Antiurolithiasis, Anti-Inflammatory And *In Silico Docking Studies Of Karpura Shilajit*

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ABSTRACT

Urolithiasis is the condition where urinary calculi are formed anywhere within the excretory organ, bladder, and/or ureters. Asphaltum commonly called *Karpura shilajit* is known for different activities like antimicrobial, antidiabetic, antiulcer, fungitoxocity, estrogenic, anti-inflammatory, antihyperglycemic, antilithiatic activity and antioxidant activity. The aim of study is to evaluate anti-lithiatic activity by ethylene glycol ammonium chloride (0.75%) model for 28 days and *in vitro* anti-inflammatory activity by protein denaturation method using the mineral extract where cystone (500 mg/kg, *p.o.*) used as a standard drug in the present study. Preliminary Phytochemical screening resulted in the presence of flavonoids, saponins, carbohydrates, terpenoids and steroids. The Shilajit extract significantly restored creatinine, urea, uric acid, calcium, phosphate, oxalate, sodium and potassium levels in Ethylene glycol ammonium chloride induced urolithiasis model. Histopathological examination further confirmed the induction of lithiasis with crystal deposition in the sections of kidney treated with ethylene glycol-ammonium chloride. The mechanism by which the extract exhibited this effect is possibly through an anti-inflammatory, nephroprotection. *Karpura shilajit* had shown significant effect on urine volume, urine pH, urine excretion, sodium and potassium levels. The steroids, terpenoids and flavonoids isolated from Gas Chromatography-Mass Spectroscopy are docked with prostaglandin synthase inhibitor for anti-inflammation activity and Glycolate oxidase / Lactate dehydrogenase inhibitor. Overall results explain us that *Karpura shilajit* has proven nephroprotective activity by ethylene glycol induced model along with anti-inflammatory activity i.e., by controlling renal stone formation and increasing urine flow and reduction in impaired renal tubules.

Keywords: *Karpura shilajit*, Ethylene Glycol, Antilithiatic, anti-inflammatory, Docking.

1. INTRODUCTION

Lithiasis, often known as calculus, seems to be a disorder wherein acid salts and hard, tiny mineral fragments accumulate in either organ or duct of the body. Renal lithiasis can be defined as the consequence of an alteration of the normal crystallization conditions of urine in the urinary tract. (Grases *et al.* 2006). Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis (Alelign & Petros 2018). In our study lithiasis is induced by ethylene glycol –ammonium chloride model where cystone is the standard drug used. In addition to being uncomfortable as they try to pass through the tissue, the renal caliculi's restricted urine flow may also result in inflammation and increased pressure. Inflammation is the body's first response to infection or injury and is critical for both innate and adaptive immunity (Kany *et al.* 2019). Diclofenac sodium inhibits the enzyme cyclooxygenase (COX). The therapeutic effects of NSAIDs are attributed to the lack of these eicosanoids. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. One such example is the herbal mineral extract called Shilajit. It is widely used in traditional medicinal system of India has been reported to possess antioxidant, anti-inflammatory, memory enhancer and anti-ageing property. It is known as a rich source of humic substances, including fulvic acid (Suvarchala *et al.* 2022). The innumerable medicinal properties and therapeutic uses of Shilajit prove its importance as a valuable medicinal substance (Nareshrao & Talekar 2019). The aim of the study is to evaluate the antilithiatic activity of Karpura shilajit, docking studies of bioactive compounds obtained from GC-MS studies of *Karpura shilajit* and in *silico* ADME analysis of docked compounds by molinspiration to calculate the properties and to predict bioactivity.

2. MATERIALS AND METHODS

2.1 Identification of Chemical Constituents

Selected mineral was screened for the presence of various phytoconstituents like glycosides, alkaloids, terpenoids, volatile oils etc., and also chemical compounds such as carbohydrates, protein and lipids that exert a physiological and therapeutic effect.

2.2 Acute Toxicity Testing

Studies were carried out in order to check the toxic effects of the extract. The study performed as per organization for economic cooperation and development (OECD) to evaluate the acute oral toxicity by up and down procedure. Animal's inbred colony of adult wistar albino rats (150-200 gm) are used for the present study. They were kept in polypropylene cages at 25 ± 2°C, with relative humidity 45- 55% under 12-hour light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed and water ad. libitum. All the pharmacological experimental protocols were approved by the IAEC.

