



Research Article

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Tal Sindoor: an *in vivo* study

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ABSTRACT

Tal Sindoor is one of Ayurvedic metallic preparations processed by Kupipakwa method (special processing). As per the present day scenario intake of formulations prepared with heavy metals like mercury and mineral compound like arsenic trisulphide are considered highly toxic. Tal Sindoor is prepared with Shuddh (purified) Parad (mercury), Gandhak (sulphur) and Haratal (arsenic trisulphide). With the myth that Rasoushadhis (metallic preparations) are toxic, the need for the study was persuaded to elucidate the safety of diligently processed Tal Sindur at therapeutic dose prescribed in classics. Study was conducted for 14 days on Wistar strain albino rats. Selected Wistar strain albino rats were divided into two groups. Group-I being vehicle control group, albino rats were administered with 0.5ml of compound consisting of 3 parts of de-ionized water and 2 parts of honey. Group-II rats were administered with 0.5ml of compound vortexes with test compound Tal Sindoor at therapeutic dose of 250mg/Kg/day in 3 parts of de-ionized water and 2 parts of honey. During or after the study either morbidity or mortality was not observed. Food and water intake, body weight, normal activity, behavioural changes and other toxicological changes were evaluated daily for both groups during administration of drugs. After the study haematological, biochemical parameters, lipid-peroxidation and histopathological changes were evaluated for both the groups. Statistics of body weight and above mentioned parameters were not significant. Histopathological examination of liver and kidney revealed normal histology for both groups.

Keywords: Tal Sindoor, Heavy metal toxicity, Metallic preparations, Lipid-peroxidation, Kupipakwa.

INTRODUCTION

Ayurveda not only deals with herbal medicines but also includes preparations with metals, mineral and herbal poisons. Kupipakwa Rasayana is one of Murchita Parada yoga. Tal Sindoor^[1] is Sagandha (with sulphur), Sagni (drug processed by heating) and Kantastha (final product accumulates at neck of bottle) Kupipakwa Rasayan with Parad, Gandhak and Haratal as its ingredients at proportions equal quantity by weight. Processed drugs acquire the therapeutic properties due to transformation of chemical bonding that differentiates it from the raw drug compound. Classically processed preparations are used since time immemorial and renowned for their clinical efficacy. To evaluate and analyse the perfection of pharmaceutical processing, many tests and methods were explained in the literature. Till date the literary study suggest not much reference regarding this preparation, Tal Sindoor. To understand the rationale of Acharyas this study was proposed to evaluate the toxic effects of arsenic compound in processed form of Tal Sindoor under the light of science with different facet.

MATERIALS AND METHODS

All ingredients were collected according to physical characteristics of raw drugs enumerated in classics. Parad^[2], Gandhak^[3] and Haratal^[4] were purified as described in classics. Tal Sindoor was prepared as described in Rasendra Sambhava^[4]. Tal Sindoor was prepared in Rasashatra department of JSS Ayurveda medical college. The final product was subjected to various chemical analyses with Ayurvedic and modern parameters to confirm the quality. After obtaining positive results further *in vivo* study of Tal Sindoor was conducted [Figure-1].

Ethical Clearance for the Study: Animal studies were conducted according to the Institute Animal Ethical Committee regulations approved by the 'Committee for the Purpose of the Control and Supervision of Experiments on Animals' (CPCSEA). Clearance of experimental design by the Institutional Ethical Committee for albino rats was taken at Defence Food Research Laboratory (DFRL). The study was approved with register number 28/1999/CPCSEA and conducted in DFRL, Mysore.

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Instruments used:

Independent cage for each rat, weighing machine for rats, metler balance, vortex the mixture, tuberculin syringe (1ml), 16-18 gauges bent balled needle, Eppendorf tips and pipettes.

Procurement and selection of animals: Healthy rats of Wistar Strain were used for study, reared in the DFRL animal facility, Mysore. A total of 12 Wistar Strain albino rats weighing between 140-160 g and 3-4 week of age were selected randomly from the stock colony.

