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Research Article

NEPHROPROTECTIVE ACTIVITY OF *SHILAJATU & GOKSHURA KWATHA BHAVITHA SHILAJATU*: A COMPARATIVE EXPERIMENTAL STUDY

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ABSTRACT

Shilajatu (*Asphaltum punjabinum*) is a well-known drug mentioned in brihatrayees and laghutrayees. *Acharya Charaka* says that there is no *sadyarupa vyadhi* (curable disease) that cannot be treated with *shilajatu.* The therapeutic values of *shilajatu* can be increased by undergoing *bhavana* (impregnation) like *samskara w*ith suitable drugs. So, to analyse the variations caused by *samskara* process, *bhavana* was given with *gokshurakashaya* for *shilajatu* and its effect in nephrotoxicity was evaluated. **Objectives**: To evaluate the efficacy of *shilajatu* and *gokshura kwatha bhavitha shilajatu* in nephroprotective activity. **Methods**: Nephroprotective activity of *shilajatu* was checked on cisplatin induced renal damage in wistar albino mice. Animals were grouped into four categories and group specific drugs were administered for 10 consecutive days. On 8th day an hour after treatment with group specific drugs, Cisplatin20mg/kg was injected intra-peritoneally to all the groups except in normal control mice group. Nephrotoxicity was assessed by biochemical, antioxidant and histological changes in kidney after 72 hrs of cisplatin injection. **Results:** By the virtue of the *'mutrala'* and *'rasayana'* properties and *'tikta rasa'* the drug *'shilajatu'* (*Asphaltum punjabinum*) is known to act as nephron-protective.*'Gokshurabhavitashilajatu'* (*Asphaltum punjabinum* impregnated with decoction of *Tribulus terrestris*) is comparatively more effective in reversing the nephrotoxicity induced degenerative changes as 'samskara' enhanced the properties of the drug.

Key Words: *Samskara*; *bhavana,* impregnation, cisplatin

INTRODUCTION

*Samskara*is a process in which the nature of raw drug is converted according to the requirement of the formulation[1]. *Bhavana*is one among such *samskaras* defined in Rasasastra. During *bhavana* process the raw material will be impregnated with specific liquids for specific duration with suitable method of trituration.

Shilajitu, also known as *Asphaltum punjabinum*, is a pale brown to blackish exudation, of variable consistency, obtained from layers of rocks in mountain regions of the world, especially the Himalayan ranges. One or the other form of *shilajit*h has been used for thousands of years, under the indigenous system of medicine.

Bhavana with suitable liquid medium is advocated in classics to increase the potency of *Shilajatu and* induce new therapeutic values.[2]

Gokshura (*Tribulus terrestris*), a classically mentioned prominent drug, possesses *rasayana* properties along with specific indications in *mootravaha srotas* (urinary system) and *mootravaha-sroto*-vikaras[3] (diseases of urinary system). There are classical references that do speak on usage of *gokshura* along with *Shilajatu* in *mootravaha-sroto-vikara* for better therapeutic benefits. [4]

Cisplatin (Cis–diamminedichloroplatinum – 11) is the chemotherapeutic agent of choice in the treatment of several tumours, particularly testicular and ovarian. Unfortunately Cisplatin is also one of the most toxic anticancer drugs, causing nephrotoxicity[5].Hence a nephron-protective agent against this toxicant would be a therapeutic boon.

MATERIALS & METHODS

The two test drugs *shilajatu* (*Asphaltum punjabinum*) and *gokhusra kwatha bhavita shilajatu* (*Asphaltum punjabinum* impregnated with decoction of *Tribulus terrestris*) were processed in *Rasasastra & Bhaishajya kalpana* practical Hall. S.D.M .College of Ayurveda, Udupi and taken up for the study. The chemical cisplatin, the required reagents and other requirements of the experimental study were procured from standard and reputed firms. The agents which are used to induce nephrotoxicity like Cisplatin (1mg/1ml) were purchased from local market, manufactured by well-known establishment (Fresenius Kabi Oncology Ltd. Brand name – Cisplatin injection BP, KEMOPLAT).

