where males are more prone than women. Various treatment modalities are being implemented till this decade with their respective adverse effects and recurrence rates. Current research is investigating on phytotherapy which is being considered as the safer, cost-effective therapy in treating kidney stones with low recurrence rates. Hence, We have evaluated antiurolithiatic activity of methanolic extract of whole plant of *Solidago virgaurea* on male albino wistar rats. MESV (250, 500 and 750 mg/kg) treatment doses were initiated from 15th day. The present study, results and the significant values reveals that the MESV has got a potential antiurolithiatic activity against experimentally induced renal calculi in rats. It can be concluded that the results indicate that the administration of MESV (750 mg/kg) reduced the biochemical parameters than the 250 and 500 mg/kg MESV and can be stated that the 750 mg/kg is an effective dose for the treatment of urolithiasis. This can be attributed by the chemical constituents like saponins, flavonoids, glycosides, polysaccharides and diuretic action.

1. Introduction

One of the most common form of renal disorders prevalent from ancient times is Urolithiasis. Urolithiasis/Renal calculi/Kidney stones is defined as the formation of mineral crystal aggregates in the urinary system mainly, kidneys, (nephrolithiasis) ureters, urinary bladder. Hence the name Urolithiasis. Urolithiasis is itself a problem whereas its recurrence is serious challenge for its therapy.

*a) Types:* The type of kidney stone depends on its chemical composition and pathogenesis, the chemical composition in turn depends on the abnormalities of various chemicals in urine. According to this, there forms five types of kidney stones namely calcium stones (calcium oxalate and calcium phosphate), uric acid (a breakdown product of purines) stones(urate), Struvite stones (magnesium phosphate), cystine stones and drug induced stones (guaiphenesin, triamterene etc.). The percentage prevalence of calcium stone is 80%, struvite stones is 10-15%, uric acid stone is 3-10%, cystine stone 2% and drug induced stone is 1%.

*b) Epidemiology:* Urolithiasis occupies third position in common urological diseases. According to geographical area and socio-economic conditions, age and sex distribution, type of lithiasis and stone location its epidemiology varies. Review of previous epidemiological surveys is showing that the prevalence rate ranges between 4% and 20% in economically developed countries. Both males and females are affected but predominantly observed in males than females in 2:1 ratio approximately. Males are more prone to stone formation because high levels of testosterone which functions as a promoter for stone formation in addition to their high dietary intakes. where estrogen acts as an inhibitor. Increasing incidence of nephrolithiasis in women might be due to life style changes and obesity. Tropical and subtropical countries show high prevalence rate than frigid countries.

*c)Etiology:* Calculogenesis (formation of stones) is a multifactorial complex process that includes intrinsic (age, sex and heredity) and extrinsic(geography,climate,dietary mineral composition and water intake) factors which deviate the balance between promoters and inhibitors of calculogenesis. Calcium stones are formed when calcium levels in the urine
are high with respect to hypercalcemia owing to parathyroid tumors and metabolic disorders. Calcium stones also readily form when urine is highly alkaline. Calcium oxalate stones are mostly observed in people who are on high oxalate diet (vegetarians).

Struvite stones are formed when urease levels in urine are raised due to chronic urinary tract infections due to proteus, klebsiella, Enterobacter and pseudomonas species which make urine alkaline favoring the phosphate aggregation. Diets high in purines like animal meat and fish results in hyperuricosuria, low urine volume and pH leading to the formation of uric acid stones. Defect in rBAT gene on chromosome 2 and mutations in SLC3A1 and SLC7A9 results in impaired cystine absorption by renal tubules and hence hyper cystinuria favoring cystine stone formation. Patients on antiretroviral therapy prone to stone formation due to protease inhibitor such as indinavir sulphate. Other drug inducers of stones are triamterene, guaiphenesin, atazanavir etc... Deposition of calcium oxalate among all the minerals is the major causative factor of kidney stones.