2.3 Experimental Protocol

Animal procurement Wistar albino rats (Approx. 150 to 200 g) were procured from Gentox Bioservices, Hyderabad, and present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg, No. 1175/PO/Re/S/08/CPCSEA).

2.4 In vitro evaluation of Antilithiatic Activity

2.4.1 Homogenous precipitation method

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous preci pitation: Equimolar solution of calcium chloride dihydrate (AR) in distilled water and sodium oxalate (AR) in 10 mL of 2N H2SO4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Precipitate freed from traces of sulphuric acid by ammonia solution. It was washed with distilled water and dried at 60ºC for 4 hours.

Step-2: Preparation of semi-permeable membrane from eggs: The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole was made on the top and the contents squeezed out separately from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened

condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7-7.4.

Step-3: Estimation of calcium oxalate by Titrimetry: weighed exactly 1 mg of the calcium oxalate and 10 mg of the extract/compound/ standard and packed together in semi evaluation permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100 mL 0.1M TRIS buffer. One group served as negative control (contained only 1 mg of calcium oxalate). Conical flask, of all groups in an incubator, preheated to 37°C for 2 h, for about 7-8 h. The contents of semi-permeable membrane from each group were removed into a test tube. Added 2 mL of 1N sulphuric acid and titrated with 0.9494N KMnO4 till a light pink colour end point obtained.

1 mL of 0.9494N KMnO4 = 0.1898 mg of 4 Calcium

The amount of undissolved calcium oxalate was subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually dissolved the test substance(s) (Suvarchala *et al.* 2021).

2.5 In vitro Evaluation of Anti-inflammatory Activity

2.5.1 Protein denaturation Assay

2.5.1.1 Preparation of reference drug (positive control)

NSAID (Diclofenac sodium) was used as reference drug. It was crushed into fine powder. About 0.2 g of drug powder was measured using a digital analytical balance (Shimadzu corporation, Japan) and was added to 20.0 ml of distilled water. The solution was mixed well using a vortex.

2.5.1.2 Serial dilutions

Serial dilution from 1000 µg/ml to 0.01µg/ml was performed for shilajit and for reference drug (Diclofenac sodium). All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg albumin (from fresh hen's egg). Then 2 ml of extract from each different concentration were mixed gently with reaction mixtures. A similar procedure was used for reference drug (Diclofenac) and they were used as positive controls for this study. In addition, distilled water was used as negative control.

2.5.1.3 Inhibition of protein denaturation

Reaction mixtures were incubated in a water bath at 37°C ± down 2°C for 15–20 min, and later, it was heated at 70°C at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool at room temperature for 15 min. Absorbance of reaction mixture before and after denaturation was measured for each concentration (1000 µg/ml, 100µg/ml, 10µg/ml, 1 µg/ml, 0.1µg/ml and 0.01µg/ml) at 680 nm using a colorimeter. Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula (Raju et al. 2018).

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.6 In vivo Evaluation of Antilithiatic Activity

2.6.1 Ethylene glycol – ammonium chloride induced urolithiasis

2.6.1.1 Experimental animal groups

The animals are divided in to five groups containing six animals in each group. Group I is Control received (0.5%) gum acacia, Group II (Disease control group), III (*Karpura shilajit* (100 mg/kg), IV *Karpura shilajit* (200 mg/kg) & V cystone (500 mg/kg) received 0.75% v/v ethylene glycol for 28 days and 1% w/v ammonium chloride for first 3 days in distilled water additionally Group III received *Karpura shilajit* (100 mg/kg, bd. wt, p.o) for 15-28 days, Group IV received Karpura shilajit (200 mg/kg, bd. wt, *p.o*) for 15-28 days and group V received cystone (500 mg/kg, bd. wt, *p.o*) for 15-28 days.

