Medicine and Medical Sciences 12(1): 012-021, February 2024
DOI: 10.15413/mms.2024.0101
ISSN 2682-5759

Academia Publishing

Research Paper

Experimental evaluation of Vanga Sindoora for its nephroprotective activity

Accepted 17th January, 2024

Drjayalaxmim Laxmi

Department of Rasa shastra and Bhaishajya Kalpana, Sri Siddarameshwara Ayurvedic Medical College, Bidar, Karnataka, India

* Corresponding authors email: drjayalaxmim@gmail.com

ABSTRACT

The most potent and fast-acting *Rasaaushadhis* (metal-mineral preparations) have been repeatedly criticized for their heavy metal toxicity. To rule out this myth in scientific era *Vanga sindoora sagandha bahirdhuma kupipakwa rasayana* (rejuvenating traditional preparation using a glass bottle and purified forms of tin, sulphur and others as ingredients) which possesses qualities like *Deepana* (appetizer), *Balya (*strenhening), *Dhatusthairykara (*tissue rebuilding*), Sarva prameahahara*(anti diabetic), was selected and prepared as per the classics and assessed for nephroprotective efficacy in gentamicin induced nephrotoxicity protocol in Wister strained albino rats. The assessment was done by analyzing blood parameters S.Urea, S.creatinine, S.electrolytes and histopathological study of kidneys. The data was analysed by one way Annova test. The results of the test group showed a significant improvement in renal function in both hematological and histological reports compared to the standard group. *Vanga sindoora* dose of 2.25 mg/0.2kg (Human Dose 125 mg) was more effective than the *Vanga sindoora* group dose 4.5 mg/0.2kg (Human Dose 250 mg) and had the best Nephro Protective effect.

Key words: Albino rat, Gentamicin, Nephrotoxicity, *Vangasindoora.*

INTRODUCTION

Rasashastra (Ancient Indian Metallurgy) aims to use available potent metallic-mineral ingredients for the cure of several acute and chronic illness. *Rasaushadhi* is considered organically toxic, but instead has an organically protective effect when prepared according to classical guidelines. *Vanga* (tin) is one of such metal used in many disorders including urinary system disorder. *Vangasindoora1*, a rejuvenating medication, is mentioned in the Rasendra Sambhava (classical text) and contains ingredients such as purified forms of tin[2], mercury[3], sulphur[4] and ammonium chloride[5], which are indicated in the treatment of diabetes and the strengthening of essential body tissue elements. Research has demonstrated a relationship between nephrotoxicity and gentamicin used against gram negative bacteria. *Vangasindoora*, on the other hand, is advocated for its quality as Dhatu Sthairyakara. Therefore, the present study was designed to evaluate the effect of *Vanga sindoora* on gentamicin induced nephrotoxicity in Wistar strained albino rats[6].

MATERIALS AND METHODS

Vanga sindoora was prepared according to the classics of the teaching pharmacy. Gentamicin injection of a single batch number was purchased from local market (Abott co.). Institutional Ethics Committee approval was granted with Reg. 112/1999/CPCSEA obtained for experimental studies.

Animals used: Wister strained albino rats male.

Chemicals: 10% Formalin, Ether.

Procedure

Healthy adult Wister strained albino rats of 90-120 days old, weighing from 150-250g were taken for the experimental study. The animals were chosen using both inclusive and exclusive criteria from the Central Animal

House in Bangalore. The animals were maintained under strict laboratory condition with controlled temperature, humidity, light and dark cycles. After being observed for their activities 7 days prior to actual experimental study, they were also fed a balanced pellet diet and water as prescribed by CFTRI, Mysore (Central Food Technological Research Institute). A maximum of three animals were housed per cage. Animals under different groups of experimental species were caged separately.

Inclusive criteria

Adult healthy male albino rats weighing 150-250g, between 90-120 days.

Exclusive criteria

Albino rats unhealthy, less than 150/more than 250g, age below 90 days and above 120 days and rats underwent other experimental trials were excluded.