d) Pathophysiology: Calculi tend to form when there are excessive amounts of relatively insoluble salts in the filtrate or when urine becomes highly concentrated due to insufficient fluid intake. Pathogenesis of stone formation is regarded as the complex process. Various theories have been reviewed stating the formation of crystal stones such as free particle theory, fixed particle theory, Randall’s plaque hypothesis. However stone formation depends on the imbalance in the level of urinary inhibitors and promoters of crystallization. All types of stones show similar mineral phase but differs in the sequence of stone formation which is dependent on type of stone, urine chemistry and level of respective promoter or inhibitor. In short, the sequence of events that promotes stone formation in common are as follows. Increased level of promoters-building up of oxidative stress-stress injury to the cells-crystal nucleation-interaction of crystals and cells-crystal growth-retention/adhesion of crystals-stone formation. Symptoms are manifested when the stones try to move downwards among the urinary tract and create friction.

e) Treatment: This disease is treated by medical and surgical methods. Medical therapy includes, NSAIDS, Thiazide diuretics, allopurinol, medical expulsive therapy, potassium citrate, sodium cellulose phosphate, D-penicillamine, acetohydroxyamide whereas surgical methods include lithotripsy and percutaneous nephrolithotomy. These methods have reported several side effects such as tubular necrosis and fibrosis. To overcome these effects, medicinal plants could be beneficial and WHO also gave importance to the use of herbal remedies for the treatment urolithiasis due its low-cost high safety.

Solidago is the genus of around 100 to 120 species of flowering plants belonging to the family Asteraceae. Commonly solidago is known as golden rods. Species from this genus contains saponins, terpenoids, phenolic glycosides, phenolic acids and high amounts of flavonoids. These chemical constituents have been reported for their antimicrobial, analgesic, astringent and some species like S. Virgaurea for antiseptic and in the treatment of urolithiasis, chronic nephritis and rheumatism. Their medicinal activities could be due to their characteristic chemical constituents. Hence, we have selected the species S. Virgaurea for its antiurolithiatic activity.

2. Materials and Methods

Collection of plant material:
The whole plant of solidago virga-aurea is collected from the forest area of Tirupati and the botanical identity of the plant was confirmed and authenticated by Dr.K.Madhavashetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupathi-517502, A.P.India.

Preparation of extract:
The whole plants were cleaned, shade dried and the dried plant material was powdered and sieved through a sieve no#60. This powered was accurately weighed about 500 gm and was loaded in a Soxhlet’s apparatus which was extracted with methanol at about 40-50oC until the appearance of colourless solvent in the siphon tube of extractor. The extracted solvent was concentrated in a heating mantle and dried in desicators. The yield was 8.9 gm of methanolic extract. The dried extract was stored in an air tight container.

Phytochemical screening:
Test for flavonoids
Alkaline reagent test: Test solution when treated with sodium hydroxyl solution shows increase in the intensity of yellow colour which becomes colorless on addition of few drops of dilute acid.

Shinoda test: To the test solution, few magnesium turnings and concentrated hydrochloric acid added dropwise pink scarlet, crimson red or occasionally green to blue colour appears after few minutes indicates presence of flavonoids.

Zinc hydrochloride test: Test solution treated with a mixture of zinc dust and concentrated hydrochloric acid gives red colour after few minutes if flavonoids are present

Test for saponins:
Ferric chloride test: Test solution is treated with ferric chloride solution blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Phenzone test: To the extract solution about 0.5 gm sodium acid phosphate is added. Warmed and filtered. To the filtrate 2% phenzone was added which gives coloured bulky precipitate.

Test for chlorogenic acid: Treat the test solution with aqueous ammonia and expose it to the air gradually green colour is developed Tannins precipitate on treatment with potassium dichromate or chromic acid.

Test for poly saccharides:
Molisch’s test: To the test solution add few drops of alcoholic a-naphthol
then add few drops of concentrated sulphuric acid through sides of the test tube, purple violet ring appears at the junction.

