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TOXICOLOGICAL EVALUATION OF *RASA-SINDOOR* IN ALBINO RATS

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ABSTRACT

Ayurvedic formulations containing heavy metals are used therapeutically since ages. However the safety of these formulations is questioned on time to time by modern counterparts as metals in their elemental form are very toxic. The present study was carried out with an aim to screen out toxicity (if any) of *Rasa-Sindoor*, an *Ayurvedic* formulation on kidney and its functioning in albino rats (Wistar strain). Total 30 albino rats were taken for the study and they were divided into 5 groups, 1st group was control group while other 4 groups were administered two samples of *Rasa-sindoor* i) *Hingulottha Parada Rasa-sindoor and ii) Shodhita Parada Rasa-sindoor* in two different doses (50mg/kg and 100mg/kg respectively) orally for 28 days consecutively. Effect of test drugs on kidney was evaluated on biochemistry (Renal function test) and post-mortem histo-pathological parameters. This study revealed normal behaviour, no mortality, no significant changes in renal functions test and no drug related morphological changes in histo-pathological examination. It was found that in 28 days of study, in comparison to control group *Rasa-sindoor* treated groups did not have any adverse effect on kidney.

Keywords: *Rasa-sindoor, Hingulottha Parada,* Heavy metals, Toxicity

INTRODUCTION

The *Ayurvedic* medicinal system has been in vogue since the *Vedic* period. It emphasizes on the maintenance, promotion of health and curing the diseases. In a report revealed by World Health Organisation (WHO), it is indicated that many people in developing countries still rely on herbal medicine.[1] In some *Ayurvedic* formulations heavy metals are integral part are in use for centuries.[2] *Ayurveda* never advised to take minerals/metals in their original form in which they occur in nature as these are mixed with lot of impurities which are toxic for our body. Various pre-pharmaceutical processes known as *Shodhana* like detoxification, trituration, heating etc. have been described in the texts to render them useful to incorporate in medicines. These *Shodhana* process removes unwanted part from raw material and separate out impurity. So the elements present in final product have no toxicity. *Ayurveda* also suggested various methods of administration and do's and don'ts for the patients taking these medicines, when followed all these as suggested then there will be least scope for toxic symptoms to develop.

Rasa-sindoor is therapeutically very effective in *kaphaja roga* (disease due to *kapha*), *Balakhasya* (loss of strength), *Dhatukhasya* (tissue wasting), *Hrd-daurbalya* (weakness of heart), *Prameha* (Diabetes), *Shula* (colicky pain).[3] This

formulation is also being in use for centuries in different conditions like *Rajyayakshma* (Tuberculosis), *Rakta-pitta* (Bleeding disorders), *Rasayna* (Immuno-modulator), *Vrishya* (Aphrodisiac), *Pandu* (Anaemia) etc. with different *anupana* (adjuvant or vehicle).[4]

A lot hue and cry has been made after an article published in JAMA about heavy metals content in *Ayurvedic* drugs preparations.[5]So it has become very important for all *Ayurvedic* physicians to have knowledge of toxicity profile of all *Ayurvedic* drugs which are used in clinical practice and especially in case of drug containing metals and minerals. Since *Rasa-sindoor* (RS) is a mercurial preparation, its safety profile is being questioned by modern physicians.

The present study was designed with an aim to screen out toxicity (if any) of two different samples of *Rasa-sindoor* (RS) prepared from two different methods of *Shodhana* (detoxification and purification), at 50mg/kg (5 times) and 100mg/kg (10 times to the therapeutic dose) dose level on Kidney and it's functioning in albino rats. The two different samples of *Rasa-sindoor* taken for study were i) *Hingulottha Rasa-sindoor* (HRS) and ii) *Shodhita Parada Rasa- sindoor (*SP-RS). The article present the result of 28 days repeated oral dose toxicity study of RS treated groups (both HRS and SP-RS) on kidney and its functioning in albino rats in comparison to control group, by observing changes (if any) in biochemical and Histo-pathological study.

MATERIAL AND METHODS

The present experimental study was conducted in the Pharmacology and Toxicology department of Apollo College of Veterinary Medicine, Jaipur, after getting approval of the Institutional Animal Ethical committee (IAEC) with Registration No.-886/ac/05/CPCSEA dated 06/09/12.

