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"Study of Toxicity of Rasa Sindoor on Liver at 5 times & 10 times of Therapeutic dose- an Experimental Observation"

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Abstract:

Formulations containing heavy metals has been integral part of treatment in Ayurveda and are in use since ages. It is known fact that metals in their elemental forms are always toxic, so the safety of these formulations are questioned by our modern counterparts. However, the fact is that metals are used in drugs after a prolonged and laboured procedure of purification known as "*Shodhana*". The present study was carried out with an aim to screen out toxicity (if any) of *Rasa-Sindoor,* an *Ayurvedic* formulation containing Mercury on Liver and its functioning in albino rats (Wistar strain). Since the study was to observe toxic effect (if any) on liver, so the dose was the fixed to 5 times and ten times the therapeutic dose. 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 Liver was evaluated on biochemistry (Liver function test) and post-mortem histo-pathological parameters. No mortality was found in 28 days of study, in either of control group and *Rasa-sindoor treated* groups ,however a significant change in SGOT, SGPT, ALP values were observed in some treated groups.

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

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INTRODUCTION:

The foremost emphasis of Ayurveda is Maintenance, Promotion of health and cure of diseases. A recent report of WHO(world health organisation) has revealed that people in developing countries relay on Herbal medicine as peferance[1]. Heavy metals are integral part of some *Ayurvedic* formulations which are in use for centuries[2]. Metals/ Minerals as occur in nature in compounds forms with a lots of impurities which are very toxic to human bodies and are never advised to take in their original form. Various pre-pharmaceutical processes known as *Shodhana* like detoxification, titration, heating etc. have been described in the Ayurvedic texts to render them useful to incorporate in medicines. These *Shodhana* process removes unwanted parts from raw material and separate out impurity. So the elements present in final product have no toxicity. The methods of administration of these drugs and a lists of do's and don'ts are also mentioned in texts for patients who is undergoing treatment, when followed all these as suggested then there will be least or no scope for toxic symptoms to develop and adversely affect the body. After an article published in JAMA about heavy metals content in *Ayurvedic* drugs preparations, a lot of hue n cry were made[3]. So it has became a great need of time for all Ayurveda 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. These data regarding toxicity of Herbo-mineral formulation will not only helpful for providing efficacy and safety of the drugs but also but also likely damage to body or organs which can occur if taken not cautiously. The present research work is on one such drug known as "*Rasa-sindoor*", since it's main component is Mercury and Sulphur so it become quite essential to study its toxicity profile. Cinnabar-containing preparations have been used extensively in Indian and Chinese systems of medicine for treatment of chronic ailments like syphilis, high fever, pneumonia, insomnia, nervous disorders, deafness, and paralysis of the tongue. Contrary to Western medicine, which does not promote the use of mercury due to its toxic effects, Indian and Chinese traditional practitioners believe that mercury-based formulations have potent therapeutic efficacy, while there is no toxicity due to the unique and repeated purification processes employed during preparation. However, lack of proper pharmaco-vigilance and widespread self-medication has resulted in undesirable effects to certain sections of the consumers of these preparations, which have contributed to the negative publicity for these forms of medicine.

Therapeutic use: *Rasa-sindoor* is 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]It 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)[5]. 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 for a period of 28 days on liver and it's functioning in albino rats. Since the study is to observe the RS toxicity, so the dose was fixed at 5 times and 10 times of therapeutic dose as no toxicity was reported on therapeutic dose. (Singh Anil Kumar et.al 1992)[6]. The two different samples of *Rasa-sindoor* taken for study were i) *Hingulottha Parada Rasa-sindoor* (HRS) and ii) *Shodhita Parada Rasa-sindoor (*SP-RS). This article present the result of 28 days of repeated oral dose toxicity study of RS treated groups (both HRS and SP-RS) on Liver 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*[7](Cinnabar) i.e *Hingulottha Parada Rasa-sindoor* (HRS) as mentioned in the classical text[8].

2. RS prepared after the *Samanya Shodhana* [9]and *vishesa shodhana* [10] 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 and were randomly divided into 5 groups (6 rats per group) [Table 1]. 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.

Table 1: Showing group code and dose administered

S.No.	Group code	Dose in mg/kg/day
I	Control group	10.8
II	HRS-5x	50
III	HRS-10x	100
IV	SP-RS-5x	50
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 : The study was conducted strictly following OECD guidelines. The rats were divided into 5 groups consisting of 6 rats in each group (table 1). The rats were

given daily vehicle control group (20%gum acacia) and HRS-5x ,HRS-10x(group II,III) and SP-RS -5x and 10x (group IV &V) to the therapeutic dose (table no. 1) dissolved in 20 % gum acacia by oral gavage method, once daily for 28 days consecutively . 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 (BT) and after completion of dosing in both control and treated groups (AT).(Table2)

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 Liver function test viz. S. Bilirubin, S.G.O.T.(AST), S.G.P.T.(ALT), Alkaline phosphate[11]. Animals were sacrificed on 29th day by overdosing of anaesthetic agent viz. chloroform; on autopsy Liver 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.[12]Finally the sections slides were mounted with Distyrene Plasticizer and Xylene (DPX) and examined under microscope.