**Test for volatile oils:**
To the test drug add Sudan III solution, red color obtained by the globules indicate presence of volatile oil or essential oils. To the test drug add few drops of Tincture alkane, red color indicates presence of essential oils.

**Experimental animals:**
Male albino wistar rats weighing in between 150-160 gm were procured in the Albino labs. Animals are maintained under 12 light and 12 hrs. dark and they were well-ventilated. Male rats were selected because lithiasis is a male predominant disorder as males contain testosterone which is a promoter of urolithiasis, urinary system of humans resembles that of male rats and the previous literature show that the males are more prone to lithiasis than that of females.

**Acute toxicity study:**
Acute toxicity study was performed as per the OECD-423 guideline using albino rats prior to the evaluation of antiurolithiatic activity. Male albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various strength of extracts was administered orally at the dose of 250mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 400,500 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h and LD<sub>50</sub> of the methanolic extract of Solidago virgaurea was noted.

**Estimation of parameters:**

**Urine analysis:** On 29th day all the animals were kept in individual metabolic cages in order to collect urine. Once the urine is collected it is measured for its volume and its pH is measured using pH meter. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40C. Urine was analyzed for oxalate, calcium, inorganic phosphate, uric acid, magnesium and citrate levels using diagnostic kits obtained from Span Cogent diagnostics (Surat, India), S.D.Fine chemicals (Mumbai, India), Cystone (Surat, India) and MESV (Mumbai, India).

**Kidney analysis:** All the animals from each group were taken and sacrificed by cervical dislocation; abdomen was cut and kidneys were isolated. Isolated kidneys were cleaned of extraneous matter and one of them was preserved in 10% neutral formalin. Urine was centrifuged at 2000 rpm for 10 min and the supernatant was separated. The supernatant was analyzed for calcium, inorganic phosphate, oxalate levels [6]

**Enzyme Assay:** A portion of kidney was taken from all the groups and 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) [23]

**Acute toxicity study:**
Acute toxicity study was performed as per the OECD guideline using albino rats prior to the evaluation of antiurolithiatic activity. Male albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various strength of extracts was administered orally at the dose of 250mg/kg and observed for 14 days.

**Results:**

**Table 1:** Treatment schedule for the induction of disease and curative regimen

<table>
<thead>
<tr>
<th>Group</th>
<th>Induction treatment</th>
<th>Dose</th>
<th>Induction period</th>
<th>Curative regimen</th>
<th>Duration of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Control</td>
<td>water and food</td>
<td>-</td>
<td>28 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II Disease</td>
<td>Ethylene glycol</td>
<td>0.75%v/v, p.o</td>
<td>28 days</td>
<td>Cystone 750mg/kg</td>
<td>From 15&lt;sup&gt;th&lt;/sup&gt; to 28&lt;sup&gt;th&lt;/sup&gt;day</td>
</tr>
<tr>
<td>III Standard</td>
<td>Ethylene glycol</td>
<td>0.75%v/v, p.o</td>
<td>28 days</td>
<td>MESV 250mg/kg</td>
<td>From 15&lt;sup&gt;th&lt;/sup&gt; to 28&lt;sup&gt;th&lt;/sup&gt;day</td>
</tr>
<tr>
<td>IV Test</td>
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<td>0.75%v/v, p.o</td>
<td>28 days</td>
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</tr>
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<td>Ethylene glycol</td>
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<td>28 days</td>
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</tr>
</tbody>
</table>

**Statistical analysis**
Experimental data was expressed as mean ± Standard error of mean (SEM). Statistical analysis was performed by one-way ANOVA (Analysis of variance) followed by using Dunnet’s method for comparison of different dosage groups. All the raw data were recorded in appropriate formats and summarized in tabular form, wherever necessary. Statistical Analysis was made using the Graph Pad Prism Instant Inc., 5755 Software.