Dose

Human dose of Test Drug =125mg per day.
Conversion factor for rat of average body weight of 200 g =0.018.
Therapeutic dose in rat per 200 g body weight was calculated as 125*0.018=2.25mg.
High dose in a rat per 200g body weight was calculated as 112.5/5=4.5mg.

Experimental protocol

The study was designed to last 10 days because the paper that was referred to for it had a 10-day duration. Twenty four healthy Wister strained albino rats (100-250g) were selected for this experiment. These rats were divided into four groups comprising six animals in each group. Each group was marked with different colored stain from one to six. The cage of each group was labeled with the group name and weight in grams. All rats were weighed and the results recorded accordingly.

Group (1) – Normal Control Group - 6 rats.
Group(2)–Inducercontrol - 6rats (Gentamicin).
Group (3) -Test 1 *Vangasindoora* TED - honey with deionized water.
Group (4) Test 2 *Vangasindoora TED10*- honey with deionized water.

On zero day (first day morning) 0.5 ml blood Sample was collected by retro orbital puncture from rats of all groups for estimation of S.urea, S.creatinine and electrolytes. No medicine was given in animals of all groups on zero days. The required test compound was weighted on the mettler balance as per standard procedures[7] on a butter paper. The weighed test substance was poured into a centrifuge tube that held 10–15 ml, and the mixture was vortexed. The calculated dose was used to determine the appropriate volume to be supplied.

Dilution of honey with deionized water

Honey (1.20 ml) was mixed with 1.8 ml of water to prepare a total of 3 mL of trial drug solution.

Control group: Animals honey was diluted with deionized water in a ratio of 2:3 and 0.5 ml was injected into each rat.

Test 1 group: The 3 ml of diluted honey was mixed with 13.5mg of *Vangasindoora* and divided into 6 parts of 0.5 ml, that is (2.25 mg/0.5 ml).

Test 2 groups: The 3 ml of diluted honey was mixed with 27 mg of *Vangasindoora* and divided into 6 parts of 0.5 ml, that is, 4.5 mg /0.5 ml.

Group 1: From 2nd day, animals were given Vehicle (normal water) in the dose of 30 ml/kg twice a day for 10 days.

Group 2: Animals were treated with Gentamicin only in dose of 80mg/kg once in a day for 10 days. Gentamicin was administered by intraperitoneal injection once daily.

Group 3: Animals were given TED- 2.25 mg, that is, 0.5 ml of prepared solution taken in the tuberculin syringe (1ml) with 18 gauge bent balled needle along with gentamicin in dose of 80mg /kg intra peritoneal once a day for 10 days.

Group 4: Animals were given TED 10 4.5mg, that is, 0.5 ml of prepared solution taken in the tuberculin syringe (1ml) with 18 gauge bent balled needle, along with gentamicin in dose of 80mg /kg once a day for 10 days.

On the 10th day 12 h after the vehicle drug administration, all the animals were sacrificed by overdosing of anesthetic ether and blood was collected by cardiac puncture. Serum was separated from the blood and the level of S.urea, S.creatinine and electrolytes were estimated by a diagnostic kit. Elevation of S.Urea and S.creatinine level electrolyte disturbance was taken as the index of nephro toxicity. Then ventral midline incision was administered to the rats abdomen, the kidneys were identified and released from the surrounding tissue. Right and left kidneys were collected from animals of all four groups, preserved in 10% formalin and examined histologically at the Hubli Scan Centre, Hubli, Karnataka.

Table 1: Observation on weight.

Group	Weight on 1st day	Weight gain at the end of 11th day
Control	210 g	30 - 40g
Standard	200g	30-50g
T1	190g	10 -20g
T2	190g	30 -40g

Table 2: Comparison of four groups (Control group, Standard group, Test group 1 and Test group 2 with respect to Urea (mm/dl) levels at baseline and 10th day by one way ANOVA.

Groups	Difference from baseline to 10th day	
	Mean	SD
Control group	19.65	8.62
Standard group	68.85	27.12
Test group 1	49.12	26.51
Test group 2	58.24	27.73
F-value	4.7021	
P-value	**0.0121***	

Observations

All rats had *ad libitum* access to food and water, and their body weight was measured and recorded daily and dosed accordingly (Table 1).

