Research Article

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EVALUATION OF ANTIHYPERURICEMIC ACTIVITY OF SHODHITA SHILAJATU ON POTASSIUM OXONATE INDUCED HYPERURICEMIC RAT MODEL

Sangameshwar [1]*, H.M. Chandramouli [2], Surekha S. Medikeri [3], Shobha G. Hiremath [2]

[1]PG Scholar, Department of Rasashastra and Bhiashajya Kalpana, Government Ayurveda Medical College, Bengaluru, Karnataka, India

[2]Emeritus Professor, Department of PG studies in Rasashastra and Bhiashajya Kalpana, Government Ayurveda Medical College, Bengaluru, Karnataka, India

[3]Professor and Head, Department of PG studies in Rasashastra and Bhiashajya Kalpana, Government Ayurveda Medical College, Bengaluru, Karnataka, India

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***Corresponding author**
E-mail: sangameshvk@gmail.com

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ABSTRACT

Vatarakta a Mahavatavyadhi is a burning problem in present era. Here, both vata and rakta are responsible for complex effects in the joints. The status of Vatarakta (gout) is often compared with Gout in the allied sources due to the outstanding similarities. The present study has been conducted to evaluate Antihyperuricemic activity of Shodhita Shilajatu following its 7-days repeated oral administration on potassium oxonate induced hyperuricemic rat model. Antihyperuricemic activity of Shodhita (purified) Shilajatu (*Asphaltum punjabinum* - Black bitumen or mineral pitch) was studied in hyperuricemia induced in wistar albino rats using potassium oxonate. The uric acid levels in serum and urine were measured and histopathological study of Kidneys was evaluated. In potassium oxonate induced hyperuricemia, standard drug allopurinol and trial drug significantly lowered the serum uric acid levels. Urine analysis shows the presence of uric acid crystals in both the groups. When the results were compared it was found to be higher in the test group which suggests better uric acid excretion efficacy of Shilajatu. Histological examination of the kidney sections from all the groups showed moderate changes in Potassium oxonate administered control group which were significantly reduced in reference standard and moderately reversed in test drug administered group.

Keywords: Antihyperuricemic activity, Shodhita Shilajatu, potassium oxonate.

INTRODUCTION

Vatarakta (gout) a mahavatavyadhi[1] is a burning problem in present era. Here, in both vata and rakta (blood) dhatu (tissue) are responsible for complex effects in the joints. The chief complaints in the patient will be severe joint pain and inflammation with onset at Hasta pada mulagata sandhi (smaller joints in extremities)[2] and it migrates to all other joints in a way similar to that of Akhuvisha (Rat poison)[3].

The status of Vatarakta is often compared with Gout in the allied sources due to the outstanding similarities. The fundamental biochemical hallmark of gout is hyperuricemia which results from increased production or decreased excretion of uric acid or from a combination of the two processes[4,5]. Reported prevalence of this Gouty Arthritis is 2.0 to 2.6 per 1000 patients, usually between the age group of 25-50 years. The prevalence of gout is around 1 % with a strong male predominance (10:1)[6]. The underlying metabolic disorder in gout is an excessive concentration of uric acid in the blood. Urate lowering therapy is the main approach in the treatment of gout. The target level of serum uric acid is <6.8 mg/dL to dissolve the urate crystals and inhibit gout attack.[7,8] The most important approach in the treatment of gout is the development of xanthine oxidase (XO) inhibitors, Allopurinol is the most common clinically used XO inhibitor prescribed for the treatment of gout[9]. Allopurinol can cause the side effects, such as nephrolithiasis, allergic reaction and increase the toxicity of 6-merkaptopurin[10]. Thus, the development of novel hypouricemic agents with greater efficacy and a broader safety profile is greatly needed. In the Phalasruti (indication) of Shilajatu (*Asphaltum punjabinum* - Black bitumen or mineral pitch) it is mentioned that it eradicates Prabala Vatarakta along with other diseases [11.] Shilajatu is classically known for nephro protective activity and used clinically, however there is less data available on efficacy in hyperuricemia and underlying mechanism. So the present study was planned to explore antihyperuricemic effect of Shodhita Shilajatu following its oral administration at TED dose[12] along with biochemical and histo pathological evaluation in wistar albino rats.