Statistical 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.)

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Table3:-Effect of Rasa-Sindoor samples on various parameters of Liver Function test in Albino rats.

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)
S.G.O.T.		208.3 ± 3.639	203.16 ± 3.859	201.66 ± 4.3101	174.66 ± 7.6317	185.83 ± 7.790**
S.G.P.T.		55.33± 5.660	49.16± 3.477	58.83± 3.3107	81.83± 2.7495	51.166± 3.092***
Alkaline Phosphatase		462.16 ± 59.567	516.6 ± 25.549	500 ± 15.426	375.16 ± 14.90	380.3 ± 22.261*
Total Bilirubin		0.7166 ± 0.4013	0.733 ± 0.04943	0.633 ± 0.066	0.533 ± 0.0494	0.65 ± 0.4281
Bilirubin	Direct	0.3166±0.0307	0.35 ± 0.0428	0.366 ±0.0494	0.25 ± 0.0894	0.233 ± 0.02108*
	Indirect	0.25 ± 0.0224	0.30 ± 0.03651	0.30 ± 0.2233	0.266 ±0.0333	0.266 ±0.033*
	Albumin	3.9±0.1386	4.283±0.0401	4.116±0.0400	3.533±0.1763	3.806±0.1650**

Table 4: Showing summary of % change in Bio-chemical parameters of LFT of Albino rats in HRS, SP-RS treated group in comparison to control

Group	SGOT	SGPT	ALP	T.B.	D.B.	I.B.	T.P.	S. Albumin
Control								
HRS5x	2.46↓	11.20↓	11.77↑	2.28↑	10.5↑	20.0↑	9.01↑	9.82↑
HRS10x	3.18↓	6.32↑	8.18↑	11.6↓	15.60↑	20.0↑	1.74↑	5.53↑
SP-RS-5x	16.14↓	47.81↑	18.82↓	25.6↓	21.0↓	6.4↑	9.98↓	9.41↓
SP-RS-10x	10.78↓	7.52↓	17.71↓	9.29↓	26.40↓	6.4↑	7.59↓	2.41↓
F-value	6.536	11.66	4.246	2.071	3.186	5.04	3.186	5.045
p-value	<0.01	<.001	<0.05	>.05	<0.05	<.01	<0.05	<0.01
Result	**	***	*	NS	*	**	*	**

***p<0.001- highly significant, **p<0.01-very significant, *p<0.05-significant(T.B.-Total Bilirubin, D.B.-Direct Bilirubin, I.B.-Indirect Bilirubin, T.P.-total protein)

- There was marginal to moderate decrease observed in SGOT values in all the groups. The change in the mean value is found to be very significant.
- A significant increase (47.89%) in the value of SGPT was observed in SP-RS-5x group to the control one. The increase was statistically highly significant ($p<0.001$)
- A marginal increase in ALP value 11.77% & 8.18% was noted in both HRS groups 5x & 10x respectively but at the same time a moderate decrease in the ALP value 18.82 & 17.71% was observed in SP-RS-5x and SP-RS-10x treated groups. These changes were found to be statistically significant.
- A marginal increase in D. Bilirubin value 10.5% & 15.60% was noted in both HRS treated groups 5x & 10x respectively but at the same time a moderate decrease in the D. Bilirubin value i.e., 21.0%& 26.4% was observed in SP-RS-5x and SP-RS-10x treated groups. These changes were found to be statistically significant.
- A marginal increase in the value of Albumin of HRS-5x (9.82%) and HRS-10x (5.53%) in compare to control group was found. The value of Albumin declined in SP-RS-5x (9.41%) and SP-RS-10x (2.41%) compare to the control group. The variation in the data was found statistically very significance ($p<0.01$).(Table3&4)

Table 5: showing summary of results of post ANOVA Tukey-Kramer multiple comparison test for various parameters of LFT

Parameters comparison	SGOT	SGPT	ALP	D.Bilirubin	I. Biliirubin	S. Albumin
Control v/s HRS-5x	NS	NS	NS	NS	NS	NS
Control v/s HRS-10x	NS	NS	NS	NS	NS	NS
Control v/s SP-RS-5x	**	**	NS	NS	NS	NS
Control v/s SP-RS-10x	NS	NS	NS	NS	NS	NS
HRS-5x v/s HRS-10x	NS	NS	NS	NS	NS	NS
HRS-5x v/s SP-RS-5x	*	***	*	NS	NS	**
HRS-10x v/s SP-RS-10x	NS	NS	NS	NS	NS	NS
SP-RS-5x v/s SP-RS-10x	NS	**	NS	NS	NS	NS

