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EXPERIMENTAL APPRAISAL OF NEPHRO-PROTECTIVE ACTIVITY OF *VARUNADI LOHA*

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ABSTRACT

It's a known fact that Iron is nephro toxic, causing various urinary diseases which when untreated results in fatal condition like renal failure. But *Ayurveda*, after subjecting to various *Samskaras* (processing), has used *Loha* (~Iron) in *bhasma* (calx) form in numerous formulations to treat urinary diseases. These diagonally opposite perceptions form the very base of the present work where in *Varunadi loha*, a formulation containing *Loha bhasma*, described in *Bhaishjyaratnavali* was studied for its nephro protective activity using Sprague Dawley rats as an experimental model. Significant decrease in serum creatinine and serum urea in curative group was suggestive that the drug *Varunadi loha* was delivering its best to improve the lost kidney function.

Keywords: *Varunadi loha*, nephro protective, serum creatinine

INTRODUCTION

It is estimated that 1, 00,000 new patients of end stage renal disease (ESRD) enter renal replacement programs annually in India[1]. In the absence of any registry in our country, these figures were based on estimates from rest of the world, tertiary care centre data and collective experience of nephrologists[2]. In an initial survey conducted by Mani et. al[3], in the rural population of Chennai from South India, the evidence of CKD short of renal failure was 0.7%. In a population based study from Bhopal in Central India, Modi et al[2] have reported the average crude and age adjusted incidence of rates of stage 5 CKD (ESRD) as 151 and 232 per million population. In a community based study by Agarwal et al[4], form Delhi in Northern India, the prevalence of earlier stages of CKD was reported to be 7852 per million populations.

According to recent research works, iron is nephro toxic and damaging the kidney, leading to various urinary disorders resulting in renal failure in some cases. But the classics of *Rasashastra* have described numerous herbo-metallic preparations, with iron as its integral ingredient, to be used in urinary disorders. The intriguing contrast of these diagonally opposite perceptions forms the very basis of the present work. *Varunadi loha* is an Iron-containing herbo-metallic preparation described in *Bhaishjyaratnavali*.[5] The indications for *Varunadi Loha* have recommended its usage in various urinary disorders. Hence, *Varunadi Loha* was selected as the drug of choice in the present study.

AIMS AND OBJECTIVES

To prepare Varunadi *loha* according to AFI and conduct a pre-clinical study

MATERIALS AND METHODS

In the pharmaceutical study, *loha bhasma* of 60 *putas* was prepared as per the guidelines mentioned in Ayurvedic Formulary of India[6]. In the same manner *Abhraka bhasma* of 20 *putas* was prepared. All the herbal contents of this formulation were powdered and mixed with *bhasmas* uniformly. Three samples of *Varunadi loha* were prepared for the purpose of standardization.

In Analytical study, organoleptic characters and physicochemical characters were studied. XRD was done to detect the compounds present in the final product. ICP-AES was done to detect the percentage of elements and heavy metals present in the *Varunadi loha*.

In the experimental study, Sprague dawley Swiss albino rats were selected. They were grouped into five groups i.e. control, disease control, prophylactic, curative and safety. Experimental model was established by injecting Gentamycin. The trial drug was given for 21 days and biochemical parameters were studied. After completion of experiment the animals were sacrificed humanely and tissues of liver and kidney were sent to histopathology laboratory.

Mixing of *churna* and *bhasma* to the combination of *Varunadi loha*

Materials: *Varun, Aamlaki , Dhataki , Haritaki , Prishniparni, loha bhasma, abhrak bhasma*

Equipments: Spatula, steel vessel, plastic gloves, gas stove, measuring cylinder etc.

Procedure: Powders of five drugs were taken into a steel vessel and mixed all contents with spatula. After proper mixing it was transferred into tray. Named as Sample I. Same procedure was followed again for Sample II and Sample III. After mixing all the samples were powdered again and weighing done.