MATERIALS AND METHODS

Place of work: SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India.

Animals - Healthy Wistar albino rats of either sex weighing between 150-250g were used for the study. Animals obtained from animal house a part of research lab, SDM Research Centre, Udupi. The rats were maintained under normal husbandry conditions and exposed to 12h night and 12h day and with ideal laboratory condition in terms of ambient temperature and humidity. Animals were fed with standard laboratory pellet feed supplied by Sai durga feeds, Bengaluru and water *ad libitum*. The experiment was conducted after obtaining the permission from the institutional ethics Committee in accordance with the guide line formulated by CPCSEA (IEC No: SDMCRA/IEC//BNL/RSBK) and Approval no. (01)

Dose fixation

The dose for the rats will be calculated by using the standard conversion method.
i.e., Human dose ×0.018×5/kg body weight[13].

Drugs and chemicals

Potassium oxonate was used as inducing agent, manufactured by Alfa–Aesar, Bond street ward hill, MA-01835. Allopurinol was used as reference standard, manufactured by Glaxo Smith Kine Pharmaceuticals Limited, Mohabewala industrial area Dehradun.

Purification of Shilajatu

In the present study Shilajatu was purified by Suryatapi (with the aid of sunlight) method, as mentioned in Rasa Tarangini.[14]

Test compound preparation

Potassium oxonate (PO) (250 mg/kg body weight, IP) was used for the induction of hyperuricemia and allopurinol (180mg/kg, orally) served as reference standard, were dissolved in 0.9% saline solution. Trial drug were dissolved in 10 ml of distilled water using 50mg of CMC (Carboxymethyl cellulose) and mixed well and administered orally. For the Normal control group and positive control group, the vehicle was prepared using 100mg of CMC, added with 20ml of distilled water and mixed well, (1ml /100g) and administered orally.

Experimental design

Animals were divided into four groups containing six animals in each group. First group served as normal control administered orally with 0.5%CMC and received regular rat food and drinking water *ad libitum*. Second group administered with CMC and diet for 7 consecutive days and served as disease control. Third group administered with Allopurinol (180mg/kg) orally for 7 consecutive days and served as reference standard. Fourth group administered with test drug Shodhita Shilajatu at therapeutic dose (625mg/kg–56.25mg/kg), for 7 consecutive days and served as trial group. The test drug and reference standard were administered according to the body weight by oral route with the help of rat feeding needle attached to syringe.

Collection of blood, urine and tissue samples

On 7th day for group 2,3,4, after an hour of administration of specific drug, potassium oxonate (250mg/kg, IP) and for investigation all rats were anaesthetized with diethyl ether and blood was collected from retro-orbital plexuses after 3hours and 24hours for estimation of serum biochemical parameters[15,16,17].

After collecting blood, animals were kept in a separate metabolic cage for 24 hours for urine collection on 8th day urine samples are collected and kept for analysis. Then animals were sacrificed by over dose of diethyl ether. The abdomen was opened by midline incision and kidneys were dissected out carefully and cleaned off the extraneous tissue. Kidneys were weighed and one kidney of each animal was transferred to 10% formalin solution and sent for histo-pathological studies.

Parameters studied

1. Ponderal changes**:** Body weight on initial day and before sacrifice, weight of kidney, the weight of kidney was expressed in terms of absolute value.
2. Urine analysis**:** like urine volume, urine pH and presence of crystals in urine.
3. Serum parameters: Analyzed for parameters Urea, Creatinine, and Uric acid in auto analyzer

Histopathology

The kidney was transferred to 10% formalin and sent to a commercial laboratory for preparation of slides. The slides with sections obtained were scanned through Trinocular Carl Zeiss's microscope (Germany) under different magnifications. Changes, if any in the cyto architecture were noted down.