Test drug: Two different samples of *Rasa-sindoor* (RS) were prepared following different methods of *Shodhana* (purification and processing) as mentioned. Coded as mentioned below

1. RS prepared by *Parada* extracted from *Shuddha* (processed) *Hingula*[6] (Cinnabar) i.e *Hingulottha Parada Rasa-sindoor* (HRS) as mentioned in the classical text[7].
2. RS prepared after the *Samanya Shodhana*[8] and *vishesa shodhana*[9] of *Parada* viz. *Shodhita Parada Rasa-sindoor* (SP-RS).

Test animal and housing: Total 30 Wistar Albino rats of both sexes weighing 100-200g were selected for the study. They were kept in colony cages in Animal house of Apollo College of Veterinary Medicine, Jaipur at an ambient temperature of (24±5°C) and at a relative humidity of 55-65% in 12 hrs light and 12 hrs dark sequences. They were fed with standard rodent pellet diets and tap water throughout the study. Animals were allowed to acclimatized one week prior to commencement of experiment. Animals were randomly divided into 5 groups (6 rats per group) [Table 1].

Table 1: Showing group code and dose administered

Sl No	Groups code	Dose in mg/kg/day
I	Control	10.8
II	HRS-5x	50.0
III	HRS-10x	100
IV	SP-RS 5x	50.0
V	SP-RS-10x	100

HRS-5x: *Hingulottha Parada Rasa-sindoor* 5times of therapeutic dose, HRS-10x: *Hingulottha Parada Rasa-sindoor* 10 times of therapeutic dose, SP-RS 5x: *Shodhita Parada Rasa-sindoor* 5 times therapeutic dose, SP-RS-10x: *Shodhita Parada Rasa-sindoor* 10 times therapeutic dose

Experimental design

This study was conducted strictly following OECD guidelines. The repeated oral dose toxicity was conducted for 28 days on 30 albino rats. The rats were divided into 5 groups consisting of 6 rats in each group. The rats were given daily vehicle control (20%gum acacia) and HRS and SP-RS in 5times (5x) and 10 times (10x) to the therapeutic dose (table no. 2) dissolved in 20 % gum acacia by oral gavage , once daily for 28 consecutive days. Animals were observed for mortality and general clinical and behavioural changes viz. routine activity, irritability, food intake and external appearances etc.

The body weights of rats were observed before the commencement of trial and after completion of dosing in control and treated groups. Blood samples were collected on 29th day prior to euthanasia through puncturing the retro-orbital plexuses under chloroform induced anaesthesia. Blood was collected in centrifuge tube for renal function test viz. Blood urea, S. Creatinine, S. Uric acid, S. Phosphorus, S. Electrolytes.[10]

Animals were sacrificed on 29th day by overdosing of anaesthetic agent viz. chloroform; on autopsy kidney were observed, collected, weighted and preserved in 10 % neutral buffered Formalin solution. Tissue were then trimmed and dehydrated in ascending grades of alcohol. The tissue sections were finely cut to 3-5µm in microtone and stained with Hematoxylin and Eosin.[11]Finally the sections slides were mounted with Distyrene Plasticizer and Xylene (DPX) and examined under microscope.

Stastical analysis

All data are expressed in mean± S.E.M. Paired "t-test" were applied to assess change in the body weight within the group. Drug treated groups were compared to control group using one way Analysis of Variances (ANOVA) followed by post hoc Tukey and Kramer multiple comparison tests. A difference with a $p < 0.05$ was accepted as statistically significant.

OBSERVATIONS AND RESULTS

No mortality was observed in control and treated groups, neither any treatment related clinical signs were observed. All the animals were well oriented and active during and after the trial period.

No significant ($p > 0.05$)change was observed in the weight of rats after 28 days although there was a marginal increase in the weight of rats of both the control and treated groups, but that was not due to effect of drug [Table 2].