2.6.1.2 Procedure

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis in rats. It was used to evaluate antilithiatic effect of *Karpura shilajit* in wistar albino rats. This is a chronic

model of urolithias where treatment period was 28 days. All the animals in different groups placed individually in metabolic cages for 24 hours with free access to drinking water, Analysed for urine volume and urine pH on 0th, 14th and 21st day. A drop of concentrated Hydrochloric acid was added to urine and stored at 40ºC. Under anaesthesia blood was withdrawn from retro orbital sinus on 0th, 7th, 14th, 21st and 28th day a sample was centrifuged at 3000 rpm for 15 min. Serum obtained was analysed for creatinine, BUN, uric acid, calcium, phosphate, oxalate, sodium and potassium (Suvarchala *et al.* 2020).

2.7 Histopathology of Kidney

For the histopathological examinations, the kidney tissue samples from the animal were fixed in 10% formalin for at least 24 h. Then the paraffin sections were prepared and cut into 5-µm thick sections in a rotary microtome. The sections were stained with Haematoxylin-eosin dye. The histopathological examination of slides were performed under plain and polarized light microscope and photographed by camera. Histopathological changes, aggregation of calcium oxalate crystals and stones in the kidney tissues were recorded (Suvarchala *et al.* 2020).

2.8 Statistical Analysis

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, disease control and standard. By using Dunnett's test, significant values were expressed as p= 0.0001, p< 0.0001, p< 0.0005, p= 0.001.

2.9 Molecular Docking Studies

Molecular docking is a kind of bioinformatics modelling which involves the interaction of two or more molecules to give the stable adduct. Depending upon binding properties of ligand and target, it predicts the three-dimensional structure of any complex. In study mcule software is used for docking and visualized in discovery studio (Suvarchala *et al.* 2019).

2.10 Ramachandran Plot

Generated by PROCHECK validation server showing the stereo chemical quality of the protein 5AIS & 7M2O. Ramachandran plot has been generated from PROCHECK validation server was used to access the quality of the model by looking into the allowed and disallowed regions of the plot (Suvarchala et al. 2022).

2.11 Statistical Analysis

Values are expressed as Mean ± SEM, (n=6). All the groups were compared with control, diabetic control and standard by using Dunnett's test. Significant values were expressed as control group (**=p<0.01, *=p<0.05), diabetic control (A= p<0.01, B = p<0.05) and standard (a = p< 0.01, b = p< 0.05), ns- non significant.

3. RESULTS AND DISCUSSION

Karpura shilajit was explored for its antioxidant, antilithiatic and anti-inflammatory activity using suitable in vitro and in vivo models. All the results obtained in the study were included below.

3.1 Preliminary Identification Tests

Identification tests of the mineral extract revealed the presence of terpenoids, sterols, saponins, alkaloids, carbohydrates.

3.2 Acute Toxicity Study

Administration of *Karpura shilajit* at 2000 mg/kg dose showed no mortality (death rate) or no evidence of adverse effects implying that Karpura shilajit is nontoxic. No changes observed in behavioural outline, clinical signs and body mass of mice throughout 14 days of study. This shows that *Karpura shilajit* is safe to use at dose of 2000 mg/kg.

3.3 In-vitro Evaluation of Antilithiatic Activity

3.3.1 Homogenous precipitation method

Step 1: Preparation of experimental kidney stones

A)

Step 2: Preparation of semipermeable membrane from eggs

B)

C)

D)

Step 3: Estimation of calcium oxalate by titrimetry

E)

F)

G)

H)

Fig.1: In vitro antilithiatic activity by Homogenous precipitation method

A)Preparation of sodium oxalate and CaCl2., B) Eggs placed in HCl overnight, C) Contents of the egg squeezed out, D) Semi permeable egg membrane placed in ammonia solution, E) Calcium oxalate + 10 mg control/ Shilajit/ Cystone packed in membrane, placed in conical flask containing Tris buffer, F) Suturing of the membrane with contents in it, G) Conical flasks placed in incubator for 2 h at 37ºC, H) estimation of percent dissolution of calcium oxalate by titrimetric.