Rats were initially irritated by injections and oral medications, but gradually became less irritable during the course of experimental study. Test 1 and Test 2 groups showed more bed wetting as compared with the control and standard groups. On 3rd and 4th day, both test groups showed slight loose motion during handling. They were carefully observed for the whole day and no further loose motions were observed. The control group showed no changes in their behavior, while the standard group became dull at the end of the experiment as compared with the test groups.

Precautions

The rat cage was kept clean and dry, and the bed was replaced when necessary. Animal handled with proper care. To prevent contamination from rats to humans and vice versa, gloves were used. All animals were weighed and recorded daily before administration of medicines and dose given accordingly. While administering the test drug with honey, it was diluted with deionized water in accordance with the CCRAS guidelines for toxicity/safety profile evaluation of bhasma/rasa kalpas. This facilitated the ease of administration and maintenance of drug concentration. Care was taken to avoid intra abdominal injury during gentamicin injection and as no drug remained in the needle by giving 0.5 ml of distilled water orally, after each oral dosage. Each time needle pricked and pulled back little to confirm no fluid/blood seen in syringe which confirms proper injecting method. Proper dosage was maintained using insulin syringe for injecting standard drug.

Data analysis

The data was expressed as Mean ± SD. Statistical comparison was performed by Annova one way test followed by Turkey's post test and paired T test and p value <0.05 was considered as significant (Tables 2 to 11).

RESULTS

Blood was collected on days 0 and 10, serum was separated, and serum urea, S.creatinine, and S.electrolyte content were evaluated. On the 10th day, the rats were sacrificed and kidneys were removed for histopathological studies. The results showed that concomitant administration of *Vangasindoora* significantly reduced gentamicin-induced elevated levels of serum creatinine, S.urea and electrolytes in albino rats. Histopathological examination of gentamicin –treated rats revealed degenerative changes in glomeruli and tubules. On the other hand, simultaneous administration of *Vangasindoora* along with gentamicin protected the kidney tissues against nephrotoxic effects of gentamicin as evidenced from amelioration of histopathlogical changes and significant kidney biochemical parameters (Table 12, and Figures 1 to 5).

Table 3: Comparison of baseline and 10th day with respect to Urea (mm/dl) levels in four groups (Control group, Standard group, Test group 1 and Test group 2) by paired t test.

Groups	Time point	Mean	Std.Dv.	Paired t	p-value
Control group	Baseline	52.16	2.36	-5.5831	0.0025*
	10th day	71.81	9.52		
Standard group	Baseline	55.47	3.87	-6.2183	0.0016*
	10th day	124.32	24.74		
Test group 1	Baseline	51.43	10.82	-4.5392	0.0062*
	10th day	100.55	18.09		
Test group 2	Baseline	50.72	13.40	-5.1454	0.0036*
	10th day	108.96	21.49		

Table 4: Comparison of four groups (Control group, Standard group, Test group 1 and Test group 2) with respect to Creatinine (mm/dl) levels at baseline and 10th day by one way ANOVA.

Groups	Difference from baseline to 10th day	
	Mean	SD
Control group	-0.06	0.48
Standard group	0.18	0.31
Test group 1	0.08	0.45
Test group 2	0.24	0.58
F-value	0.4454	
P-value	0.7232	

Table 5: Comparison of baseline and 10th day with respect to Creatinine (mm/dl) levels in four groups (Control group, Standard group, Test group 1 and Test group 2) by paired t test.

Groups	Time point	Mean	Std.Dv.	Paired t	p-value
Control group	Baseline	2.86	0.34	0.2889	0.7843
	10th day	2.81	0.55		
Standard group	Baseline	2.69	0.33	-1.3711	0.2287
	10th day	2.86	0.21		
Test group 1	Baseline	2.55	0.48	-0.4232	0.6897
	10th day	2.63	0.07		
Test group 2	Baseline	2.44	0.09	-0.9888	0.3681
	10th day	2.68	0.52		

Histopathological changes

The results of the histopathological changes are shown in Table 6.