Experimental animals i.e. wistar albino mice of either sex weighing between 30 – 40g were used for the study. Animals were procured from animal house attached to the department of pharmacology &toxicology S. D. M. Centre for research in Ayurveda and Allied Sciences, Udupi. Twenty four Albino mice were selected and allotted to four groups of six mice each. Six mice were housed in each cage made up of poly – propylene with stainless steel top grill. The dry husk was used as bedding material and was changed frequently to protect from infections. Animals were maintained at Standard laboratory conditions such as temperature at 25 to 27° C, humidity of 50-55 % and 12 hour light and dark cycles. Animals were fed with standard laboratory

pellet feed supplied by 'Saidurga feeds', Bengaluru and water *adlibitum.*

The Institutional Animal Ethical Committee has approved for experimentation on animals with reference no SDMCRA/IAEC/SDM/RS- 06.

The acute oral toxicity study was carried out as per OECD guidelines 425 using AOT software. *Shilajatu* and *gokshura kwatha bhavitha shilajatu* were made into solution and dosed in the following order 175, 550 and 2000mg/kg body weight. After the dosing the animals were observed for 14 days for mortality. The LD 50 was determined using AOT software.

By AOT study, $LD_{50}$of *shoditha shilajatu* (purified *Asphaltum punjabinum*) and *gokshura kwatha bhavitha shilajatu* (*Asphaltum punjabinum* impregnated with decoction of *Tribulus terrestris*) was calculated as 2000 mg/kg. $1/5^{th}$ of LD_{50} is 400mg/kg.
Dose fixation was done by standard dose conversion formula, which is equal to-Human dose × 0.018 × 5/Kg body weight. Here the dose of *shilajatu is* 5 *gunja* (1 *Gunja* = 125mg). Therefore 625 × 0.018 × 5/Kg body weight= 56.25mg/Kg body weight= 11.25 mg/ 200 gm. body weight

The control, standard drug and test drug were administered according to the body weight of the animals by oral route. Inclusion criteria was selection of adult albino mice having weight in between 30-40 g; and randomly selected from both sexes. Exclusion criteria was rejection of mice, which were used for other studies previously.

As a part of Methodology the selected animals were grouped into four different groups randomly, irrespective of sex and each group comprised of six animals.

Table 1: Grouping of experimental animals i.e. swiss albino mice

Group1	Normal control group
Group 2	Negative control (Cisplatin 20 mg/kg)
Group 3	Cisplatin 20 mg/kg + *Shoditha shilajatu*
Group 4	Cisplatin 20 mg/Kg + *Gokshura kwatha bhavitha shilajatu*

Shoditha shilajatu i.e. trial drug 1 was administered orally by taking 400mgs of the above said drug along with 50 CMC and 10 ml distilled water. They were mixed well and administered orally. In the similar way *gokshura kwatha bhavitha shilajatu* was also administered orally.

The procedure adopted for assessment criteria was by grouping the mice into four different groups. Group specific drugs were administered for 10 consecutive days. On 8^{th}day an hour after drug administration a single dose of Cisplatin20mg/ Kg body weight was injected intra-peritoneally to all the groups except normal control albino mice. After 48 hours i.e. on 10^{th} day an hour after test drug administration, the animals were sacrificed and blood was collected from retro – orbital puncture and kidney tissue was collected 10% formalin used for histopathological examination.

Biochemical parameters were Serum analysis, Lipid peroxidation and Histopathology of Kidney.

The findings were statistically analysed and expressed as mean ± SEM and the data will be analysed by one way ANOVA followed by Dunnet's multiple 't' test as a post HOC test. Graphic Pad version 3 was used for this purpose.