Table 2: Showing effect of drugs on body weight before and after treatment

Groups (n=6)	B.T. wt(g)	A.T. wt(g)	% of Change	t value	p value
Control	137.5±17.97	167.5±19.74	21.8↑	0.686	>0.05 (N.S.)
HRS-5x	116.67±8.33	137.5±10.7	17.85↑	0.823	>0.05 (N.S.)
HRS-10x	137.5±15.48	150±17.08	9.09↑	2.231	>0.05 (N.S.)
SP-RS-5x	167±15.48	179.17±18.73	7.28↑	1.663	>0.05 (N.S.)
SP-RS-10x	143.33±20.28	170.83±15.02	19.20↑	1.324	>0.05 (N.S.)

Data represented as Mean±S.E.M, NS-Not significant, ↑-increase, HRS-*Hingulottha parada Rasa-sindoor, SP-RS: Shodhita Parada Rasa-sindoor,* 5x- 5times of therapeutic dose, 10x-10times of therapeutic dose.

A significant ($p<0.05$) decrease in the weight of kidneys of rats was observed in HRS-5x (50mg/kg) and HRS-10x (100mg/kg) groups but on inter group comparison the change in the weight in these two group were found not significant ($p>0.05$) to that of control group rats [Table 3 and 4]. Hence it was not considered as treatment related effects.

Table 3: Showing effect of drug samples on kidney wt. in different groups (ANOVA)

Group (n=6)	Absolute wt. (g)	% of Change	F value
Control	2.216±0.1137		F=5.381 p=0.02*
HRS-5x	1.616±0.1600	27.07↓	
HRS-10-x	1.666±0.0881	24.8↓	
SP-RS-5x	2.633±0.2403	18.81↑	
SP-RS-10x	2.266±0.2629	2.25↑	

Data represented in Mean± S.E.M.,↓-decrease,↑-increase,*=$p<0.05$ – Significant

Table 4: Effect of drugs on absolute wt. of kidney (ANOVA test followed by Tukey-Kramer multiple comparison test)

Comparison	Mean Difference	q value	p	Result
Control v/s HRS-5x	0.60	3.225	>0.05	NS
Control v/s HRS-10x	0.55	2.953	>0.05	NS
Control v/s SP-RS-5x	-0.4169	2.239	>0.05	NS
Control v/s SP-RS-10x	-0.04	0.2685	>0.05	NS

Data represented in Mean± S.E.M., ↓-decrease, ↑-increase, NS-$p>0.05$– not Significant

There were no significant ($p>0.05$) changes observed in Renal function test i.e. Blood Urea, S. Creatinine, S. Uric acid, S. Electrolyte on 28 days study in treated group rats when compared with control group except for S. phosphorus level which was found significantly ($p<0.05$) change in SP-RS-10x (100mg/kg) group [Table 5].

Table 5: Investigation of Renal functions test of rats of treated and control group

Bio-chemical parameters		Control group	Test drug groups RS in(mg/kg)			
			HRS-5x (50mg/kg)	HRS-10x (100mg/kg)	SP-RS-5x (50mg/kg)	SP-RS-10x (100mg/kg)
Blood Urea		21.5 ± 0.8465	18.66 ± 1.406	19 ±0.632	19.66 ± 0.8027	19.666 ± 0.8028
S. Creatinine		0.416±0.0307	0.416±0.0307	0.35 ± 0.0428	0.35 ± 0.0428	0.35 ± 0.0428
S. Uric acid		4.63 ± 0.4862	3.988 ±0.060	4.5 ± 0.301	3.66 ± 0.3412	4.03 ± 0.2616
S. Phosphorus		4.03± 0.061	3.983±0.1306	3.5 ±0.3447	4.0 ±0.3200	2.91±0.2343*
S. Electrolyte	Na^+	140±1.788	140.3±1.819	140±2.88	143.1±1.775	141.8±2.13
	K^+	4.83±0.231	4.4±0.3065	3.8±0.250	4.7±0.2828	4.2±0.8725
	Cl^-	102.3±0.614	101.3±0.615	102±0.577	103±1.414	100.3±0.813

Data represented in Mean±S.E.M., * $p<0.05$-significant

However histo-pathological study of Kidney of both samples of RS treated groups did not reveal any treatment-related or dose-dependent change [Figure 1 – 5].