DISCUSSION

In the evaluation of all four groups based on Renal blood parameters and histopathological findings, gentamicin was shown to cause nephrotoxicity in the Standard, Test1, and Test2 groups. The animals in the control group, that were fed and given water along with vehicle control honey, did not exhibit any significant changes in their histopathological or blood parameters. Animals in the standard group received only 80 mg/kg of gentamicin injection once daily and showed no signs of kidney failure

Table 6: Comparison of four groups (Control group, Standard group, Test group 1 and Test group 2 with respect to Sodium (mEq/l) levels at baseline and 10th day by one way ANOVA.

Groups	Difference from baseline to 10th day	
	Mean	SD
Control group	5.83	1.72
Standard group	-7.83	1.17
Test group 1	7.50	0.84
Test group 2	9.00	2.28
F-value	140.7763	
P-value	**0.00001***	

*p<0.05.

Table 7: Comparison of baseline and 10th day with respect to Sodium (mEq/l) levels in four groups (Control group, Standard group, Test group 1 and Test group 2) by paired t test.

Groups	Time point	Mean	Std.Dv.	Paired t	p-value
Control group	Baseline	134.00	4.56	8.2958	**0.0004***
	10th day	128.17	3.66		
Standard group	Baseline	128.33	1.51	-16.4131	**0.00001***
	10th day	136.17	1.17		
Test group 1	Baseline	135.67	1.03	21.9578	**0.00001***
	10th day	128.17	1.47		
Test group 2	Baseline	137.83	3.31	9.6676	**0.0002***
	10th day	128.83	1.47		

*p<0.05.

Table 8: Comparison of four groups (Control group, Standard group, Test group 1 and Test group 2) with respect to Potassium (mEq/l) levels at baseline and 10th day by one way ANOVA.

Groups	Difference from baseline to 10th day	
	Mean	SD
Control group	0.37	0.17
Standard group	0.40	0.07
Test group 1	0.34	0.17
Test group 2	0.28	0.55
F-value	0.1798	
P-value	0.9089	

and/or renal failure. Test1 group animals received both gentamicin 80 mg/kg and Trial drug *Vangasindoora* in TED 2.25 mg/0.2kg showed significant improvement in renal impairment as compared with Standard Group. The animals in the Test 2 group that received both gentamicin (80 mg/kg) and the trial drug *Vangasindoora* in TED 10 (4.5 mg/0.2 kg) showed more improvement than the animals in the Test 1 group, and had less renal impairment than the standard group. Therefore, it can be concluded that the dose of 2.25 mg/0.2 kg (human dose 125 mg) in test group 1 is more effective and has the best nephro-protective effect than the Test 2 group with dose of 4.5 mg/0.2kg (Human Dose 250 mg). Test 1 and Test 2 groups were found to be statistically significant with a P value of 0.0121-0.2698 in response to Urea, Na, K, Cl and clinical assessment. Although the P value was not statistically significant, it was

Table 9: Comparison of baseline and 10th day with respect to Potassium (mEq/l) levels in four groups (Control group, Standard group, Test group 1 and Test group 2) by paired t test.

Groups	Time point	Mean	Std.Dv.	Paired t	p-value
Control group	Baseline	5.01	0.20	-5.3002	**0.0032***
	10th day	5.38	0.16		
Standard group	Baseline	5.18	0.28	-13.8310	**0.00001***
	10th day	5.58	0.22		
Test group 1	Baseline	4.69	0.17	-5.0460	**0.0039***
	10th day	5.03	0.03		
Test group 2	Baseline	5.16	0.58	-1.2405	**0.2698**
	10th day	5.43	0.09		

*p<0.05.

Table 10: Comparison of four groups (Control group, Standard group, Test group 1 and Test group 2) with respect to Chloride (mEq/l) levels at baseline and 10th day by one way ANOVA.