Table 1: Effect of *Karpura shilajit* on percent dissolution calcium oxalate by homogenous precipitation method

Group	Percent dissolution calcium oxalate (%)
Blank	0
Karpura shilajit	73.5±0.49
Cystone	64.25±0.54

The *in-vitro* antilithiatic activity of Shilajit was carried out by using homogenous precipitation method. Blank group showed no percent dissolution of calcium oxalate was found to be 0 %. Shilajit has shown increase in percent dissolution calcium oxalate to 73.5 %. The potential of the extract was comparable to that standard cystone and percent dissolution of calcium oxalate value was found to be 64.25 %.

3.3.2 In vitro evaluation of anti-inflammatory activity

3.3.2.1 Protein denaturation assay

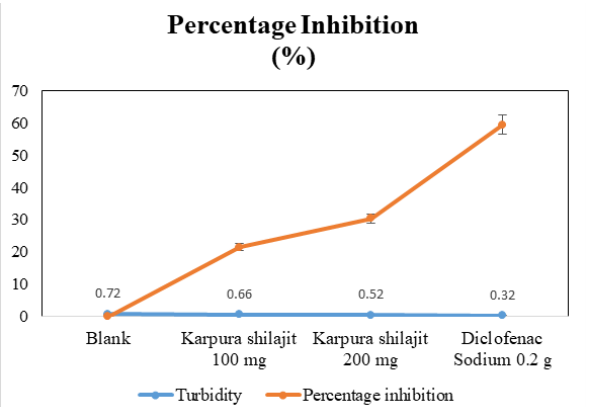


Fig.2: Effect of Karpura shilajit and Diclofenac sodium on turbidity and percentage inhibition in invitro Protein Denaturation assay method.

The *in-vitro* antilithiatic activity Karpura shilajit was carried out by using Protein Denaturation assay. Blank group showed high turbidity so the percent inhibition was found to be 0 %. Shilajit has shown increase in percent inhibition, decrease in turbidity with increase in dose, Shilajit 100 - 21.51 % and Shilajit 200 – 30.37 % . The potential of the extract was comparable to that standard Diclofenac sodium and percent inhibition value was found to be 59.49.

3.4 *In vivo* evaluation of antilithiatic activity

3.4.1 Ethylene glycol – ammonium chloride induced urolithiasis

In lithiatic disease control group the creatinine, uric acid, BUN, calcium and potassium levels were increased and sodium level was decreased after the administration of ethylene glycol-ammonium chloride and it was known to be significant in comparison with normal control. In treatment groups the *Karpura shilajit* 100 mg/kg and 200 mg/kg produced significant increase in sodium levels and significant decrease in creatinine, uric acid, phosphate, BUN, calcium and potassium levels respectively and showed results compared with disease control group. The dose of 200 mg/kg had shown better response than the 100 mg/kg dose which was comparable to standard Cystone shown in Table 2, Figure 3

Table 2: Effect of *Karpura shilajit* on serum creatinine, Uric acid, BUN, Sodium, potassium and calcium levels in ethylene glycol- ammonium chloride model.

	Control	Disease control	Karpura shilajit 100 mg/kg	Karpura shilajit 200 mg/kg	Cystone 500 mg/kg
Creatinine (mg/dL)	0.67±0.03	6.17±0.23^{a}	2.90±0.09^{a*B}	1.80±0.08^{a*ns}	1.62±0.10^{a*}
Uric acid (mg/dL)	0.94±0.19	4.80±0.27*	2.53±0.14^{a*D}	1.69±0.14^{a*ns}	1.49±0.14^{*a}
Potassium (mEq/L)	3.73±0.11	20.1±0.94*	6.48±0.25^{a*ns}	6.14±0.33^{a*ns}	5.78±0.28^{*a}
BUN (mg/dL)	33.5±0.69	106.83±0.84*	54.33±0.60^{a*A}	47.5±0.91^{a*C}	43.12±0.72^{*a}
Sodium (mEq/L)	140.8±0.80	76.5±0.81*	126.6±0.54^{a*A}	138.16±0.86^{*aA}	140.16±0.70^{*a}
Calcium (mg/dL)	10.15±0.2	15.31±0.21*	12.43±0.27^{a*C}	11.59±0.32^{a**ns}	10.76±0.42^{**a}