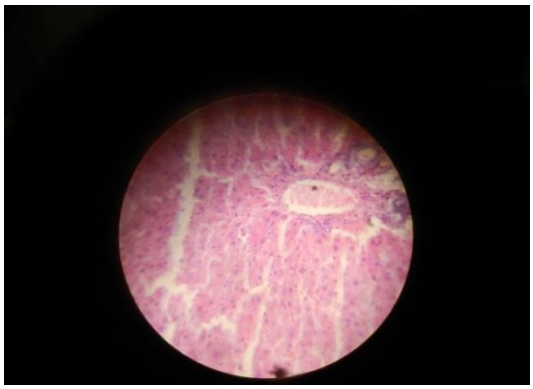
*p<0.05-significant, **p<0.01-very significant, ***p<0.001highlysignificant

Table (5) shows the Tukey-Kramer multiple comparison test results which depicts

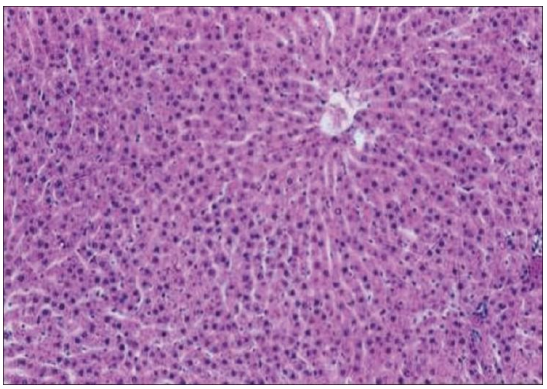
- A very significant ($p<0.01$) decrease in SGOT value in SP-RS-5x treated group in comparison to control group.
- Also, a significant decrease in the value of Serum SGOT in SP-RS-5x group was observed in comparison to HRS-5x group. Among other group sets there is not any significant change observed.
- A highly significant ($p<0.001$) increase in SGPT is found in SP-RS-5x group when compared with HRS-5x group.
- A very significant ($p<0.01$) increase is found in SGPT value of SP-RS-5x group when compared to control group and SP-RS-10x group respectively.
- There is a significant increase ($p<0.05$) in ALP value of HRS-5x group in comparison to SP-RS-5x group. Among other group sets comparison there is not any significant change observed.
- In sets of group comparison there is not any statically significant change is observed in Direct Bilirubin.
- The increase observed in Indirect Bilirubin the treated groups(table 4) did also possess no significance in any sets of group comparisons
- a very significant ($p<0.01$) decrease in Albumin value was observed in SP-RS-5x group in comparison to the HRS-5x group. Among other sets of comparison no significance change was observed.
- **Histo-Pathological Studies:** In treated group Microscopic examination of liver: HRS-5x HRS-10x, SP-RS-5x groups shows normal cyto-structure, no specific pathology was identified in any rats, however in SP-RS-10x group two out of six rats mild congestion of sinuses were noted in liver.

Microscopic Pictures Of Liver of albino rat of all trial group(fig. 1-5)

I) Control Group rat liver



(II) HRS-5x group rat liver



(III) HRS-10x group rat liver

(IV) SP-RS-5x group rat liver

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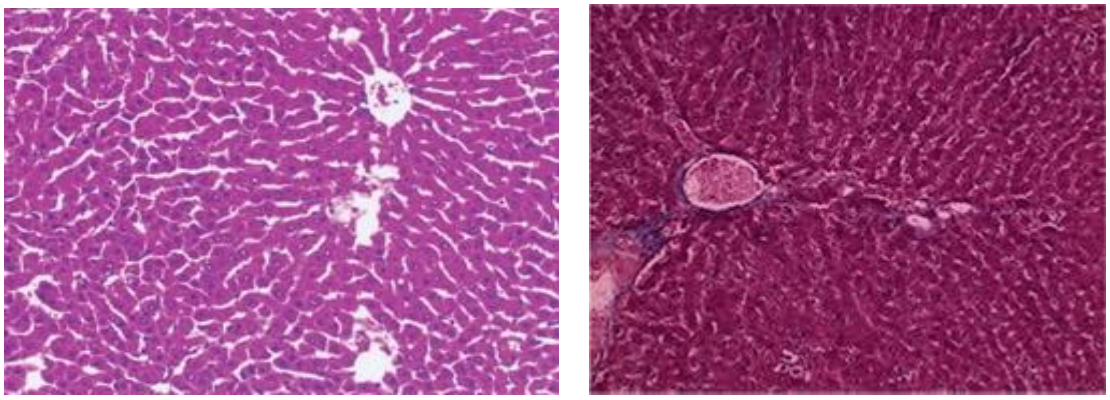
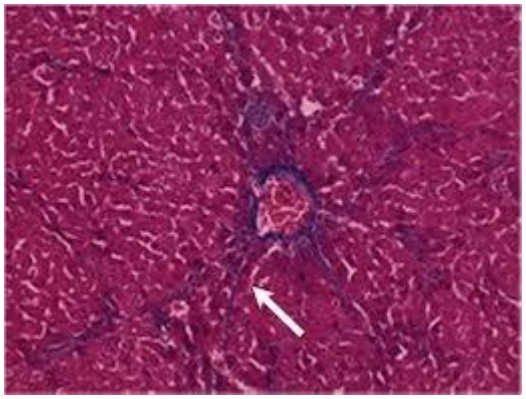