Table 1: Ingredients and their ration in *Varunadi loha*

Drug	Ratio	Quantity in grams		
		Sample 1	Sample 2	Sample 3
Varun	8 parts	96	96	96
Aamlaki	4 parts	48	48	48
Dhataki	4 parts	48	48	48
Haritaki	2 parts	24	24	24
Prishniparni	1 part	12	12	12
Loha	1 part	12	12	12
Abhrak	1 part	12	12	12
Varunadi loha		248	248	248

ANALYTICAL STUDY

Analytical study of physic-chemical parameter of prepared *Varunadi loha* was conducted at the laboratory of Shree Dhootpapeshwara Ltd. XRD and ICP-AES test were performed at the laboratory of Dept. of Earth Sciences, IIT, Bombay.

Table 2: Organoleptic characters of *Varunadi loha.*

Parameters	*Varunadi loha 1*	*Varunadi loha 2*	*Varunadi loha 3*
Colour	Light Brown	Light Brown	Light Brown
Odour	Odourless	Odourless	Odourless
Taste	*Tikta ,kashaya*	*Tikta ,kashaya*	*Tikta, kashaya*
Touch	Rough powder	Rough powder	Rough powder

Table 3: Physico-chemical parameters of three samples of *Varunadi loha*

Parameters	Varunadi Loha I	Varunadi Loha II	Varunadi Loha III	Mean
Ash (%)	17.69	16.67	16.89	17.08
Acid Insoluble Ash (%)	5.18	4.92	4.97	5.02
Water Soluble Extractive (%)	28.11	28.39	29.68	28.73
Alcohol Soluble Extractive (%)	22.55	21.14	21.65	21.78
Loss on Drying (%)	9.68	9.55	9.47	9.57

Table 4: Identification of compound present in *Varunadi loha* by XRD

VL 1	VL2	VL3
Mica	Mica	Mica
Hematite	Hematite	Hematite
Magnetite	Magnetite	Magnetite

As *varunadi loha* contains *abhraka* and *loha bhasma*, compounds like Mica, Magnetite, and hematite were detected in XRD report. Some small peaks of other compounds were also observed in XRD graph.

Table 5: Showing elements present in *Varunadi loha* by ICP-AES

Elements	VL-1	VL-2	VL-3
Fe	5.46%	5.89%	5.70%
S	0.17%	0.59%	0.88%
Mg	0.73%	0.42%	0.56%
Al	0.55%	0.80%	0.64%
Si	1.88%	2.01%	1.62%
Hg	1.15 ppm	1.05ppm	1.01ppm
As	5.0ppm	4.82ppm	4.86ppm
Pb	9.4ppm	9.1ppm	9.6ppm
Cd	ND	ND	ND

VL= varunadi loha, ppm=parts per million, Fe=Iron, S=Sulphur, Mg=Magnesium, Al=Aluminium, Si=Silica, Hg=Mercury, As=Arsenic, Pb=Lead, Cd=Cadmium, ND= Not detected

In ICP-AES report, in all the three samples of *Varunadi loha*, Iron was present in the maximum percentage while elements like Sulphur, Magnesium, Aluminium, Silica were traced in the whole compound. Heavy metals like Mercury, Arsenic and Lead were present in permissible limit. However, Cadmium was not detected. Hence, *Varunadi loha* is safe to administer for experimental trial. Variation in three different samples is due to sensitivity and accuracy of an instrument. Instrumental and manual error also accounted for variation in results.

EXPERIMENTAL STUDY

The research protocol for an Experimental study was presented before Institutional Animal Ethical Committee (IAEC) at National Toxicology Center (NTC), Pune. After getting the clearance from IAEC; the trial was started in the Animal House of NTC, Pune. (IAEC Clearance no.-RP 128-181211).

1. Experimental animals – Sprague Dawley albino rats
2. Experimental model – Gentamycin (100 mg/kg) for 8 days intra peritoneal
3. Trial drug – *Varunadi loha* (sample 1)
4. Preparation of suspension – *Varunadi loha* in water
5. Route of administration – Oral forceful feeding
6. Dose calculation –

Human Dose x 0.018(conversion factor for rats) x 5

1.5x 0.018x5=0.135gm/kg (i.e.135mg/kg)

It can be stated as 13.5 mg/100g body wt of rats.