The values were expressed as mean ± SEM (n=6), analysis was performed with one way ANOVA followed by Dunnett's multiple comparison test against control (*= p=0.0001, **= p<0.001, ***= p<0.0001), against disease (a= p=0.0001, b= p<0.0001) and against Cystone 500 (A= p=0.0001, B= p<0.0001, C= p<0.001, D= p=0.001). ns = non-significant

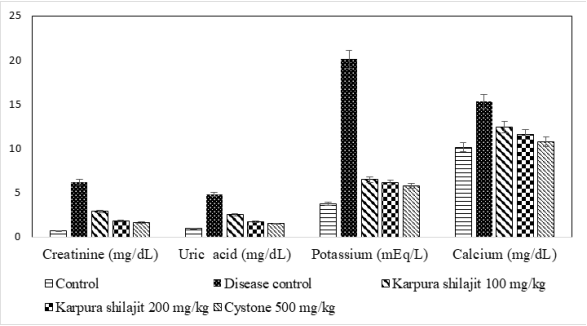


Fig.3: Effect of Karpura shilajit on serum creatinine, Uric acid, potassium and calcium levels in ethylene glycol- ammonium chloride model.

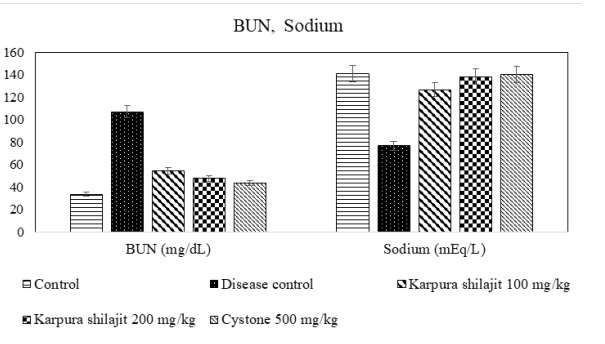


Fig.4: Effect of Karpura shilajit on serum BUN and Sodium levels in ethylene glycol- ammonium chloride model

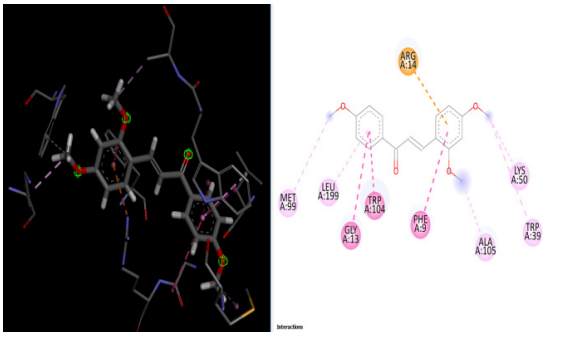
3.5 Molecular Docking Studies

To perform docking, initially the protein was downloaded from PDB and molecular docking performed in mCule software and visualized in discovery studio. Some compounds exhibited good binding ability with prostaglandin synthase inhibitor (PDB ID: 5AIS) for anti-inflammation activity and glycolate oxidase/lactate dehydrogenase inhibitor (PDB ID: 7M2O) antilithiatic activity were given [Fig. 5 &6] and docking results with glide score (Table 3).

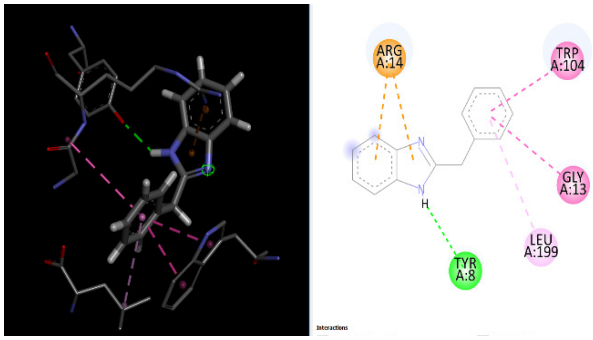
Table 3: Glide scores of *Karpura shilajit* constituents with 5AIS and 7M2O protein