Statistical analysis

The experimental data were expressed as Mean ± SEM (Standard Error of Mean).
The data obtained was analyzed using Graph pad In sat version 3.05 by 't' test for comparison between groups and rest of the data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett multiple 't' test as post hoc test for determining the level of significance of the observed effects. 'p' value of less than 0.05 was considered statistically significant.

RESULTS
Biochemical parameters
Serum uric acid

Table 1: Effect of Shodhita Shilajatu on serum uric acid (after 3h)

Groups	Serum Uric acid (mg/dl)	% change
Normal control	1.08 ± 0.07	
Potassium oxonate (PO) Control	2.61 ± 0.19**	141.1↑@
Allopurinol (Standard)	0.93 ± 0.28**	64.36↓#
Shodhita Shilajatu (T)	1.96 ± 0.13	24.90↓#

Data: MEAN ± SEM, **P<0.01 @-Compared with normal control #-compared with PO control

Table 2: Effect of Shodhita Shilajatu on serum uric acid (after 24h)

Groups	Serum Uric acid (mg/dl)	% change
Normal control	1.03 ±0.04	
Potassium Oxonate (PO) Control	1.8 ± 0.15**	74.75↑@
Allopurinol (Standard)	0.88 ± 0.21**	51.11↓#
Shodhita Shilajatu (T)	1.11 ± 0.13**	38.33↓#

Data: MEAN ± SEM, **P<0.01, @-Compared with normal control, s#-compared with PO control

Effect of Shodhita Shilajatu on serum uric acid (3h & 24h) is depicted in the table 1 and 2. The data obtained after 3hours of blood collected sample it was observed that, there is significant increase in serum uric acid in positive control was found, Further it was observed that, there is significant decrease in standard group due to standard drug Allopurinol (64.36%) and there was a significant increase of uric acid in positive control group (141.1%) while Test group (24.90%) decrease was observed which was statistically non-significant.

The data obtained after 24hours of blood collected sample, it was observed that, there was significant increase of serum uric acid in positive control group (74.75%) while in the test group (38.33%) decrease was observed which was statistically significant.

Blood urea

Table 3: Effect of Shodhita Shilajatu on serum urea (after 3h)

Groups	Urea (mg/dl)	% change
Normal control	36.66 ± 1.08	
Potassium Oxonate (PO) Control	30.33 ± 2.51	17.26↓@
Allopurinol (Standard)	56 ± 12.53*	84.63↑#
Shodhita Shilajatu(Test drug)	26.6 ± 6.21	12.29↓#

Data: MEAN ± SEM,*P<0.05, @-Compared with normal control, #-compared with PO control

Table 4: Effect of Shodhita Shilajatu on serum urea (after 24h)

Groups	Urea (mg/dl)	% change
Normal control	37 ± 1.15	
Potassium Oxonate (PO) Control	25.4 ± 1.43	31.35↓@
Allopurinol (Standard)	64.86 ± 14.82**	155.3↑#
Shodhita Shilajatu (Test drug)	37.2 ± 2.55	46.45↑#

Data: MEAN ± SEM,**P<0.01 ,@-Compared with normal control , #-compared with PO control

Effect of Shodhita Shilajatu on serum urea (after 3h & 24h) is depicted in table 3 and 4,The data obtained after 3hours of blood collected sample it was observed that, there was a non-significant decrease of blood urea in positive control group (17.26%) and increased blood urea in standard group (84.63%), which was statistically non-significant. While test drug (12.29%) decrease blood urea was observed this was statistically non-significant.

The data obtained after 24 hours of blood collected sample it was observed that, there was a non-significant decrease of blood urea in positive control group (31.35%) while in the test drug (46.45%) increase was observed which was statistically non-significant.