Groups	Difference from baseline to 10th day	
	Mean	SD
Control group	1.00	1.10
Standard group	1.17	1.60
Test group 1	0.83	2.14
Test group 2	1.50	1.87
F-value	0.1643	
P-value	0.9191	

Table 11: Comparison of baseline and 10th day with respect to Chloride (mEq/l) levels in four groups (Control group, Standard group, Test group 1 and Test group 2) by paired t test.

Groups	Time point	Mean	Std.Dv.	Paired t	p-value
Control group	Baseline	102.33	0.82	2.2361	0.0756
	10th day	101.33	0.82		
Standard group	Baseline	102.83	2.32	1.7838	0.1345
	10th day	101.67	1.75		
Test group 1	Baseline	101.33	0.82	0.9552	0.3833
	10th day	100.50	1.52		
Test group 2	Baseline	103.67	1.86	1.9640	0.1067
	10th day	102.17	0.75		

found to be significant in the preclinical assessment of S.creatinine. This may be attributed the lower number of creatinine values, which are only single digits with noticeable differences.

Other experimental studies related to gentamicin induced nephrotoxicity also report that Gentamicin definitely brings significant pathological changes in renal functional parameters as well as histopathological changes.[8]

Vanga: It is indicated in Mutravahaya srotovikaras as well. All the qualities, that is, Mehaghna and Mutravaha srotovikarahara, Dhatusthairyakara property of shodhita

Table 12: Averages of each group of S.Urea, S.Creatinine , Na, K, Cl value.

Group	S.Urea 0Day	S.Urea 10 Day	S.Cre 0day	S.Cre 10Day	Na 0Day	Na 10day	K 0day	K 10day	Cl 0day	Cl 10day
C	52.16	71.81	2.86	2.80	134	128.16	5.00	5.37	102.33	101.33
S	55.47	124.32	2.68	2.86	128.33	136.16	5.18	5.57	102.83	101.66
T1	51.43	100.55	2.54	2.62	135.66	128.16	4.69	5.03	101.33	100.5
T2	50.71	108.95	2.44	2.67	137.83	128.83	5.15	5.43	103.66	102.16

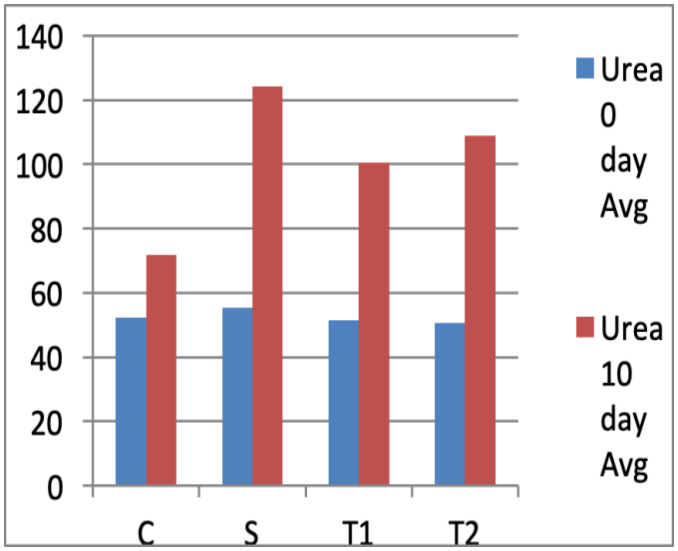


Figure 1: Comparative Average S.Urea (mg/dL).

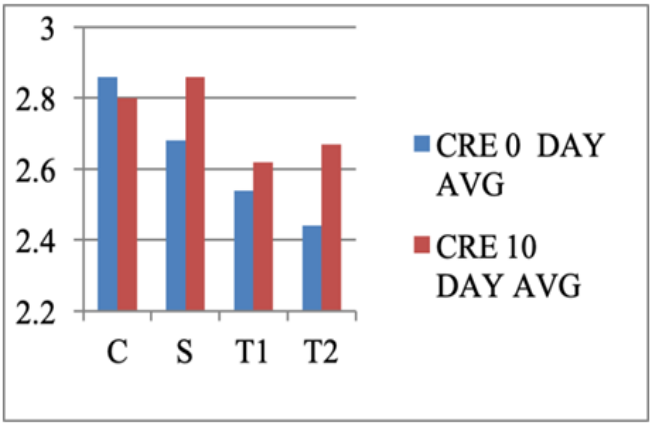


Figure 2: Comparative Average S.Creatinine(mg/dL).