RESULTS

The effects of 'test drugs' i.e. *shoditha shilajatu* and *gokshura kwatha bhavitha shilajatu* on 'serum urea is documented in the table below;

Table 2: Effect of test drugs on serum urea

GROUPS	UREA(mg/dl)	% CHANGE
Normal Control	35.75 ± 3.55	
Cisplatin Control	187.64± 24.75 **	424.86↑ @
Cisplatin+ *Shoditha shilajatu*	104.2 ± 20.56	44.46 ↓#
Cisplatin + *Gokshura kwatha bhavitha shilajatu*	81.8 ± 4.46*	56.4↓#
@ Compared with normal group; # Compared with Cisplatin control; DATA: MEAN ± SEM, **P < 0.01, *P < 0.05		

The data shows that there was increase in serum urea level in cisplatin control group when compared to the normal control group the observed increase was found to be extremely significant. It reveals that there was decrease in serum urea level in T1groups when compared to cisplatin control group, the observed increase was found to be statistically non-significant. It also reveals that there was decrease in serum urea level in T2 groups when compared to cisplatin control group, the observed increase was found to be statistically significant.

The effects of 'test drugs' i.e. *shoditha shilajatu* and *gokshura kwatha bhavitha shilajatu* on 'serum creatinine' is documented in the table below.

Table 3: Effect of test drugs on serum creatinine

GROUPS	CREATININE (mg/dl)	% CHANGE
Normal Control	0.62 ±0.07	
Cisplatin Control	1.93 ± 0.35**	211.29 ↑ @
Cisplatin + Shoditha shilajatu	1.12 ± 0.04	41.96 ↓ #
Cisplatin + *Gokshura kwatha bhavitha shilajatu*	0.84 ± 0.09*	56.47 ↓ #
DATA: MEAN ± SEM, **P < 0.01,* P < 0.05		

The data shows there was increase in serum creatinine level in cisplatin group when compared to the normal control group, the observed decrease was found to be statistically very significant. It reveals that there was decrease in serum creatinine level in T1 group when compared to cisplatin group, the observed decrease was found to be statistically non-significant. It also reveals that there was decrease in serum creatinine level in T2 group when compared to the cisplatin group, the observed decrease was found to be statistically significant.

The effects of 'test drugs' i.e. *shoditha shilajatu* and *gokshura kwatha bhavitha shilajatu* on 'uric acid' is documented in the table below.

Table 4: Effect of test drugs on serum uric acid

GROUPS	URIC ACID (mg/dl)	% CHANGE
Normal Control	1.28 ± 0.12	
Cisplatin Control	2.97 ± 0.27**	132.03↑ @
Cisplatin + *Shoditha shilajatu*	1.44 ± 0.06**	51.51 ↓ #
Cisplatin + *Gokshura kwatha bhavitha shilajatu*	1.74 ± 0.15**	41.41 ↓ #

The data shows there was increase in serum uric acid level in cisplatin control group when compared to the normal control group, the observed increase was found to be statistically very significant. It reveals that there was decrease in serum uric acid level in T1 group when compared to the cisplatin group, the observed decrease was found to be statistically very significant. It also reveals that there was decrease in serum uric acid level in T2 group when compared to the cisplatin group, the observed decrease was found to be statistically very significant.

The effects of 'test drugs' i.e. *shoditha shilajatu and gokshura kwatha bhavitha shilajatu* on 'lipid peroxide' is documented in the table below.

Table 5: Effect of test drugs on lipid peroxide

GROUPS	LIPID PEROXIDE (mg/dl)	% CHANGE
Normal Control	0.41 ± 0.03	
Cisplatin Control	0.48 ± 0.04	17.07 ↑ @
Cisplatin + *Shoditha shilajatu*	0.40 ± 0.06	16.6 ↓ #
Cisplatin + *Gokshura kwatha bhavitha shilajatu*	0.56 ± 0.01	16.6 ↑ #

The data shows that there was increase in lipid peroxide in cisplatin control when compared to the normal control group the observed increase was found to be statistically non-significant. It reveals that there was decrease in lipid peroxide value in T1 group when compared to the cisplatin control group the observed decrease was found to be statistically non-significant. It also reveals that there was increase in lipid peroxide value in T2 group when compared to the cisplatin control group the observed increase was found to be statistically non- significant.

The effects of 'test drugs' i.e. *shoditha shilajatu and gokshura kwatha bhavitha shilajatu on* change in 'weight of kidney' is documented in the table below.