Serum creatinine

Table 5: Effect of Shodhita Shilajatu on serum creatinine (after 3h)

Groups	Serum Creatinine (mg/dl)	% change
Normal control	0.26 ± 0.03	
Potassium Oxonate (PO) Control	0.23 ± 0.03	11.53↓@
Allopurinol (Standard)	0.38 ± 0.10	65.21↑#
Shodhita Shilajatu (Test drug)	0.24 ± 0.04	4.347↑#

Data: MEAN ± SEM @-Compared with normal control #-compared with PO control

Table 6: Effect of Shodhita Shilajatu on serum creatinine (after 24h)

Groups	Serum Creatinine (mg/dl)	% change
Normal control	0.41 ± 0.15	
Potassium Oxonate (PO) Control	0.25 ± 0.03	39.02↓@
Allopurinol (Standard)	0.23 ± 0.09	8↓#
Shodhita Shilajatu (Test drug)	0.34 ± 0.06	36↑#

Data: MEAN ± SEM @-Compared with normal control #-compared with PO control

Effect of Shodhita Shilajatu on serum creatinine is depicted in table 5 and 6, The data obtained after 3 hours of blood collected sample, was observed that, there was a non-significant decrease of serum creatinine in positive control group (11.53%) and increase of serum creatinine in standard group (65.21%), test drug group (4.34%) increase was observed which was statistically non-significant. The data obtained after 24hours of blood collected sample it was observed that, there was a non-significant decrease of serum creatinine in positive control group (39.02%) while in the test drug group (36%) increase was observed which was statistically non-significant.

Urine parameters

Table 7: Effect of Shodhita Shilajatu on urine volume

Groups	Urine volume (ml)	% change
Normal control	9 ± 1.36	
Potassium Oxonate (PO) Control	5.25 ± 1.22	41.66↓@
Allopurinol (Standard)	9.43 ± 2.02	79.61↑#
Shodhita Shilajatu (Test drug)	7.4 ± 1.69	40.95↑#

Data: MEAN ± SEM @-Compared with normal control #-compared with PO control

Table 8: Effect of Shodhita Shilajatu on urine Ph

Groups	Urine pH	% change
Normal control	8.83 ± 0.16	
Potassium Oxonate (PO) Control	7.83 ± 0.16**	11.32↓@
Allopurinol (Standard)	8.16 ± 0.16	4.214↑#
Shodhita Shilajatu (Test drug)	8.83 ± 0.16**	12.77↑#

Data: MEAN ± SEM,**P<0.01 @-Compared with normal control #-compared with PO control

A careful analysis of the result related to urine volume, there was non-significant decrease of urine volume in positive control group (41.66%) and non-significant increase of urine volume in standard (79.61%) and trial drug group (40.95%) when compared with positive control group.

Urine analysis

Table 9: Effect of Shodhita Shilajatu on uric acid crystals /cumm in urine deposit

Groups	Uric acid crystals (cumm)	%change
Potassium Oxonate (PO) Control	4426.26 ± 2144.40	
Allopurinol (Standard)	3583.33 ± 118.79	19.04↓
Shodhita Shilajatu (Test drug)	6698 ± 267.22	51.32↑

The result obtained from urine analysis shows that the deposition of uric acid crystals is increased in test drug (51.32%) on the contrary is decreased in standard group (19.04%).

Ponderal changes
Body Weight

Table 10: Effect of Shodhita Shilajatu on Body weight

Group	Body Weight gain (g)	% change
Normal control	9.64 ± 0.24	---
PO control	5.08 ± 2.37	47.30@↓
Allopurinol (Standard)	15.06 ± 3.28	196.4↑#
Shodhita Shilajatu (T)	17.15 ± 4.76	237.5↑#

Data: MEAN ± SEM, **P<0.01 @-compared with normal control #-compared with PO control

The data shows that there was decrease in body weight in PO control group when compared to the normal control group, the observed decrease was found to be statistically non-significant. Increase in body weight in standard, trial group when compared to the PO control group, the observed increase was found to be statistically non -significant.