**3. Results and Discussion**
Urolithiasis is quite common disease and now-a-days it has become growing global problem with an increasing incidence and prevalence which appears more pronounced in industrialized countries [17]. Urinary stone is a solid, hard non-metallic crystalline mineral material formed and found in the kidney and urinary tract [5]. Stone formation occurs as a result of the successive physicochemical events such nucleation, crystallization, growth, aggregation, precipitation, retention and crystal attachment [43].

**Experimental design:** Chronic administration of ethylene glycol (0.75%v/v) for 28 days. Standard drug cystone and the plant drug (Methanolic extract of Solidago virgaurea) is started from 15<sup>th</sup> day to till 28<sup>th</sup> day which serves as curative regimen.

**Enzyme Assay:** A portion of kidney was taken from all the groups and 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) [23]

**Serum analysis:** On the 30<sup>th</sup> blood was withdrawn from each rat by retro-orbital puncture and centrifuged at 8000 rpm for 20 min. The supernatant serum was separated and analyzed for its creatinine, Blood urea nitrogen (BUN), and uric acid.

**Kidney analysis:** All the animals from each group were taken and sacrificed by cervical dislocation; abdomen was cut and kidneys were isolated. Isolated kidneys were cleaned of extraneous matter and one of them was preserved in 10% neutral formalin. Wet weight and dry weight of each kidney were recorded after its removal. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. Using REMI C-24BL Homogenizer. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was separated. The supernatant was analyzed for calcium, inorganic phosphate, oxalate levels [6].

**Evaluation of antiurolithiatic activity:**

**Calcium Oxalate Nephrolithiasis:** CaOx kidney stones are produced in rats by the induction of chronic hyperoxaluria by using ethylene glycol (0.75% v/v for 28 days) respectively. Healthy male albino wistar rats (36) weighing about 150-160 gm were taken and were divided into six groups where each group contains six animals. All groups received regular rat food and drinking water. All animals are weighed before and after the study period.

**Acute toxicity study:** Acute toxicity study was performed as per the OECD-423 guideline using albino rats prior to the evaluation of antiurolithiatic activity. Male albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the various strength of extracts was administered orally at the dose of 250mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 400,500 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h and LD<sub>50</sub> of the methanolic extract of Solidago virgaurea was noted.

**Estimated of parameters:**

**Urination analysis:** On 29th day all the animals were kept in individual metabolic cages in order to collect urine. Once the urine is collected it is measured for its volume and its pH is measured using pH meter. A drop of concentrated hydrochloric acid was added to the urine before being stored at 40C. Urine was analyzed for oxalate, calcium, inorganic phosphate, uric acid, magnesium and citrate levels using diagnostic kits obtained from Span Cogent diagnostics (Surat, India), S.D.Fine chemicals (Mumbai, India), Cystone (Surat, India) and MESV (Mumbai, India).
Glycolic acid is further oxidized to glyoxalic acid which is further oxidized to oxalic acid by glycolate oxidase or lactate dehydrogenase [6]. In the current study, feeding with 0.7% ethylene glycol through drinking water alone to the disease control group resulted in the significantly elevated levels (p<0.01) of oxalate, calcium and phosphate and the values are found to be (3.24±0.04, 3.95±0.04, 7.31±0.38) when compared to normal control (0.46±0.06, 1.17±0.01, 3.80±0.03) respectively. When standard drug cystone 750mg administered as a curative regimen to the calculi induced rats' oxalate, calcium and phosphate levels are significantly reduced (p<0.01) and the values are (1.22±0.08, 1.53±0.01, 4.23±0.12) respectively.

On treatment with 250 mg/kg of MESV it has shown that significant reduction (p<0.01) of oxalate and calcium levels but it was no significant excretion of phosphate as compared to diseased control group.