Study Protocol

Pharmacological activity of *varunadi loha* had been segmented in following parts:

- Toxicity study: Oral acute toxicity study

Drug Efficacy Study

- Establishment of Animal model
- Pilot study
- Main study

Toxicity Study: The oral acute toxicity study was performed to study the acute toxic effects of the drug. Six Sprague dawley albino rats of both sexes weighing 150-250g were used for the study. Suspension of *varunadi loha* in the dose of 2000mg/kg was administered orally to overnight fasted rats. After administration of the drug, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were kept under observation up to a period of two weeks. After 14 days of observation, it was found that all the rats were alive and there were no any toxic effects seen on rats. So it was concluded that the drug *varunadi loha* is safe for rats when administered in 2000mg/kg dose.

Drug Efficacy Study

Table 6: Description of groups included in the trial for the pilot study

Group		Specificity	Time
1	Normal control	Animals were on regular feed and water	RF=Day 0 –Day 23
2	Disease control	Animals received gentamycin in the dose of 100 mg/kg for 8 days	G=Day 0 – Day 8 RF= Day 9 – Day 23
3	Prophylactic	Animals received gentamycin 100 mg/kg for 8 days followed by *varunadi loha* soon after.	G= Day 0 – Day 8 VL=Day 0 – Day 23
4	Curative	Animals received gentamycin 100 mg/kg for 8 days then received *varunadi loha*	G= Day 0 – Day 8 VL=Day 9 – Day 23
5	Safety	Animals received only *varunadi loha* for 23 days	VL=Day 0- Day 23

RF- Regular feed, G-Gentamycin, VL-Varunadi loha

Results: Gentamycin induced the nephrotoxicity after 8 days when given in the dose of 100 mg/kg. In control group no changes have been occurred. In disease control group, sr. creatinine and sr. urea levels were raised than the normal and sr. creatinine level was maintained up to 23rd day while sr. urea level increased significantly. In group 3 i.e. prophylactic group, all the animals died after 8 days. This might have occurred due to drug interaction between *varunadi loha* and Gentamycin. So in the main study prophylactic group was omitted.

Main study: For the main study total 24 Sprague Dawley albino rats of both sexes were divided in 4 groups i.e. six rats in each group. Study was planned for 29 days.

Table 7: Explaining group description included in the trial for main study

Group	Animals	Group	Specificity	Time
1	6	Normal control	Animals were on regular feed and water	RF=Day 0 –Day 28
2	6	Disease control	Animals received Gentamycin in the dose of 100 mg/kg for 8 days	G=Day 0 – Day 8 RF= Day 9 – Day 28
3	6	Curative	Animals received Gentamycin 100 mg/kg for 8 days then received *varunadi loha*	G= Day 0 – Day 8 VL=Day 9 – Day 28
4	6	Safety	Animals received *varunadi loha* for 21 days	VL=Day 0- Day 28

RF- Regular feed, G-Gentamycin, VL-Varunadi loha

Histopathological studies

The vital organs like kidneys and liver were carefully dissected and transferred to 10% of Formalin solution for preservation. Later tissues were sliced from kidneys and Liver and sent to a commercial laboratory for preparation of histopathological slides. The slides were scanned in trinocular carl zeiss's microscope (Germany) under different magnifications. Changes if any, in cytoarchitecture were noted down.

Observations and Results

Table 8: Histopathological changes in Liver and Kidney

Group	Liver changes	Kidney changes
Normal control	No abnormality detected in liver.	No abnormality detected in liver.
Disease control	Congestion was observed	Swollen tubular epithelium with hyaline cast was observed. Tubular necrosis detected.
Prophylactic	Mild to moderate congestion was noticed	Swollen tubular epithilium with few hyaline casts. Focal interstitial haemorrhage seen.
Curative	Mild congestion noticed in some rats	mid congestion & swollen tubular epithilium
Safety	No abnormality detected	Mild congestion observed.

Observations and results statistically

Group 1 (Normal control)

Table 9: Showing significance of serological and hematological parameters of normal control group

Parameters	Mean		% of Change	SD	SE	t	p	Results
	BT	AT						
Sr. creatinine	0.43	0.43	3.85	0.075	0.030	0.542	>0.05	N.S.
Serum urea	38.13	37.24	2.321	3.199	1.306	0.678	>0.05	N.S.
Hb	8.88	9.13	-2.814	0.281	0.115	-2.179	>0.05	N.S.
WBC	9.08	9.13	-0.55	0.176	0.072	-0.696	>0.05	N.S.
RBC	6.18	6.00	2.938	0.395	0.161	1.126	>0.05	N.S.
PCT	0.31	0.33	-7.837	0.015	0.006	-3.991	<0.05	S.
PLT	531.33	533.67	-0.44	4.321	1.764	-1.323	>0.05	N.S.
HCT	31.97	32.15	-0.574	0.546	0.223	-0.823	>0.05	N.S.