(v) SP-RS-10x liver rat showing congestion at sinuses



DISCUSSION :

In present study (table 5) a highly significant ($p<0.001$) increase in SGPT value is observed between groups. In both HRS-5x &10x group a non-significant change was observed. In SP-RS-5x group animals very significant ($p<0.01$) increase in SGPT value was found when compared to control group animals. The increase in SGPT values in SP-RS-5x treated group was found highly significant ($p<0.001$) in comparison to HRS-5x group (table6). On the other hand a very significant ($p<0.01$) fall in SGOT value is noted in SP-RS-5x group treated animal. The decrease in the value of SP-RS was very significant ($p<0.01$) when compared to control group and significant ($p<0.05$) in comparison to HRS-5x group. Rise in SGPT(ALT) value of SP-RS-5x treated group indicate that sub-acute exposure of Rasa-sindoor in five times therapeutic dose is causing inflammation or damage at hepato-cellular level as it is partially supported by findings of (Ghaleb A. Oriquat et.al 2012).[11]

Since the range of SGOT and SGPT is very vast range and in present study the experimental group size is small (6), so further detailed study on large population is required before drawing any final conclusion. In present study HRS-5x & 10x treated groups show marginal non-significant ($p>0.05$) increase in ALP compare to control group whereas SP-RS-5x & 10x group show mild insignificant ($p>0.05$) decrease compare to control group. But a significant increase ($p<0.05$) in ALP of HRS-5x is found when compared to SP-RS-5x group (table d5). Increase in ALP of HRS-5x treated group might be due inflammation of

hepatocytes due to drug. A non-significant ($p>0.05$) decrease in value of total Bilirubin was observed (table d5).Though change in the values of D. Bilirubin, I. Bilirubin between the groups appeared significant ($p<0.05$) but on multiple comparison test changes in both HRS and SP-RS 5x & 10x treated groups were observed non-significant ($p>0.05$). This shows that both preparations of Rasa-sindoor do not have deleterious effects on Bilirubin metabolism. A marginal increase was noted in S. Protein level & S. Albumin in HRS-5x & HRS-10x groups in comparison to control group and marginal decrease was noted in SP-RS-5x &10x compared in control group. But the change in S. protein was found statistically non-significant ($p>0.05$).

In both HRS-5x&10x groups a non-significant ($p>0.05$) increase was observed in S. Albumin level in compared to control group animals. In both SP-RS-5x and 10x treated groups an insignificant ($p>0.05$) decrease was noted in S. Albumin in comparison to control group. But very significant ($p<0.01$) decrease in S. albumin was observed in SP-RS-5x group animals when compared to HRS-5x group animals. The decrease might be due to liver damage cause by SP-RS-5x group but no significant was observed in histo-pathological study.

CONCLUSION:

In present study no mortality was reported in any of RS treated groups. On histo-pathological studies congestion of sinuses were observed in 2 rats of group SP-RS-10x(10 times the therapeutic dose) drug . Based on findings of the experimental study both samples of *Rasa-sindoor* (HRS and SP-RS) seem to be non-toxic on liver functioning. But in inter-group analysis it was found that SP-RS samples at higher doses (5x & 10x therapeutic dose) has shown changes in liver enzymes SGOT, SGPT and ALP levels which are statistically significant to prove that it can cause toxicity when used for longer time at higher doses when compared to HRS treated group. As previously told that no toxicity was reported on therapeutic dose (Singh Anil Kumar et.al 1992), So it may be concluded that in higher dose *Rasa-sindoor* may adversely affect the liver ,so in liver patients it should be given very cautiously and in small doses and for short period only**.** Since in present study the experimental group size is small (i.e.6 rats in each group), so further detailed study on large population is required before drawing any final conclusion.

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