Vanga may be helpful in the present Nephro Protective activity.

Parada: It possesses attributes like Dhatusthairyakara, Yogavahi and Rasayana, Tridoshaghna, Balya, Srotovahi, and Shadrasayukta. These attributes, when combined with other Dravya features, may aid in accomplishing the present study's therapeutic goal of nephroprotective activity.

Gandhaka: Shodhita Gandhaka functions as a Rasayana when paired with Parada and other Dravyas. Its other therapeutic qualities may also aid in the achievement of the study's therapeutic objective, which is the nephroprotective

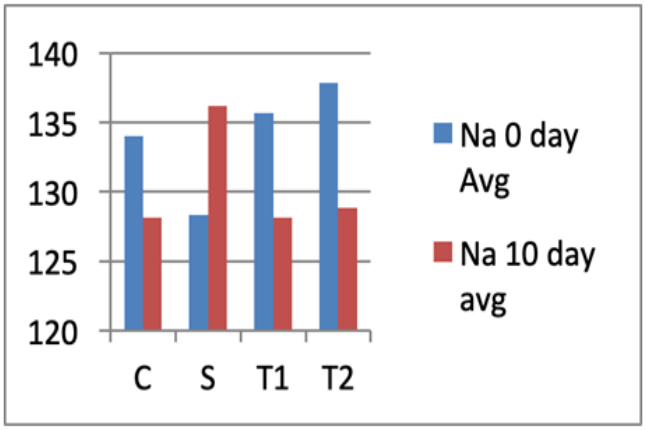


Figure 3: Comparative average S. Sodium(mEq/L).

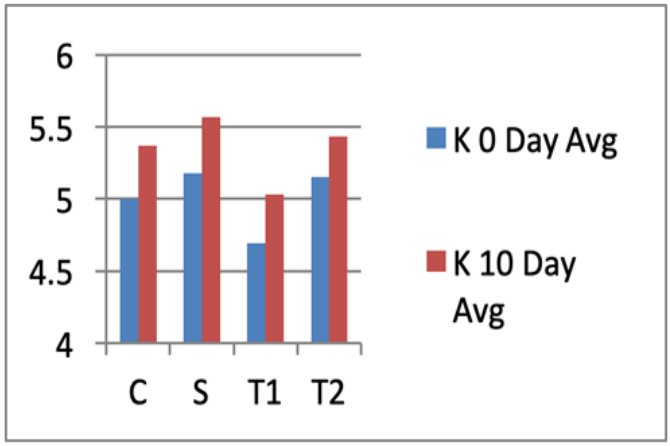


Figure 4: Comparative Average S. Potassium(mEq/L).

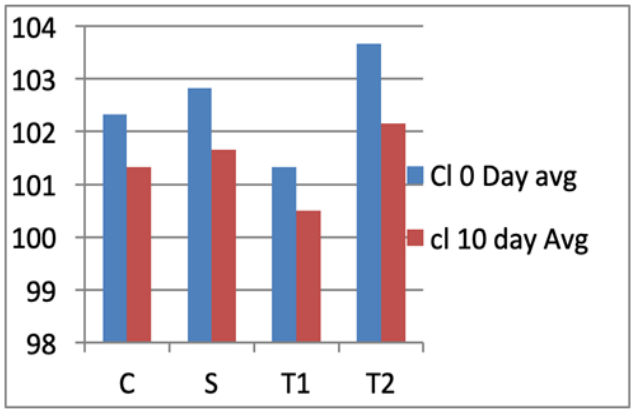
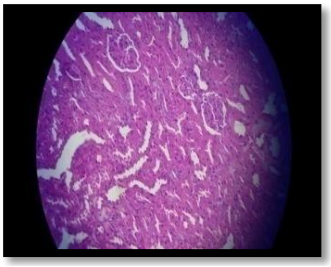


Figure 5: Comparative Average S. Chloride(mEq/L).