Treatment with 500 and 750 mg/kg of MESV it has shown significant decreased (p<0.01) levels of oxalate, calcium and phosphate. Decreased excretion of oxalate may be due to regulation of oxalate metabolism and oxalate anion transporter SLC26A6 in renal tubules [6]. Increased phosphate excretion along with oxalate stress provides an environment suitable for stone formation by forming calcium phosphate crystal deposition (Gillhotraand Christina, 2011). Treatment with MESV significantly restored phosphate level, thus reducing the risk of stone formation on other hand, Magnesium and citrate excretion levels were significantly decreased (p<0.01) in lithiatic control group and the results were (2.92 ± 0.01, 2.44 ± 0.01) as compared to normal control (4.88±0.02, 3.65±0.01). Magnesium deficiency increases citrate excretion in urine. Citrate in low concentration has the capability to form both calcium oxalate and calcium phosphate crystallization [6].

On treatment with cystone and MESV (250mg, 500mg, 750mg) significantly (p<0.01) raised the levels of Magnesium and citrate and the levels are (3.94±0.01, 3.34±0.01); (3.94±0.01, 2.64±0.01); (3.37±0.01, 2.94±0.006); (3.78±0.023, 2.3±0.02) respectively.

From this study it was noted that magnesium and citrate considered as inhibitors of stone formation. Magnesium forms complexes with oxalate and decrease supersaturation and consequently reduces oxalate concentration available for its precipitation [6]. Citrate forms soluble complex with calcium and inhibits precipitation and aggregation of phosphate and calcium oxalate [40]. This reduces the urinary macromolecules like osteopontin, and increases Tamm-Horsfall protein (THP). Urinary citrate excretion may increase urine pH which is a factor in the calcium-citrate-phosphate complex (solubility product) [6]. By treatment with MESV to ethylene glycol treated rats showed enhanced levels of citrate and magnesium when compared to disease control group. Induction of the disease was confirmed by identifying physical parameters such as less of body this may be due to the decreased intake of food due to the pain which occur due to lipid peroxidation, low urine volume, decreased urine pH and also by observing imbalance of urinary constituents like oxalate, calcium, citrate, magnesium and elevated levels of nitrogenous waste such as creatinine, uric acid etc., in case of disease control group (calculi-induced group-treatment alone with 0.7%v/v ethylene glycol).

In case of urolithiasis GFR decreases due to the obstruction of flow of urine. As the GFR greatly decreases, the nitrogenous waste products like creatinine, BUN, and uric acid get accumulated in blood (Vijaya et al., 2013). Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and modulates its crystallization also suggests its primary role in stone formation (Gillhotra and Christina, 2011). In urolithic group creatinine, BUN, uric acid significantly elevated (p<0.01) as compared to normal control. On treatment with 250 mg/kg MESV creatinine and BUN were significantly decreased (p<0.01) but the levels of uric acid were significant at (p<0.05). The levels of creatinine, BUN, uric acid was significantly reduced (p<0.01) on treatment with cystone and MESV (500 and 750 mg). Increased creatinine in blood due to decreased GFR results in the damaged of nephron structural integrity (Sarmistha and Verma, 2012).

Treatment with MESV decreased creatinine levels in serum. Increased uric acid excretion increases in hyperoxaluric rats. Kidney homogenate analysis revealed that the oxalate and calcium were significantly increased (p<0.01) with the case of calculi induced group as compared to normal but the kidney phosphate was non-significant (p<0.05) as compared to normal control. On treatment with cystone and the three doses of MESV kidney phosphate concentration was also non-significant (p<0.05) as compared to diseased control group.

Treating with 250 mg/kg MESV kidney oxalate and calcium were insignificant (p>0.05). But treatment with cystone, Oxalate and calcium were significantly decreased (p<0.01) compared to lithiatic induced rats. On treatment with MESV (500 mg/kg) they were significant at (p<0.05) the calcium levels of kidney were significantly decreased (p<0.01) with MESV (750mg/kg) as compared to lithiatic group.