N.S.- Not Significant S- Significant

Group 2 (Disease control)

Table 10: Showing significance of serological and hematological parameters of disease control group

Parameters	Mean		% of Change	SD	SE	t	p	Results
	BT	AT						
Sr. creatinine	0.38	0.72	-86.96	0.082	0.033	-10	<0.001	H.S.
Serum urea	34.19	56.25	-64.52	7.933	3.239	-6.811	<0.001	H.S.
Hb	11.167	9.7	13.134	1.461	0.597	2.458	>0.05	N.S.
WBC	7.55	8.52	-12.8	1.363	0.557	-1.737	>0.05	N.S.
RBC	7.013	5.76	17.82	0.809	0.331	3.783	<0.05	S
PCT	0.34	0.31	9.557	0.009	0.004	8.43	<0.05	S
PLT	565.17	558.83	1.121	3.615	1.476	4.292	<0.05	S
HCT	34.74	34.15	1.699	0.406	0.166	3.564	<0.05	S

H.S. – Highly Significant N.S.- Not Significant S- Significant

Group 3 (Curative)

Table 11: Showing significance of serological and hematological parameters of Curative group

Parameters	Mean		% of Change	SD	SE	t	p	Results
	BT	AT						
Sr. creatinine	0.82	0.57	30.612	0.105	0.043	5.838	<0.05	S
Serum urea	61.26	36.66	50.136	20.827	8.503	3.612	<0.05	S
Hb	11.08	10.42	6.015	0.441	0.180	3.701	<0.05	S
WBC	8.75	9.267	-5.905	1.709	0.697	-0.741	>0.05	N.S.
RBC	6.22	5.49	3.723	0.453	0.185	1.252	>0.05	N.S.
PCT	0.257	0.258	-0.194	0.018	0.008	-0.07	>0.05	N.S.
PLT	426.83	422.33	1.054	5.244	2.141	2.102	>0.05	N.S.
HCT	34.74	34.15	1.699	0.406	0.166	3.564	>0.05	N.S.

N.S.- Not Significant S- Significant

Group 4 (Safety)

Table 12: Showing significance of serological and hematological parameters of Safety group

Parameters	Mean		% of Change	SD	SE	t	p	Results
	BT	AT						
Sr. creatinine	0.42	0.38	8	0.082	0.033	1	>0.05	N.S.
Serum urea	31.98	30.36	5.051	1.214	0.496	3.259	<0.05	S
Hb	10.48	10.38	0.954	0.434	0.177	0.565	>0.05	N.S.
WBC	8.52	8.63	-1.37	0.299	0.122	-0.954	>0.05	N.S.
RBC	6.09	6.39	-4.922	0.329	0.135	-2.231	>0.05	N.S.
PCT	0.318	0.304	4.450	0.011	0.005	3.085	<0.05	S
PLT	492	493.33	-0.271	2.805	1.145	-1.164	>0.05	N.S.
HCT	34.7	33.9	2.036	1.121	0.458	1.749	>0.05	N.S.

N.S.- Not Significant S- Significant

DISCUSSION AND CONCLUSION

Pilot study of 15 days was conducted to justify the grouping of animals. Twenty S.D. rats of either sex were divided in 5 groups. The groups were control, disease control, prophylactic, curative and safety. Blood investigations were carried out at the end of day 9 and day 16. There were no changes in serum levels in control group. Serum levels were raised in disease control group. In the prophylactic group all the animals died at the end of 8th day. The reason may be drug interaction between gentamycin and *varunadi loha* or the drug overload on the liver and kidney.