Table 11: Effect of Shodhita Shilajatu on kidney weight

Groups	Kidney weight (g)	% change
Normal control	1.46 ± 0.08	
PO Control	1.26 ± 0.04	13.69↓@
Allopurinol (Standard)	1.61 ± 0.15*	27.77↑#
Shodhita Shilajatu (T)	1.09 ± 0.01	13.49↓#

Data: MEAN ± SEM, *P<0.05@-Compared with normal control #-compared with PO control

The data shows that there was decrease in kidney weight in PO control group when compared to the normal control group, the observed decrease was found to be statistically non-significant. Increase in kidney weight in standard group, the observed increase was found to be statistically significant. There was decrease in kidney weight in trail group when compared to the PO control group, the observed decrease was found to be statistically non- significant.

Histopathology

Microscopic examination of the kidney sections from potassium oxonate control group revealed tubular dilation, cell infiltration, proteus changes and few crystals in the tubules. In Allupurinol administered group these changes were found to be much less-mild cell infiltration was observed. In Shodhita Shilajatu formulation administered group mild to moderate reversal of the above changes was observed. Representative photomicrographs from different groups can be found in Plate.

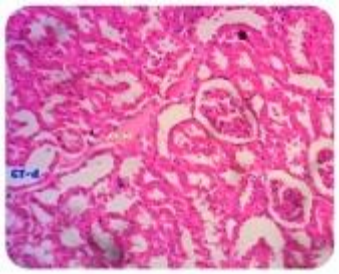

Plate: positive control

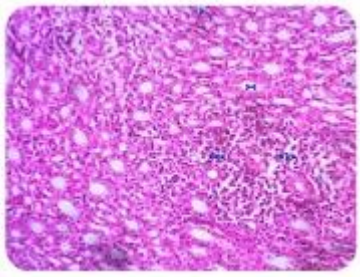

Plate: standard

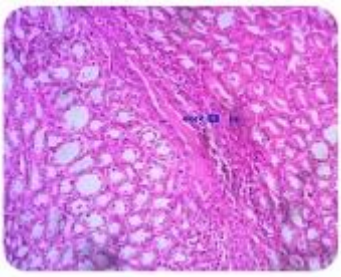

Plate: trial drug

DISCUSSION

Shilajatu a Maharasa quoted as incorporating the equivalent qualities of Rasa, Uparasa, Parada Ratna, Loha vargas (metals and minerals).[18]. Acharya Charaka even attributes that there exists no curable disease which cannot be alleviated by the use of Shilajatu[19].In the Phalasruti (indication) of Shilajatu, it is mentioned that it eradicates Prabala Vatarakta along with other diseases. Shilajatu is a known herbo-mineral compound, rich sources of organic acids and traces of minerals; which are therapeutically diverse molecules with potential therapeutic value. Certain chemical constituents, acids present in Shilajatu can act on gout.

Action of every drug depends on its Rasa (taste), Guna (property), Virya (potency),Vipaka (metabolized end product),and Prabava (influence). Shilajatu is a drug having ,Tikta (Bitter taste medicine), Kashaya rasa (astringent taste), Guru guna (property of heaviness), Sheeta virya (cold potency) and katuvipaka. Among these Tikta rasa and kashaya rasa predominant help to relieve raktadushti (toxin build up in blood) acts as raktaprasadana (blood circulation), amaharatva (removes toxic, morbid substance). Here in the present study increased Uric acid crystals were seen in Urine thereby decreasing level of Uric acid in serum. The astringent property of drug may be the cause for this action by detaching the uric acid crystals from the tissue and the same was eliminated through urine, which is considered as one of the malas (waste product) according to Ayurveda. The sheeta virya of the drug also play an important role in reducing the pitta there by reduces vitiated rakta. The action of sheeta virya of the drug is to increase the amount of urine and there by helps to expel the formed uric acid crystals from the body through urine. Guru guna is having the property of vataharatva hence attaining the action accordingly.

In this experiment as depicted in the above table1 & 2, there is significant increase in serum uric acid in positive control due to Administration of potassium oxonate, an inhibitor of uretoxidase. Further it has to be observed that, there is significant decrease in standard group due to standard drug Allopurinol and its active metabolite, oxypurinol, which inhibits the enzyme xanthine oxidase, blocking the conversion of the oxypurines hypoxanthine and xanthine to uric acid.

