

# STUDY ON IN VITRO DISSOLUTION OF CALCIUM OXALATE RENAL STONE BY SHILAJIT

<sup>1</sup>Shafiq Ahmad, <sup>2</sup>Tariq Mahmood Ansari, <sup>3</sup>Muhammad Aslam Shad.

<sup>1</sup>Pharmaceutical chemist, Department of pharmacology Nishtar Medical College, Multan, Pakistan.

<sup>2</sup>Professor of Analytical Chemistry, Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan.

<sup>3</sup>Professor of Biochemistry, Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan.

## ABSTRACT

**Objective:** This study aims to test the solubility efficiency of Shilajit in vitro for calcium oxalate renal stone. **Material & Methods:** A small stone was selected for the experiment. The weighed stone was suspended in 25 ml of aqueous extract of Shilajit for 72 hours with the interval of 24 hours. After each 24 hours, the stone was taken out, washed, dried and difference in weight was calculated. The whole procedure was carried out at room temperature. **Results:** It was found that the weight of the stone was reduced. **Conclusion:** Shilajit has the ability to dissolve the calcium oxalate renal stone.

**Keywords:** Calcium oxalate, renal stone, in vitro, Shilajit.

## ABSTRAK

**Tujuan:** Menguji efisiensi kelarutan shilajit in vitro untuk kalsium oksalat batu ginjal. **Bahan & Cara:** Sebuah batu kecil dipilih untuk percobaan. Batu yang ditimbang ditangguhkan dalam 25 ml ekstrak air Shilajit selama 72 jam dengan interval 24 jam. Setelah setiap 24 jam batu tersebut diambil, dicuci, dikeringkan dan perbedaan beratnya dihitung. Seluruh prosedur dilakukan pada suhu kamar. **Hasil:** Ditemukan bahwa berat batu berkurang. **Simpulan:** Shilajit memiliki kemampuan untuk melarutkan batu ginjal kalsium oksalat.

**Kata Kunci:** Kalsium oksalat, batu ginjal, in vitro, Shilajit.

Correspondence: Shafiq Ahmad; c/o: Pharmaceutical chemist, Department of pharmacology Nishtar Medical College, Multan, Pakistan. Phone: +92619200238. Email: biochemist111@hotmail.com.

## INTRODUCTION

Kidney stones are one of the most painful disorders to afflict humans. In the United States, the urinary stone disease is a common clinical problem with a high risk of recurrence. One out of every ten Americans suffers from kidney stone disease sometime during their lifetime. It is the third leading cause of hospitalization in the USA and the seventh leading cause for visits to urologists. Clinical treatment of stone-related disorders is estimated to cost over \$2 billion per year. Current medical treatment will not dissolve symptomatic kidney stones. Since most stones will not pass out of the body spontaneously, comminution with high-intensity shock waves (SWL) has become nearly a standard procedure for stones in the upper urinary tract. However, compelling clinical data now suggest that SWL may cause acute renal injury, a rise in diastolic blood pressure, a decrease in renal

function, and an increase in stone recurrence. This proposal seeks an alternative to SWL for kidney stone treatment.

Oral dissolution therapy of kidney stones with diuretic medicinal plants has been known for millennia by the indigenous people of America, India, and Arabia.<sup>1</sup>

The dissolution of renal calculi orally has limited indications. For example, it is used only as an adjuvant to an endourologic operation or ESWL in special situations. Chemolysis may have an important role in the management of renal stone in the future.<sup>2</sup> The choice of the kind of solution for the breakdown of the stone depends upon the composition of renal stone. Suby or Renacidin solution can dissolve calcium phosphates, though it is often tiresome and time-consuming process. These solutions, however, cannot dissolve the major urinary stone component, the calcium oxalate. Because of local toxicity, EDTA and other strong

calcium chelator cannot be used. There are enzymes, which however can digest the organic matrix of renal stone. The indication to Chemolysis of stones is rather restricted.<sup>3</sup>