On supplementation with ethylene glycol increased lipid peroxidation occurs and decreased antioxidant levels such as SOD, CAT. In the present investigation, it was found that oxalate might be the factor responsible for the cause of lipid peroxidation there by damaging of the tissue by reacting with PUFA (polyunsaturated fatty acids) in cell membranes [32]. From the study SOD levels were significantly decreased (p<0.01) with the diseased grouped rats as compared to normal group rats. On treatment with cystone and MESV of three different doses the SOD levels were significantly increased (p<0.01) when compared to calculi induced rats. CAT levels were found to be decreased significantly (p<0.05) in calculi induced rats as compared to normal. CAT levels are observed to be significant at p<0.05 compared with only cystone treated animals as compared with diseased control group while the three doses of MESV were insignificant when compared to lithiatic group.

Elevated MDA levels indicate lipid peroxidation in case of calculi induced rats and increased significantly at (p<0.01) as compared to normal control rats. On treatment with cystone and MESV (250 mg/kg, 500 mg/kg and 750 mg/kg) the MDA levels were significantly reduced (p<0.01) as compared to diseased control group. The exact mechanism involved in the effect of Methanolic extract of Solidago virga-aurea on lithiasis was unclear. However, the possible mechanism of action may be due to increased urine output, reduction of oxalate levels (i.e., fall in hyperoxaluria), increased stone inhibitors (Citrate and magnesium) and might be through antioxidant potential and decreased lipid peroxidation. The first and foremost action of the test drug (MESV) in the treatment of urolithiasis is that it shows its action through diuresis, the increased urine volume dilutes the concentration of stone forming constituents. From the previous literature it was shown that the plants rich in saponin fraction are effective in the treatment of urolithiasis through there diuretic action and anticrystallization property by disintegrating suspension of mucoproteins, promoters of crystallization, so the test drug also contain saponin which shows their potential activity against urolithiasis through reducing the levels of stone forming constituents. Saponins show their action through the elimination of nitrogenous waste materials from the body. The test drug may be effective due to the antioxidant activity i.e., due to the decreased levels of superoxide dismutase and increased levels of the catalase which ultimately reduces malonaldehyde (involved in lipid peroxidation), the reduced lipid
peroxidation reduces the attachment of the oxalates to the renal tissue due to which anti-inflammatory activity may be achieved. The flavonoids which are present in MESV may have the potent antioxidant and anti-inflammatory action through which oxidation and lipid peroxidation of the renal tubules may be monitored thus the renal tissue may be recovered from damage. Generally, Glycosides have analgesic activity, diuretic and astringent properties, so the test drug also contain glycosides as one of chemical constituent. Hence, the chemical constituents such as saponins, flavonoids, glycosides play an important role in the treatment of stone formation against ethylene glycol induced calculi in rats.

Stone formation occurs as result of successive physicochemical events such as nucleation, growth, aggregation, retention. It is a consequence of imbalance of stone promoters and inhibitors of crystallization. Lithiasis is a male predominant disorder, with a recurrence rate of 70-80% in males and 47-60% in females.

The criteria behind the selection of the plant *Solidago virgaurea* may be due to the chemical constituents present in it. They include flavonoids, diterpenes, triterpene saponins, sesquiterpenes, carotenoids, polyphenolic acids, essential oils, polysaccharides, and polyphenolic glycosides [14]. Previous literature revealed that the plants containing saponins, flavonoids, glycosides play an important role in the prevention and treatment of urolithiasis.