Table 6: Effect of test drugs on weight of kidney

Groups	Weight of kidney(g)	% Change
Normal control	0.42 ± 0.06	
Cisplatin Control	0.41 ± 0.04	2.38 ↓@
Cisplatin + ShodhithaShilajatu	0.45 ± 0.01	9.7 ↑ #
Cisplatin + *Gokshura kwatha bhavitha shilajatu*	0.42 ± 0.01	2.4 ↑#

The data shows there was increase in kidney weight in Cisplatin control group when compared to the normal control group, the observed decrease was found to be statistically non-significant. The data also shows there was increase in kidney weight in T1 and T2 group when compared to the cisplatin group, the observed increase was found to be statistically non-significant.

The effects of 'test drugs' i.e. *shoditha shilajatu and gokshura kwatha bhavitha shilajatu on* change in 'body weight' is documented in the table below (Table- 7).

Table- 7: Effect of test drugs on body weight

GROUPS	WEIGHT (g)
Normal Control	3.23 ± 0.37
Cisplatin Control	-15.1 ± 0.50**
Cisplatin + *Shoditha shilajatu*	8.33 ± 1.42 **
Cisplatin+ *Gokshura kwatha bhavitha shilajatu*	9.40 ± 1.36 **

The data shows there was decrease in body weight in cisplatin control group when compared to normal control group. The data also shows there was increase in body weight of T1&T2 group when compared to cisplatin control group.

DISCUSSION

*Shilajatu*is (*Asphaltum punjabinum*) a humus rich blackish – brown substance exudation from layers of rocks in many mountain regions of the world. It possesses properties like '*mutrala', 'rasayana' and 'yogavahitwa'*. By virtue of its *'yogavahitwa'* (ability of a drug to take in the properties of other drugs that come in contact), *shilajatu* effectively retains the therapeutic properties of the *bhavana dravya* with which it is processed, that too without leaving its own inherent properties. There by undergoing *samskara* with *gokshura kwatha* (decoction of *Tribulus terrestris*) its efficacy on *mootravaha srotas* is enhanced. As per classical references, by the virtue of the *'mutrala'* (diuretic) and *'rasayana'* (rejuvenator) properties and *'tikta rasa'* (bitter taste that mitigates vitiated pitta dosha), the drug *'shilajatu'* is known to act as nephro-protective.

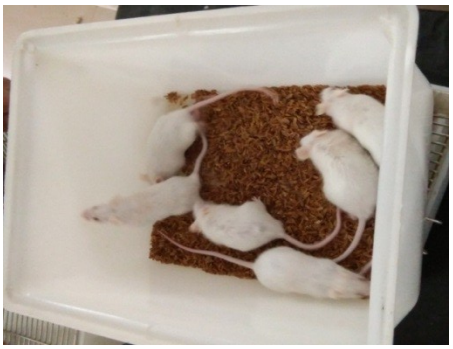
In the present study cisplatin administration caused significant elevation in the 'serum urea', 'uric acid' and 'creatinine' levels as compared to normal control. This indicates the cisplatin administered at 20 mg/kg intra peritoneal injection, readily caused renal toxicity. These cisplatin induced nephrotoxicity was significantly reversed by Test drug 2 (*gokshura kwatha bhavitha shilajatu*). Thus we can confirm the Test drug 2 has nephron-protective activity based on biochemical results. For the Test drug 1 (*shoditha shilajatu*) the parameters studied were found to be non-significant and significant only in some parameters. Non-significant decrease in lipid peroxidation indicates weak to moderate anti-oxidant activity.

Histopathological examination revealed moderate to significant degenerative changes after administration of cisplatin. These changes were found to be moderately reversed in group- 2 '*shoditha shilajatu'*. Whereas, much better protection was observed with '*gokshura kwatha bhavitha shilajatu'* (*Asphaltum punjabinum* impregnated with decoction of *Tribulus terrestris*)- indicating the importance of '*bhavana'* process for enhancing therapeutic properties.