From a historic standpoint, in 1938 Hellstrom first dissolved a struvite stone using boric acid and permanganate. In 1943, Suby and Albright developed Suby's solution G, which could be instilled into the kidney via a nephrostomy tube or ureteral catheter. In their initial report, six patients had partial or complete stone dissolution. Mulvaney then modified this solution by adding D-gluconic acid (Hemiacidrin or Renacidin®) and published the first report dissolving infection stones in nine of 13 patients, either partially or completely. However, a report of four deaths during intrarenal irrigation led to an FDA ban on renal Renacidin® use.<sup>4</sup>

Serious attempts to discover a satisfactory agent for the dissolution of urinary tract calculi began when the chemical composition of most stones was identified in the early 19<sup>th</sup> century. Since the first report of Chemolysis by Crowell in 1924, countless other urologists have used this method of eliminating all forms of urinary lithiasis. Albright et al. reported 40 years ago that a buffered solution of citric acid (pH 4.0) was effective in dissolving some phosphatic calculi, but this substance proved to be too irritating to the tissues to be clinically useful. Suby and Albright modified this solution by adding anhydrous magnesium oxide and sodium carbonate, making it more acceptable to the tissues.<sup>2</sup>

In Ayurveda, Shilajit is thought of as one of the miracle drugs. It is not a plant. It is not animal stuff either. It is a mineral pitch. It comes out the rocks of the Himalayas when they become warm in the summer season. It is believed that Shilajit has the therapeutic power of these great mountains.

It is called Shilajit in Sanskrit, Shilajita in Hindi, Hajar-ul-Musa in Arabic, Momiai in Persian, vegetable Asphalt in English, Asphaltum in Latin, and its botanical description is Bitumen mineral.

The Charaka Samhita states that "Stones of metal like gold etc., in the mountains get heated up by the sun and the exudates that come out of them in the form of smooth and clean gum is called çilājatu". According to Sharma Shilajit is not produced from metals like gold rather stones containing gold produces Shilajit.

It is thought that plants like Euphorbia royleana Boiss and Trifolium repens grow near the Shilajit rocks and they are the source of Shilajit. Current research claims that bryophytes form

Shilajit as species of mosses and liverworts were found in the vicinity of rock which exudes Shilajit. The mineral composition of the bryophytes is same as that of Shilajit.<sup>5</sup>

The biologically important classes of compounds of Shilajit include Dibenzo-alpha pyrones, phospholipids, triterpenes and phenolic acids of low molecular weight, Fulvic acids: "carrier molecules", Humins and humic acids, trace elements (Fe, Ca, Cu, Zn, Mg, Mn, Mo, P).<sup>6</sup>

Laboratory analysis of Shilajit shows that it possesses a large quantity of benzoic acid, hippuric acid, and their salts. Besides, it also contains gums, albuminoids, traces of resin and fatty acid.<sup>7</sup> Shilajit contains fresh and modified leftovers of humus. It is 10-70% of the water-soluble part of Shilajit. This humus is mixed with plant and microbial metabolites present in the rock rhizosphere of its natural environments.<sup>8</sup>

Fulvic acid is the major organic matter in aqueous extract of Shilajit. It has been reported that most of the biological and medicinal properties are due to it.<sup>9</sup>

Externally fulvic acid has been used in the treatment of hematoma, desmorrhesis, myogelosis, arthrosis, polyarthritis, osteoarthritis, and osteochondrosis. Fulvic acid has equally been used orally in the treatment of gastritis, diarrhea, stomach ulcers, dysentery, colitis, and diabetes mellitus.<sup>10</sup>

Scientists have discovered recently that fulvic acid is the finest electrolyte. It helps in human enzyme production. It has a role in the utilization of vitamins. It is also a powerful antioxidant and free radical scavenger. It has a unique ability to react with both positively and negatively charged unpaired electrons and render free radicals harmless. Free radicals are either altered to new usable compounds or eliminated from the body as waste.<sup>11</sup>