If we analyze the obtained data (table 1, 2) it can be observed that in the group administered with test drug there was significant decrease in the serum uric acid level at 24 hour with non-significant decrease being observed at 3rd hour. This is in contrast to reference standard in which significant decrease was observed at both 3rd and 24th hour. This indicates significant but late onset hypouricemic effect in this formulation. The exact mechanism behind this effect needs to be elucidated. It could be either due to attenuation of the activity of the enzymes involved in the formation of serum uric acid or its increased excretion or both the mechanism may be operative.

24hours urine sample were collected from all the groups and were observed for the presence of uric acid crystals in urine deposit (table 9). The result obtained from urine analysis shows that, uric acid crystals in urine deposit increased in test drug (51.32%) in contrast the percentage of uric acid crystals was not greater in the standard group (19.04%). This trial drug the formulation has significant excretion of uric acid. Thus the enzyme modulation and uricosuric mechanism may be operative in the observed decrease in serum uric acid level. Further, histological examination of the kidney sections from different groups showed moderate changes in Potassium oxonate administered control group which were significantly reduced in reference standard and moderately reversed in test drug formulation administered group.

Human kidney excretes nitrogenous wastes in the form of urea through urine. Decreased urea concentration is seen in liver dysfunction and increased concentration in hypo function of kidney. The data obtained after 3hours and 24hours of blood collected sample is depicted in table3&4, when the test group compare to normal control value blood urea there was no much difference was observed this signifies that may be test drug is regulating normal kidney function.

Serum creatinine is an important indicator of renal health. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidney become impaired for any reason, the creatinine level in the blood will raise due to poor clearance of creatinine by the kidneys. The data related to the above parameters (Blood urea and serum creatinine) indicate that though hyperuricemia conditions was induced by administration of potassium oxonate the effect is not sufficient to cause significant elevation in the above parameters. However, changes were observed in microscopic profile of the kidney, indicating the protocol employed is good for predicting effect of test drug on hyperuricemia condition.

A careful analysis of the result related to urine volume (table 7), pH (table 8) and uric acid crystals (table 9) indicate apparently good reversal of the changes induced by potassium oxonate. The volume was reduced to around 41 percent in potassium oxonate administered group in comparison to the normal control group. Allopurinol remarkably reversed this decrease (79.61%). The reversal was moderate in test drug formulation (40.95%). Decrease in pH indicates increased acid in the urine. This is likely due to increased excretion of uric acid in urine. Reversal of this pH decrease is indicative of decrease in uric acid

excretion. This effect was significant in the test drug administered group.

Body weight was increased non-significantly in standard and trial drug, body weight changes are considered as an important index of general health; in young rat's body weight gradually increase at a particular rate. Any increase or decrease sometimes depends upon the nutritional and general health condition. It is to be noted though consistent increase in body weight gain was observed in trial drug group indicating absence of any major tissue destruction. In fact the body weight gain, though not statistically significant, was higher in the test drug administered group indicating reversal of the decreased body weight gain observed in the PO control group.

Kidney weight was decreased non-significantly in trail drug group, Non observation of significant changes in the kidney weight the toxicant induced changes were sufficient induce hyperuricemia condition but not remarkable to cause significant ponderal changes.

CONCLUSION

Analysis of the result obtained in this study indicates that the experimental protocol employed is effective in inducing hyperuricemic condition. The test formulation was found to be effective in reversing the increased level of serum uric acid. And trial drug has significant excretion of uric acid. Thus the enzyme modulation and uricosuric mechanism may be operative in the observed decrease in serum uric acid level. Microscopic examination of the kidney sections from potassium oxonate control group revealed tubular dilation, cell infiltration, proteus changes and few crystals in the tubules. In Allupurinol administered group these changes were found to be much less-mild cell infiltration, whereas in Shodhita Shilajatu administered group mild to moderate reversal of the above changes was observed, which are significant when compared with standard drug.

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