Drug interaction can be explained as a situation in which a substance affects the activity of a drug, i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. Typically, interaction between drugs comes to mind (drug-drug interaction). However, interactions may also exist between drugs & foods (drug-food interactions), as well as drugs & herbs (drug-herb interactions). Drug interactions may be the result of various processes. These processes may include alterations in the pharmacokinetics of the drug, such as alterations in the Absorption, Distribution, Metabolism, and Excretion of a drug. Alternatively, drug interactions may be the result of the pharmacodynamic properties of the drug, e.g. the co-administration of a receptor antagonist and an agonist for the same receptor. Many drug interactions are due to alterations in drug metabolism. 248 no. of drugs are reported causing drug interaction with Gentamycin. Few of them are aluminium oxide, magnesium oxide and other minerals. In *Varunadi loha Abhraka bhasma* is composed of Aluminium, Magnesium and

Silica. So there might have drug-drug interaction between Gentamycin and *Varunadi loha*. Further research need to be done in this field. Histopathologically liver was showing congestion and tubular necrosis of nephron had occurred. So in the main study the prophylactic group was omitted. In curative group serum levels were raised at the end of 8th day but after giving *Varunadi loha* there was mild significant decrease in serum creatinine and serum urea levels. It was suggestive that the drug was acting against the diseased condition. In safety group only *Varunadi loha* was administered in its therapeutic dose for 15 days and at the end of 15th day no any adverse reaction noted. This suggested the drug has no side effects.

Main study was started with 24 animals equally divided in 4 groups. Groups were Control, Disease control, Curative and Safety. In control group the results were not significant as the rats were non diseased and kept on normal diet. In Disease control group, results were highly significant in serum creatinine and serum urea levels as the levels were raised. The handling of urea by the kidneys is a vital part of human metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons that allows for re-absorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons thus raising the osmolarity in the medullary interstitium surrounding the thin ascending loop of Henle, which in turn causes water to be reabsorbed. By action of the urea transporter 2, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. In CKDs, nephrons of a kidney failed to reabsorb urea resulting in increased urea level than the normal range. Creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine are used to calculate the creatinine clearance (CrCl), which reflects the glomerular filteration rate (GFR). Results were significant in routine hematological parameters as their levels were decreased. Decrease in Hb % was observed because Anaemia is noted as early as stage 3 CKD and is almost universal by stage 4. The primary cause is insufficient production of erythropoietin (EPO) by the diseased kidneys. Additional factors include iron deficiency, acute and chronic inflammation with impaired iron utilization ("anemia of chronic disease), and shortened red cell survival in the uremic environment. Less common causes include folate and vitamin B12 deficiency and aluminum toxicity. Platelet count was reduced due to insufficient thrombopoietic activity.[7] Low RBC and WBC counts are due to failure of erythropoietin formation system and alteration in the normal functioning of bone marrow. In Curative group results were significant in serum levels as there was moderate decrease in serum creatinine and serum urea levels after the treatment with *Varunadi loha*. The ingredients in *Varunadi loha* are having diuretic property. *Abhraka* bhasma helps to regenerate tissues in the organ. So it might have acted upon nephron for effective refunctioning. Also *Aamalaki and Haritaki*

are widely used as *rasayana*, might have helped in rejuvenation of the kidney functioning. Hb was increased moderately after the administration of *Varunadi loha*. *Loha bhasma* present in *Varunadi loha* was of helpful in the formation of erythropoietin normally. In the safety group the results were non significant indicated the drug *Varunadi loha* didn't show any adverse reaction when given in the therapeutic dose.

In the inter group comparison for serum creatinine, control vs disease control group showed highly significant result where as disease control vs safety group showed extremely significant result because serum creatinine levels were highly raised in disease control group. Rest of the group comparison for serum creatinine showed non significant results. In the inter group comparison for serum urea, control vs disease control group showed highly significant result where as disease control vs safety group showed extremely significant result. Also in disease control vs curative group, fall in serum urea level of curative group as compared to disease control group were suggestive of significant result. Inter group comparison for rest of the blood parameters showed slight changes in their values but were non significant statistically.

In histopathological studies, no abnormality was detected in the liver and kidney of control group. In disease control group mild congestion appeared in the liver and focal interstitial hemorrhage appeared in the kidney tissues because of damage occurred to nephron. In curative group mild congestion on liver of few rats appeared and swollen tubular epithelium with hyaline cast appeared in kidney. In safety group no abnormality detected in liver and kidney tissues. It was evocated histopathologically that; there was moderate improvement in curative group. Kidney tissues were damaged in disease control group due to nephrotoxicity of gentamycin.

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