CONCLUSION

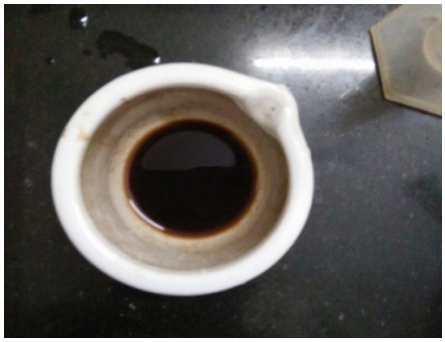
Based on the results it can be concluded that *'shodhita shilajatu'* (purified *Asphaltum punjabinum*) by the virtue of its *'mutrala'* and *'rasayana'* properties and *'tikta rasa'* acts as nephron-protective agent.*'Gokshurabhavitashilajatu'* (*Asphaltum punjabinum* impregnated with decoction of *Tribulus terrestris*) is comparatively more effective in reversing the nephrotoxicity induced degenerative changes as the *'samskara'* enhances the properties of the drug. However, its use has to be revalidated after confirming that the test formulations (both 1 and 2) do not interfere with the therapeutic activity of cisplatin.

PICTURES OF EXPERIMENTAL STUDY



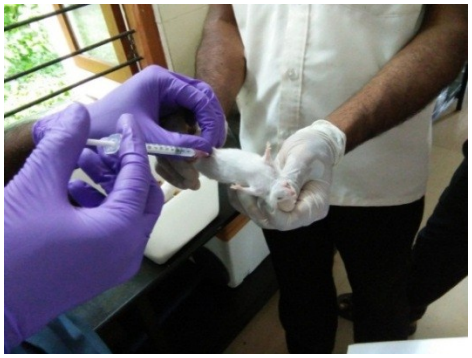
1. Grouping of rats

2. Food and water for rats

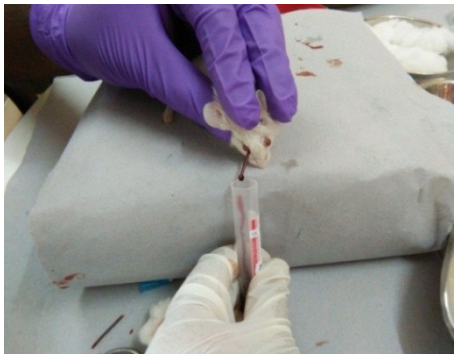


3. Stalk solution

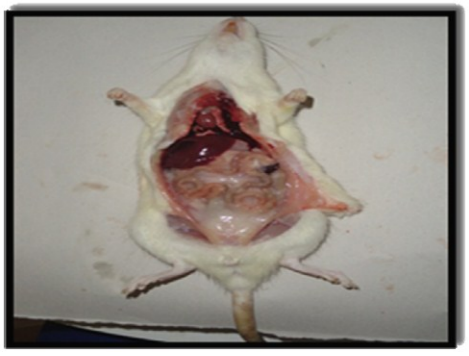
4. Administration of medicine



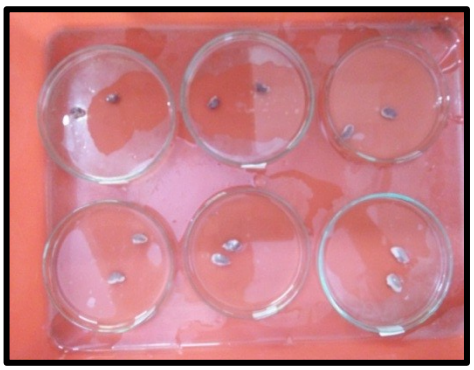
5. Intra-peritoneal injection of Cisplatin



6. Collection of blood

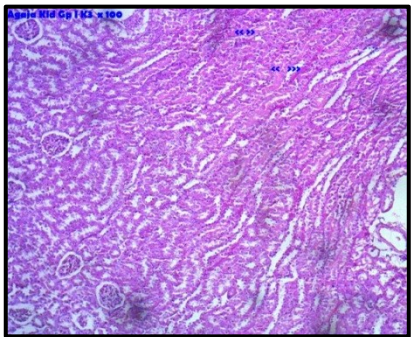
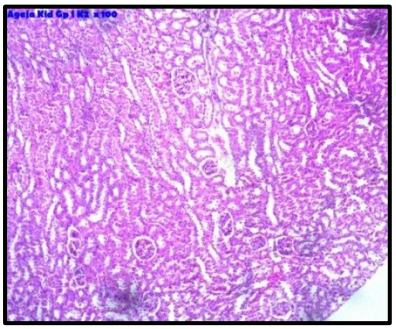