The aqueous solution of fulvic acid has the capability of dissolving minerals and metals. The fulvic acid changes them into complex fulvic acid molecular complexes. These complexes have wide range of characteristics. Fulvic acid is a natural metallic mineral chelator and turns them into readily absorbable bio-available forms.<sup>12</sup> Fulvic acid can help in the movement of metal ions that are usually difficult to transport. Fulvic acid is an exceptional natural chelator and cation exchanger and is essentially important in the nourishment of cells.<sup>13</sup> Fulvic acid and humic acids from the Shilajit are the natural organic polyelectrolyte acids. They are effective crystal growth inhibitors.<sup>14</sup> Fulvic acid

regulates calcium carbonate growth rate inhibition. Factors included in the process are structure, molecular weight, functional group character, and functional group ionization. It seems probable that the factors in fulvic acids that increase their efficiency as calcite growth inhibitors may recommend compounds or arrangements of carboxylate groups that are valuable as beginning inhibitors.<sup>15</sup>

Humic acid and fulvic acid efficiency of inhibition of growth rate are due to multiple ionized carboxylate groups. These groups are directly attached to a five member ring. Growth rate inhibition of calcium carbonate may also be due to the arrangement of carboxylate groups in the fulvic acid.<sup>15</sup>

## OBJECTIVE

This study aims to test the solubility efficiency of Shilajit in vitro for calcium oxalate renal stone.

## MATERIAL & METHODS

The aqueous extract of Shilajit was prepared by dissolving 10 grams of purified Shilajit in 100 ml of deionized water. This solution was thoroughly mixed for 3-4 hours and centrifuged at 3.000 rpm for 10 min. The supernatant thus obtained was referred to as the aqueous extract of Shilajit (AES).

The dissolution was carried out following the method of Sinha et al. with minor

modifications.<sup>16</sup> The work was carried out at Bahauddin Zakariya University, Multan, Pakistan and Multan Drug Testing Laboratory, near District Jail Multan, Pakistan in 2010-2011.

The renal stone of pure calcium oxalate composition was selected for the experiment. The stone was washed properly with distilled water. The stone was suspended in 20 ml of 0.1N NaCl solution for 24 hours then filtered and washed it with distilled water. Dried in an air oven at 80°C for 2 hours and cooled down. The stone was weighed (analytical balance, OHAUS Corp. USA) and suspended in 25 ml of the aqueous extract of Shilajit for 24 hrs. Stone was filtered, washed with distilled water, dried and weighed out. Stone was again suspended in 25 ml of aqueous extract of Shilajit for the next 24 hours. Stone was again filtered, washed with distilled water, dried and weighed out and suspended in 25 ml of each aqueous extract of Shilajit thrice for another 24 hrs. Finally, the stone was filtered and washed with distilled water and then dried and weighed out after due period. The whole procedure was carried out at room temperature.

## RESULTS

Solubility efficiency of Shilajit for calcium oxalate renal calculi at different time intervals were evaluated in this work. The weight of the renal stone chosen for the experiment was 0.365 grams. The solubility difference after 24 hours, 48 hours, and 72 hours were 0.011, 0.025, and 0.035 respectively and percentage solubility was 3.01, 6.04, and 9.58 respectively as shown in Table 1 and Table 2.

**Table 1.** Solubility differences of the renal calculi in controls and aqueous extract of Shilajit \*(AES) with time.

Solvents	Weight of the whole stone before suspension (g) (a)	Weight remained after 24 h (b)	Solubility difference (g) (a-b)	Weight remained after 48 h (c)	Solubility Difference (g) (a-c)	Weight remained after 72 h (d)	Solubility difference (g) (a-d)
Deionised water (Negative control)	1.285	1.281	0.004	1.278	0.003	1.275	0.001
Sodium citrate (Positive control)	1.142	0.762	0.38	0.611	0.531	0.423	0.719
*AES	1.354	0.975	0.379	0.834	0.62	.623	0.585

\*Aqueous extract of shilajit.