Constituents	Glide scores (KcaL/moL)	
	5AIS	7M2O
Berginin	-5.9	-7.0
Bendazol	-7.2	-8.1
2,5 Anhydrotalitol	-4.2	-5.1
Xylofuranose	-4.1	-4.7
Thymol	6.3	-6.4
Cystine	-4.0	-5.5
6-Nitroimidazo [1,2-a] Pyridine	-5.8	-5.8
Cycloheptatriene	-5.2	-4.8
2-(4-Nitrophenyl) Succinic acid	-6.6	-6.6
Tartronic acid	-4.1	-4.4
Ferulic acid	-6.5	-6.6
2,4,4-Trimethoxy chalcone	-7.5	-7.7
D-Ribitol-5-Phosphate	-5.0	-5.5
Quinoline -2-Carboxylic acid	-7.4	-6.9
Cyclopropane Carboxamide	-4.0	-4.3

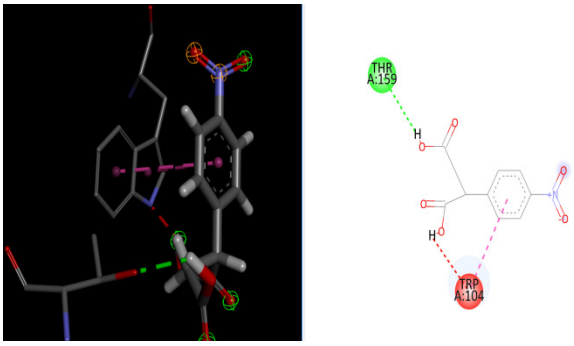
ANTI–INFLAMMATORY Protein PDB ID (5AIS)



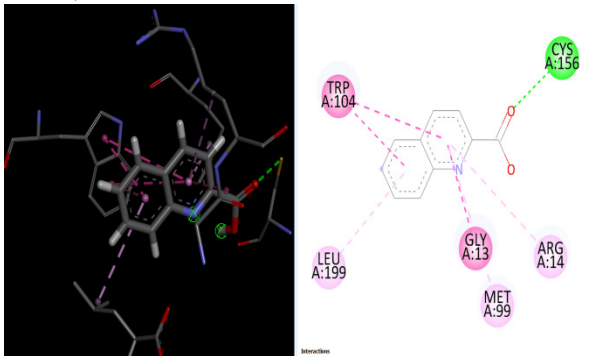
a) 2,4,4`-trimethoxy chalcone = -7.5



b) Bendazol = -7.2



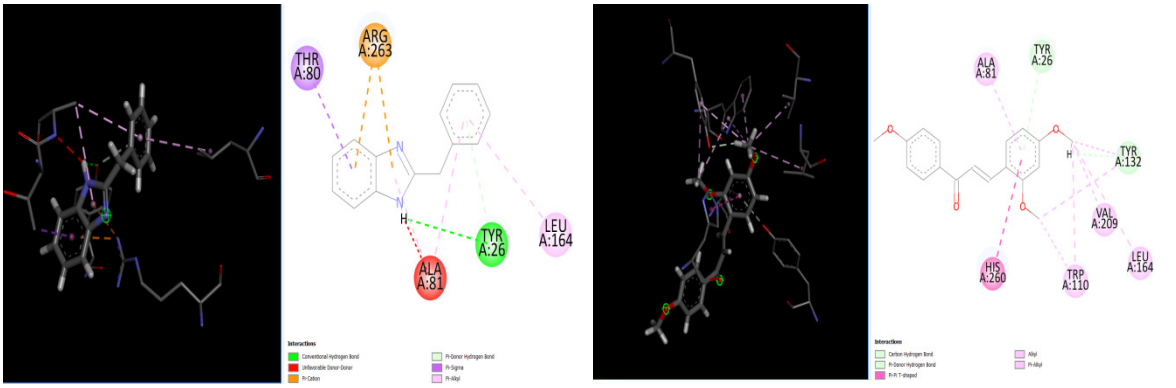
c) Ferulic acid = -6.5



d) Quinoline-2-carboxylic acid = -7.4

Fig.5: 3D & 2D structures of Prostaglandin synthase inhibitor (PDB ID: 5AIS) for anti-inflammation activity