7. Dissection of rat

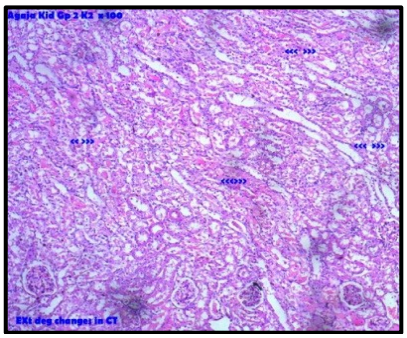
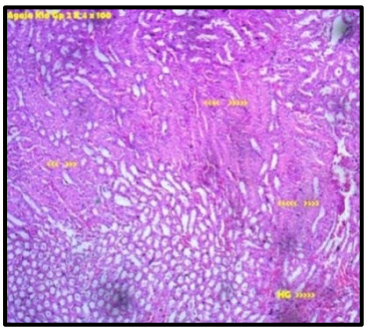


8. Dissected organs

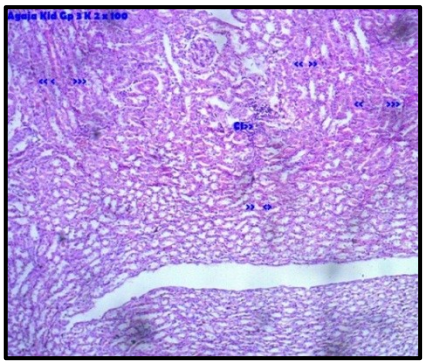
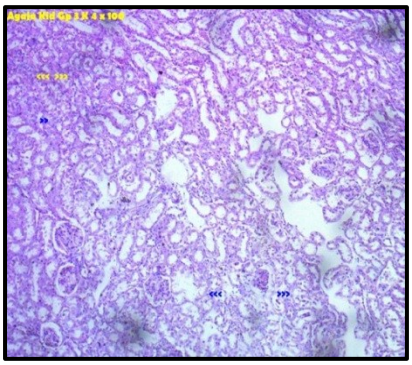
HISTOPATHOLOGY PICTURES OF KIDNEY

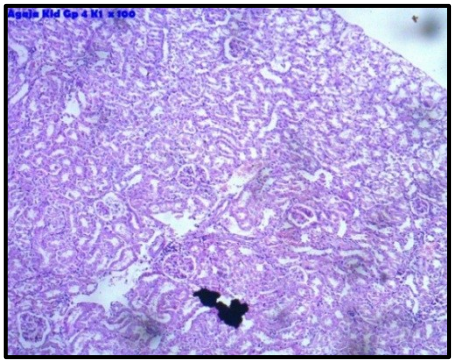
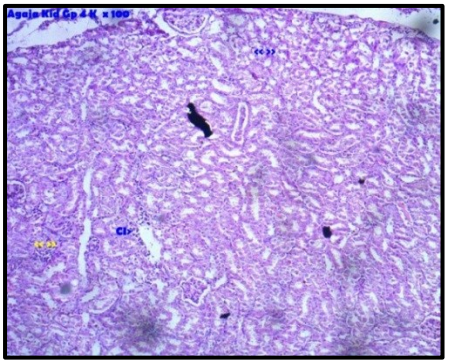
Group 1. Control group

Group 2-Cisplatin control

Group 3- Shodhitha shilajatu

Group 4- Gokshura bhavitha shilajatu

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CONTRIBUTORS: Dr. Agaja Peethambaran Leena and Dr. Ravindra Angadi contributed to study design, literature study, data acquisition and study conduction. Dr. Ravindra Angadi contributed to the conceptualization of the topic and manuscript editing.

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