**Table 2.** Percentage solubility of the renal calculi in controls and aqueous extract of shilajit \*(AES) with time.

Solvents	Weight of the whole stone before suspension (g)	Percentage solubility after 24 hours	Percentage solubility after 48 hours	Percentage solubility after 72 hours
Deionized water (negative control)	1.285	0.311	0.233	0.077
Sodium citrate (positive control)	1.142	33.27	46.49	62.95
AES*	1.354	27.99	38.40	53.99

\*Aqueous extract of shilajit.

## DISCUSSION

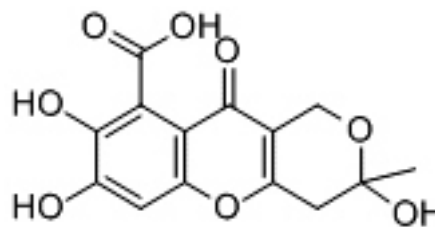
From the results, it is quite apparent that weight reduction did occur. The dissolution of calcium oxalate in the presence of Shilajit may be described as due to the chelation of  $\text{Ca}^{2+}$  ion with the major active principle of the Shilajit such as fulvic acid as it is amongst the numerous active principles of Shilajit.<sup>17</sup> Various Therapeutic actions of Shilajit have been attributed to fulvic acid and dialphabenzopyrone.<sup>18</sup>

Fulvic acid has the ability to form complexes with mono-, di-, tri- and polyvalent metal ions. These complexes are stable and water-soluble. It can also shift the metal ions that are difficult to mobilize in normal circumstances. Fulvic acids are good natural chelators, cation exchangers and very important in the nourishment of cells.<sup>12-13</sup>

From the above discussion, it may be said that fulvic acid in the Shilajit might have formed a stable complex with calcium present in calcium oxalate renal stone and sequestered it from the stone. This may be the probable mechanism of dissolution of renal calculus.

Fulvic acid, a natural organic polyelectrolyte acid, is effective crystal growth inhibitors.<sup>17</sup> It may further be concluded that the carboxylic group in the fulvic acid acts as chelator of calcium.

Fulvic acid regulates calcium carbonate growth rate. Factors included in the process are structure, molecular weight, functional group character, and functional group ionization.<sup>15</sup> The same phenomenon of sequestering the calcium from the renal calculus may be taking place in the

**Figure 1.** Fulvic acid.

dissolution process. Therefore it is gathered that the carboxylic group of the fulvic acid complexes with the calcium of the renal calculus and it is dissolved.

This finding is corroborated by the work done by Curtis et al. who established that Anticolana Valley (Fiuggi) water contains humic and fulvic acid. Keeping in view this fact, the ability of this water to dissolve kidney stone in vitro was studied.<sup>19</sup> The Anticolana Valley (Fiuggi) water, distilled water, and tap water were used in the study. A specially designed Perspex apparatus was used in the experiment. Each stone was subjected to a water flow of 2 liters /24 h. It was found that the ability of Anticolana Valley water to dissolve the human as well synthetic stone was very high as compared to that of distilled water. And distilled water was found to be more effective than that of tap water.

Fraioli et al. continued study on Fiuggi water and after an in vivo study on human beings, he found that after drinking the said water calcium oxalate monohydrate was either strongly reduced or sometimes even removed from the urinary sediment.<sup>20</sup> In this way, the risk of oxalate calculosis was greatly reduced. Organic molecules belonging to fulvic acid family are present in the fiuggi water.



These acids can make complexes with calcium ions. These acids form a film on the calcium oxalate and interact preferentially with the crystal lattice of the calcium oxalate monohydrate. These behave as pumping systems by linking the calcium ion, demolishing the crystal lattice and dissolving calcium and oxalate ions.