Anti urolithiatic activity Protein PDB ID (7M2O)



a) Bendazole: -8.1 b) 2,4,4'-Trimethoxychalcone: -7.7

c) Berginin: -7.0 d) Qunoline-2-carboxylic acid: -6.9

Fig.6: 3D & 2D structures of Glycolate oxidase / Lactate dehydrogenase inhibitor (PDB ID: 7M2O) antilithiatic activity

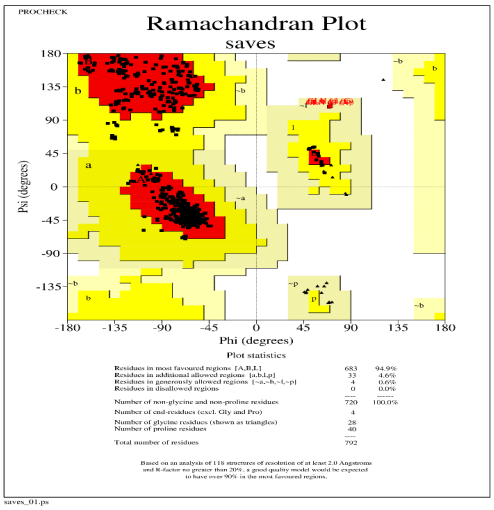
The more negative the Glidescore the more favourable is the binding. G score = glidescore,

3.6 Ramachandran plot Analysis

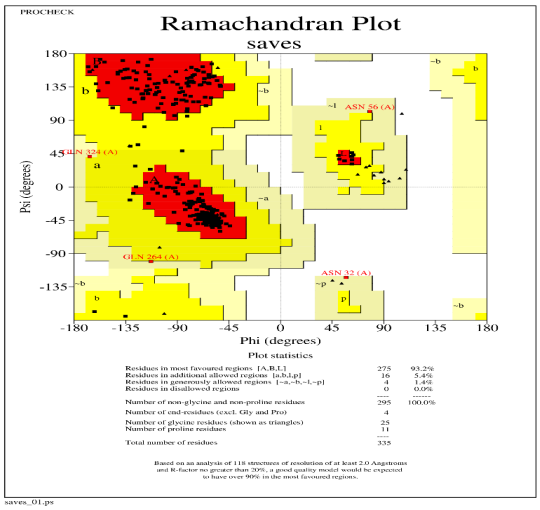
Protein 5AIS and 7M2O were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 4 and pictorial representation by fig. 7.

Table 4: Ramachandran plot status with protein 5AIS and 7M2O

Residues	5AIS	7M2O
Most favourable region (%)	94.9	93.2
Additional allowed regions (%)	4.6	5.4
Generously allowed regions (%)	0.6	1.4
Disallowed regions (%)	0.0	0.0



a) 5AIS



b) 7M2O

Fig.7: Ramachandran plot of protein 5AIS and 7M2O

4. DISCUSSION

The present work aimed with the objective of phytochemical investigation with anti-lithiatic and anti-inflammatory activity of mineral extract of *Karpura shilajit* in wistar albino rats. Preliminary phytochemical investigation of shilajit showed the presence of alkaloids, steroids, carbohydrates, saponins and terpenoids. In acute toxicity study of shilajit the maximum accepted dose was found to be 2000 mg/kg, bd.wt. (*p.o*). *In vitro* antilithiatic activity by homogenous precipitation method, shilajit and cystone dissolved calcium oxalate precipitate that is prepared. The percent dissolution of calcium oxalate is high in cystone and shilajit compared to blank.