The plant product is extracted with the methanol as a solvent using Soxhlet extraction p

Table 2: Effect of Ethylene glycol, cystone, MESV on urinary oxalate, Calcium and inorganic phosphate urinary magnesium and urinary citrate in male wistar rats against ethylene glycol induced urolithiasis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Urinary oxalate (mg/dl)</th>
<th>Urinary calcium (mg/dl)</th>
<th>Urinary inorganic phosphate (mg/dl)</th>
<th>Urinary magnesium (mg/dl)</th>
<th>Urinary citrate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>0.46±0.06</td>
<td>1.17 ± 0.01</td>
<td>3.80 ± 0.03</td>
<td>4.88 ± 0.02</td>
<td>3.65 ± 0.01</td>
</tr>
<tr>
<td>II</td>
<td>Disease control</td>
<td>3.24±0.04**</td>
<td>3.95 ± 0.44**</td>
<td>7.31 ± 0.38**</td>
<td>2.92 ± 0.01**</td>
<td>2.44 ± 0.01**</td>
</tr>
<tr>
<td>III</td>
<td>Cystone – 750 mg/kg</td>
<td>1.22±0.08**</td>
<td>1.53 ± 0.01**</td>
<td>4.23 ± 0.12**</td>
<td>3.94 ± 0.01**</td>
<td>3.34 ± 0.01**</td>
</tr>
<tr>
<td>IV</td>
<td>MESV-250 mg/kg</td>
<td>2.22±0.12**</td>
<td>3.27 ± 0.01***</td>
<td>6.67 ± 0.44**</td>
<td>3.14 ± 0.02**</td>
<td>2.64 ± 0.01**</td>
</tr>
<tr>
<td>V</td>
<td>MESV-500 mg/kg</td>
<td>1.92±0.21**</td>
<td>3.16 ± 0.25**</td>
<td>5.01 ± 0.12**</td>
<td>3.37 ± 0.01**</td>
<td>2.94 ± 0.006**</td>
</tr>
<tr>
<td>VI</td>
<td>MESV-750 mg/kg</td>
<td>1.76±0.03**</td>
<td>3.01 ± 0.10**</td>
<td>4.93 ± 0.04**</td>
<td>3.78 ± 0.02**</td>
<td>3.23 ± 0.02**</td>
</tr>
</tbody>
</table>

All of the data obtained from the experimental groups have been compared with disease control and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group I and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group

Table 3: Effect of ethylene glycol, cystone, MESV on serum creatinine, Blood urea nitrogen (BUN), serum uric acid, Oxalate, Calcium and Inorganic phosphate of kidney homogenate in male wistar rats against ethylene glycol induced urolithiasis

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum creatinine (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Kidney oxalate (mg/dl)</th>
<th>Kidney calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>0.52 ± 0.02</td>
<td>23.32 ± 0.22</td>
<td>1.41 ± 0.29</td>
<td>3.26 ± 0.53</td>
</tr>
<tr>
<td>II</td>
<td>Disease control</td>
<td>0.97 ± 0.03**</td>
<td>43.87 ± 0.41**</td>
<td>5.70 ± 0.63**</td>
<td>5.08 ± 0.20**</td>
</tr>
<tr>
<td>III</td>
<td>Cystone – 750 mg/kg</td>
<td>0.72 ± 0.02**</td>
<td>30.74 ± 0.20**</td>
<td>2.20 ± 0.01**</td>
<td>3.54 ± 0.02**</td>
</tr>
<tr>
<td>IV</td>
<td>MESV-250 mg/kg</td>
<td>0.79 ± 0.02**</td>
<td>42.28 ± 0.16**</td>
<td>3.44 ± 0.04</td>
<td>4.68 ± 0.02**</td>
</tr>
<tr>
<td>V</td>
<td>MESV-500 mg/kg</td>
<td>0.81 ± 0.02**</td>
<td>37.36 ± 0.02**</td>
<td>3.27 ± 0.53</td>
<td>4.54 ± 0.23**</td>
</tr>
<tr>
<td>VI</td>
<td>MESV-750 mg/kg</td>
<td>0.82 ± 0.02**</td>
<td>30.13 ± 0.07**</td>
<td>3.06 ± 1.11**</td>
<td>4.35 ± 0.01**</td>
</tr>
</tbody>
</table>

All of the data obtained from the experimental groups have been compared with disease control and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group I and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group