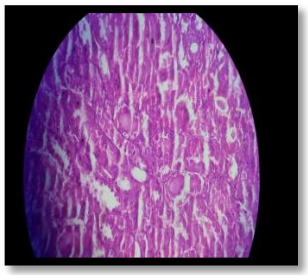
activity.

Navasadara: It has qualities such as Pachaka, Saraka, Teekshna Jatharagnideepana, Loha Dravaka and gives color to Sindoora Kalpana. It may act as Yogavahi in the preparation of Vanga Sindoora.

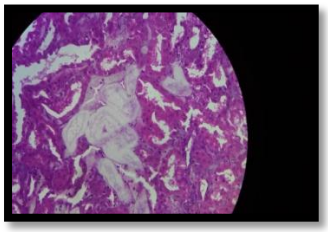
Arkaksheera: It has Laghu, Ruksha, Tikshna, and Kshara



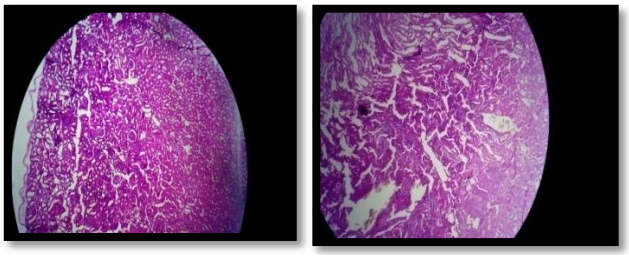
Control group showing normal histopathology



Test Group 2 showing less histopathological changes



Standard Group showing necrosis of tubules



Test Group1 showing Standard group near normal changes showing Stromal congestion

Figure 6: Histopathological changes.

guna which may be helpful in Samprapti vighatana and Sroto avarodha.

Conclusion

The present study investigated the effect of *Vangasindoora* on gentamicin-induced nephrotoxicity in albino rats. It was shown that *Vangasindoora* has an ability to reduce the development of Gentamicin induced nephrotoxicity. *Vangasindoora* may prevent Gentamicin nephrotoxicity in albino rats by rebuilding renal tissue. Based on the statistical analysis of the experiment data, it can be concluded that *Vangasindoora* is highly significant as Nephro protective rasa aushadhi. *Vangasindoora* acts as rasayana, balya, dhatusthairykara by virtue of its quality and quantity as a potent shaman aushadhi. Therefore, it can be regarded s a useful medication for treating renal disorders particularly acute and chronic renal failure brought about by various diseases including diabetes mellitus.

The misconception that rasa aushadhi are organotoxic can be disproved by this study design, leading to the conclusion that they are highly potent, safe, and effective medicines that function quickly in smaller doses when prepared according to the principles of the classics.

REFERENCES

1.Vishwanath Dwivedi, Rasendra Sambhava, 4th patala/35,Reprint,Krishnadas Academy, Varanasi 1997,

2. Sri Sadananda Sharma, Rasa Tarangini, 18th Taranga/11-12, edited by Kashinath Shastri, 11th edition, Motilal Banarasidas publication, New Delhi, 1979.

3. Ibid. Rasa Tarangini (2), 5/27-30;79.
4 .Ibid. Rasa Tarangini (2) 8/7-12;176.
5. Ibid. Rasa Tarangini (2), 14/ 3-4;326.
6. Asian Journal of Pharmaceutical and Clinical Research vol5,issue 1,2012. Effect of Simvastastin in Gentamicin induced nephrotoxicity in albino rats.
7.Guidelines for Toxicity /Safety profile evaluation of Bhasmas/raskalpas,CCras.nic.in
8. Ancient Science of Life, 2015 Jan-Mar; 34(3) Nephroprotective activity of *Bilvādiagada* in gentamicin induced nephrotoxicity in male Wistar rats.

Cite this article as:
Laxmi D (2024). Experimental evaluation of Vanga Sindoora for its nephroprotective activity. 12(1): 012-021

Submit your manuscript at
http://www.academiapublishing.org/journals/mms