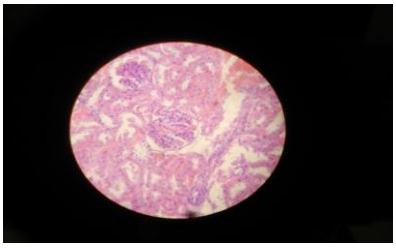

Figure 1: Control rat's Kidney

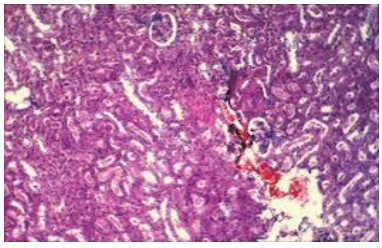

Figure 2: Kidney HRS-5x Rat

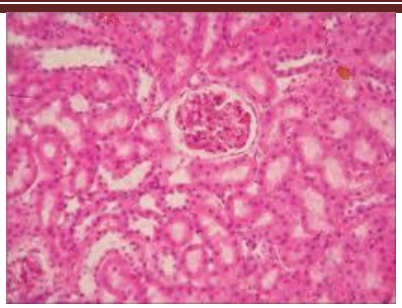

Figure 3: Kidney HRS-10 Rat

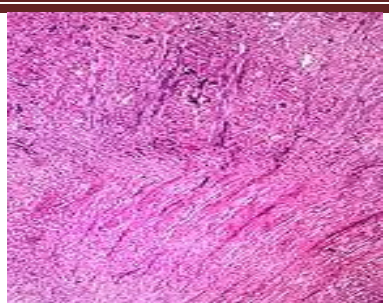

Figure 4: kidney SPRS-5x Rat

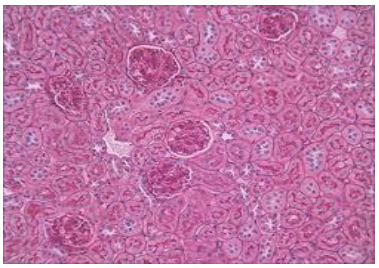

Figure 5: Kidney SP-RS-10x Rat

DISCUSSION

Ayurveda is widely practice oldest system of medicine not only in India but also worldwide. Most of *Ayurvedic* preparations are either herbal or herbal-metallic in composition.

RS is a formulation prepared from processed and purified mercury and sulphur having potent therapeutic efficacy due to the unique and repeated *Shodhana* process. However the lack of proper Pharmacovigilance and widespread self medication has resulted in undesirable effect on certain sections of consumers of these preparations which have contributed to the negative publicity for these forms of medicine.

The toxic effect of mercury is due to impure mercury, elemental mercury or improperly processed mercury. However, *Ayurvedic* formulation had mentioned strictly that metals should be subjected to *Shodhana* which attributes to purification, detoxification and restoration of its therapeutic property. Properly processed mercury shows excellent therapeutic activities in low doses without producing toxic effects in human subjects.

So, present study was initiated for evaluation of toxicological aspect of RS on the renal functioning to revalidate its pharmaceutical practices in drug development. In 28 days repeated dose toxicity in 30 albino rats that were divided into 5 different groups. No changes were observed in food intake, water consumption and the behaviour in the treated groups. The changes in values of body weights of animals of treated groups were insignificant when compared to control.

The weight of kidneys was found decreased in both HRS treated (50mg/kg and 100 mg/kg) groups but when compared to control group this change was not significant. There were no significant changes noted in kidney function test except that in the value of S. Phosphorus the reason of is matter of further study. Even no changes were observed even at cellular level on histo-pathological examination of kidney sections of treated groups when compared to control group.

The result of present study showed that RS even being mercuric preparation is non-toxic. The reason for non-toxic nature could be metals in *Ayurvedic* formulations

are not present in elemental form. The Physico-chemical state of heavy metal in the form of *Ayurvedic* medicine is totally different form the known Physico-chemical forms of metals.[12]

CONCLUSION

In present study no mortality was reported in any of RS treated groups. There were neither any morphological changes observed in kidney of treated groups nor any change in renal function test when compared to the control group. It may be concluded that drug formulations of RS does not have any toxic effect on Kidneys and its functioning, hence it is safe in animal models. It may also be concluded from present study that different *Shodahna* processes of mercury mentioned in *Ayurvedic* texts are not only effective in reducing its toxicity but also enhancing its therapeutic efficacy.

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