Table 6: Effect of ethylene glycol, cystone, *Solidago virgaurea* on SOD (Superoxide dismutase) Catalase (CAT), Malonaldehyde (MDA) in male wistar rats against ethylene glycol induced urolithiasis

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SOD (mg/protein)</th>
<th>CAT (µ mol/mg protein)</th>
<th>MDA (moles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>8.48 ± 0.05</td>
<td>3.125± 0.09</td>
<td>2.13 ± 0.06</td>
</tr>
<tr>
<td>II</td>
<td>Disease control</td>
<td>3.345 ± 0.04**</td>
<td>1±0.03**</td>
<td>6.74 ± 0.01**</td>
</tr>
<tr>
<td>III</td>
<td>Cystone – 750 mg/kg</td>
<td>6.84 ± 0.01**</td>
<td>2.95 ± 0.01*</td>
<td>4.23 ± 0.08**</td>
</tr>
<tr>
<td>IV</td>
<td>MESV-250 mg/kg</td>
<td>4.25 ± 0.01**</td>
<td>1.63 ± 0.08</td>
<td>6.03 ± 0.14**</td>
</tr>
<tr>
<td>V</td>
<td>MESV-500 mg/kg</td>
<td>5.92 ± 0.01**</td>
<td>1.93 ± 1.07**</td>
<td>4.43 ± 0.07**</td>
</tr>
<tr>
<td>VI</td>
<td>MESV-750 mg/kg</td>
<td>7.05 ± 0.06**</td>
<td>2.37 ± 0.2</td>
<td>3.95 ± 0.10**</td>
</tr>
</tbody>
</table>

All of the data obtained from the experimental groups have been compared with disease control and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group I and normal control groups. The data was analyzed statistically by one-way ANOVA followed by Dunnett test using Graph pad prism version 5.0 software. Values are significant at *#P<0.05, **##P<0.01 and ***###P<0.001. * Comparison of Diseased control, Standard and all test groups with Normal control. # Comparison of Standard and Test groups with disease control group

4. Conclusion

The main objective of the present investigation is to evaluate the anti-urolithiatic activity of *Solidago virgaurea* against experimentally induced rats.
The extracted product was under gone for its preliminary phytochemical screening and it was shown to contain flavonoids, saponins, polysaccharides and glycosides.

From acute oral toxicity studies the dose of Solidago virgaurea was fixed as 250 mg/kg as low dose, 500mg/kg as medium dose and finally 750 mg/kg as high dose.

Ethylene glycol model was selected for the screening of the anti-urolithiatic activity of methanolic extract of Solidago virgaurea. Inducing and treatment by curative regimen was conducted for 28 days with 0.75% ethylene glycol. After the completion of dosing schedule all the parameters were estimated and compared.

All the experimental raw data obtained was calculated using statistical analysis using ANOVA which was followed by Dunnet’s method and the results were tabulated.

The present study, results and the significant values reveals that the methanolic extract of Solidago virgaurea has got a potential antiurolithiatic activity against experimentally induced renal calculi in rats. It can be concluded that the results indicate that the administration of plant extract of Solidago virgaurea 750 mg/kg reduced the biochemical parameters than the 250 and 500 mg/kg Solidago virgaurea and can be stated that the 750 mg/kg is an effective dose for the treatment of urolithiasis. This can be attributed by the chemical constituents like saponins, flavonoids, glycosides, polysaccharides and diuretic action. This is because earlier studies reported that plants rich in saponins, flavonoids, glycosides play an important role in treating ethylene glycol induced urolithiasis in rats. So, it helps in the management of the nephrotoxicity. Hence here we are concluding that MESV at the dose of 750mg/kg p.o is an effective dose against ethylene glycol induced urolithiasis in male wistar rats because of chemical constituents such as saponins, flavonoids, glycosides and polysaccharides present in the plant extract.

Sound knowledge about primary immune-deficiencies should be present among health care practitioners for differential diagnosis of chronic granulomatous disease and application of test at proper time would help in timely diagnosis of disease.

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Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

References