Calace et al. isolated humic acid from the anticolana valley (fiuggi) water purified and characterized it.<sup>21</sup> Only fulvic acids were found. These are mainly composed of aliphatic chains, made of six -CH<sub>2</sub>O- groups and contain the number of carboxylic acids group responsible for their metal complexing capacity.

## CONCLUSION

Shilajit has the ability to dissolve calcium oxalate renal stone. Study on larger level is however required to establish the results.

## REFERENCES

1. Parker JM, Parker PM. The official Patient's source book on kidney stones. USA: Icon Health Publications Icon group international, Inc.; 2002.
2. Bernardo NO, Smith AD. Chemolysis of urinary calculi. *Urol Clin North Am.* 2000; 27: 355-65.
3. Oosterlinck W, Verbeeck R. Chemolysis of calcium containing urinary calculi. A review. *Acta Urol Belg.* 1994; 62: 31-7.
4. Pearl MS, Nakada SY. *Urolithiasis Medical and Surgical Management.* London, UK: Informa Health Care; 2009.
5. Talbert R. Shilajit. A Materia Medica Monograph. California. 2004; 1-17.
6. Ghosal S. Chemistry of Shilajit, an immunomodulatory Ayurvedic Rasayana. *Pure and Appl. Chem.* 1990; 62: 1285-1288.
7. Chopra RN, Chopra IC, Handa KL, Kapur LD. *Chopra's Indigenous drugs of India* 2<sup>nd</sup> edition. 1958.
8. Mukherjee B. Traditional Medicine, Proceedings of an International Seminar. Nov. 7-9 Hotel Taj Bengal, Calcutta India. Oxford & IBH Publishing. 1992. p. 308-319 New Delhi.
9. Ghosal S, Singh SK, Kumar Y, Srivastava RS, Goel RK, Dey R, Bhattacharya SK. Anti-Ulcerogenic Activity of Fulvic Acids and 4' Methoxy-6 Carbomethoxybiphenyl Isolated From Shilajit. *Phytother Res.* 1988; 2: 187-191.
10. Agarwal SP, Khanna R, Karmarkar R, Anwer MK, Khar RK 2007 Shilajit: a Review. *Phytother Res.* 2007; 21: 401-405.
11. What Is Fulvic Acid?. <http://www.supremefulvic.com/documents/pdf/1.what.is.fulvic.acid.pdf>
12. Ong H.L, Swanson, VD, Bisque RE. Natural organic acids as agents of chemical weathering (130-170). U.S. Geological Survey Professional Paper 700 c. Washington, DC: U.S. Geological Survey. 1970.
13. Schnitzer M, Khan SU. Humic substances in the environment. New York: Decker; 1972.
14. Amjad Z, Reddy M M. In *Water Soluble Polymer-Solution Properties and Applications.* New York City: Plenum Press; 1998. p. 77.
15. Reddy MM, Hoch AR. Calcite Growth Rate Inhibition by Polycarboxylic acids. *Journal of Colloid and Interface Science.* 2001; 235: 365-370.
16. Sinha MR, Dev A et al. Experimental study of urinary stones in juice of Chikku (achras zatota) fruit. *J. Chem. Pharm. Res.* 2011; 3: 231-237.
17. Wilson E, Rajamanickam V et al. Review on Shilajit used in traditional Indian Medicine. *Journal of Ethnopharmacology.* 2011; 136: 1-9.
18. Sharma P, Jha J, et al. Shilajit: Evaluation Of Its Effects On Blood. 2003; 23(3).
19. Curtis SD, Curini R, et al. Solvent Effect In vitro of Anticolana Valley Water on Renal Stones: Analytical-Instrumental Study. *Nephron.* 1999; 81: 98-102.
20. Fraioli A, Curtis DA, et al. Effect of water of Anticolana Valley on urinary sediment of renal stone formers. *La Clinica terapeutica.* 2001; 152: 347-351.
21. Calace N, D'Ascenzo G et al. Chemical analysis of water of the Anticolana Valley: isolation of humic compounds. *Nephron.* 1999; 1: 93-7.