Model used for *in vivo* lithiasis induction is ethylene glycol – ammonium chloride model which cause induction of calcium oxalate urolithiasis. Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone (Takawal *et al.* 2012). When EG is used alone, kidney crystal deposition can be quite inconsistent. To achieve uniformly high rate of kidney crystal deposition, ammonium chloride has been used in combination with ethylene glycol (Bano *et al.* 2018). EG is readily absorbed in intestine and is metabolized in the liver to oxalate leading to hyperoxaluria. Furthermore, accumulation of the calcium oxalate crystals in the kidney decreased the urinary pH, which is one of the indications of urolithiasis (Ilhan *et al.* 2014). In disease group, there is decrease in urine pH, urine volume, increased kidney weight which is due to renal impairment, *Karpura shilajit* and cystone group the results are reversed. The percent change in body weight is significantly low in disease group where as there is increasing order shilajit 100, shilajit 200, cystone and control group. Disease control group showed enhanced levels of urea, creatinine, uric acid, calcium, phosphate, electrolyte potassium and decreased sodium levels. Cystone and shilajit was found to show good nephroprotective activity by decreasing the elevated levels of urea, creatinine, uric acid, calcium, phosphate, electrolyte potassium and increased sodium electrolyte level in serum. *Karpura shilajit* 200 showed better effects than *Karpura shilajit* 100.

Steroids have been used to treat or prevent the induced mucosal inflammatory reactions that cause edema formation due to stone presence, which will eventually facilitate stone expulsion. Corticosteroids stabilize lysosomes of neutrophils by exerting anti-inflammatory and anti-edema actions; they also may exhibit their inflammation reduction by inhibiting prostaglandin release at the site of obstruction (Nouri *et al.* 2017). The potential urolithiasis preventive effect might have been contributed by the rich terpenoid content, which prevented the formation of the calcium oxalate crystals by either of the mechanisms; increased bioavailability of nitric oxide that suppressed the influx of calcium by

cGMP pathways and its nephroprotective action.

Primary hyperoxaluria (PH) produce glyoxylate reductase (GR), impairing hepatic detoxification of glyoxylate and corresponding excessive oxalate production in the liver via the action of lactate dehydrogenase A (LDHA). Oxalate produced in the liver is excreted via the kidneys, but when present at high concentrations, it complexes with calcium to form calcium oxalate salt. Deposits of calcium oxalate within the kidney can lead to inflammation. As kidney function deteriorates, concentrations of plasma oxalate increase, and calcium oxalate can accumulate. Two inhibition mechanisms have been the focus of therapeutic development for PH: glycolate oxidase and LDHA (Ding *et al.* 2021). Prostaglandin D2 (PGD2) is an allergic and inflammatory mediator produced by mast cells and Th2 cells, PG inhibitors have an anti-inflammatory effects. The compounds present in *Karpura shilajit*, are docked with prostaglandin synthase inhibitor protein (PDB ID: 5AIS) for anti-inflammation activity and Lactate dehydrogenase inhibitor protein (PDB ID: 7M2O) antilithiatic activity and Ramachandran plot is analysed.

Bendazole, 2,4,4-Trimethoxychalcone and Quinoline-2-Carboxylic acid showed good docking score when compared to other compounds for protein 7M2O & 5AIS and Ramachandran plot showed > 93% of amino acids in mostly allowed regions. In the present study the superposition of 2,4,4'-Trimethoxychalcone, Bendazole and other compounds docking found with prostaglandin synthase inhibitor protein (PDB ID: 5AIS) and lactate dehydrogenase inhibitor protein (PDB ID: 7M2O) protein have validated the accuracy of our docking study, Ramachandran plot that resulted anti-inflammatory and antilithiatic activity.

5. CONCLUSION

In the present study, *Karpura shilajit* was screened for in vitro antilithiatic activity by homogenous precipitation method which has shown increase in percent dissolution calcium oxalate. The presence of terpenoids, and sterols, flavonoids might be responsible for anti-inflammatory activity. *Karpura shilajit* was screened for in vivo antilithiatic activity by ethylene glycol ammonium chloride induced lithiasis. During histopathological examinations shilajit has shown decrease in calcium oxalate crystal deposition, with increase in dose. The results were comparable with standard and control groups. Histopathology results have revealed *Karpura shilajit's* potential in reducing the stone formation and regeneration of damaged glomeruli. Molecular docking studies confirmed the lactate dehydrogenase inhibitory effect and PG's inhibitory effect of the compounds, as they occupied the binding site and was found to possess hydrogen bonding interactions. The Glide scores order for all molecules reflected the experimental lactate dehydrogenase inhibitory effect and PG's inhibitory activity.

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