Treatment of experimental rats: The rats were housed in an acryl fiber independent cage in thermo regulated room with temperature of $25\pm 2^{\circ}\text{C}$ and were maintained in a 12 hours light/dark cycle. Rats were fed with commercial pellet diet of 15-20 gm/day (from Sri Venkateswara Enterprises, Bangalore, India) and drinking water ad libitum.

Experimental design: Twelve male Wistar albino rats were randomly divided into the following two experimental groups with six in each group: vehicle control group (Group-I) and therapeutic dose group (Group-II).

Group I – Force feeding with 0.5ml of compound consisting of 3 parts of de-ionized water and 2 parts of honey for 2 weeks.

Group II – Force feeding with 0.5ml of compound vortexes with test compound Tal Sindoor in 3 parts of de-ionized water and 2 parts of honey.

Dosage schedule: The required dose for rat was calculated by using the standard dose calculation procedure from recommended clinical dose.

Table 1: Observations before and after the *in vivo* study

Observation	Before administration	After 14 days of administration	
		Group-I	Group-II
Food intake	Normal	Normal	Normal
Water intake	Normal	Normal	Normal
Activity	Normal	Normal	Normal
Colour of eyes	Red	Red	Red
Colour of fur	White	White	White
Stool colour	Black	Black	Black

Table 2: Results of haematological parameters of both groups

Group	WBC ($\times 10^3$)	RBC ($\times 10^6$)	Hb gm/dl	HCT	MCV	MCH	MCHC
Group I	5.10 \pm 1.28	7.78 \pm 0.68	12.75 \pm 4.18	45.32 \pm 3.14	58.3 \pm 1.13	17.83 \pm 0.82	31.17 \pm 0.59
Group II	4.13 \pm 0.97	8.70 \pm 0.73	15.17 \pm 1.72	49.25 \pm 5.85	59.0 \pm 1.82	18.17 \pm 0.68	30.82 \pm 0.89

Table 3: Biochemical parameters of Group I and Group II

GROUP	SGOT	SGPT	Creatinine	ALP	Urea	Uric acid	Total Bilirubin	Direct Bilirubin
Group-I	22.88 \pm 2.65	6.60 \pm 0.89	0.40 \pm 0.05	1.03 \pm 0.08	35.83 \pm 3.37	1.17 \pm 0.19	0.14 \pm 0.18	0.06 \pm 0.02
Group-II	26.18 \pm 2.93	7.37 \pm 1.38	0.41 \pm 0.04	1.24 \pm 0.39	34.67 \pm 3.33	0.89 \pm 0.18	0.05 \pm 0.03	0.06 \pm 0.04

Determination of Thiobarbituric acid-reactive substances (TBARS) assay:

TBARS as malon di aldehyde (MDA mmol/cm/g) was analyzed by Buege and Aust (1978). Liver and kidney tissues (100mg) were homogenized in 2ml of phosphate buffer (pH 7.0). TCA (10%), 0.5ml and 2ml of TBA mixture were added to tissue homogenate (0.5ml).^[5] The TBA mixture contained TBA (0.35%), SDS (0.2%), FeCl₃ (0.05mM) and BHT in glycine-HCl buffer (100mm, pH 3.6). The above reaction mixture was boiled at 100°C for 30 minutes and then allowed to cool. The mixture was centrifuged at 8000 rpm for 10 minutes and the absorbance was measured at 532 nm.^[6]

Conversion formula as per SOP: The highest possible human therapeutic dose of Tal Sindoor is 250 mg/ human/ day (1-2 Ratti) was converted to the dose in rats as mg/kg was administered to Wister Albino rats orally for 14 days (Gosh, 1984)

- Human dose is 125 mg, BD, i.e. 250 mg
- Total clinical dose (a) x conversion factor (b) 0.018 = (c) per 200 gm of rat

$$125 \text{ m} \times 2(a) \times 0.018 (b) = 4.5 \text{ m} (c) / 200 \text{ g of rat}$$
$$4.5 \times 1000/200 = 22.5 \text{ mg / kg.}$$

Experimental Doses Calculated as per the standard procedures are:

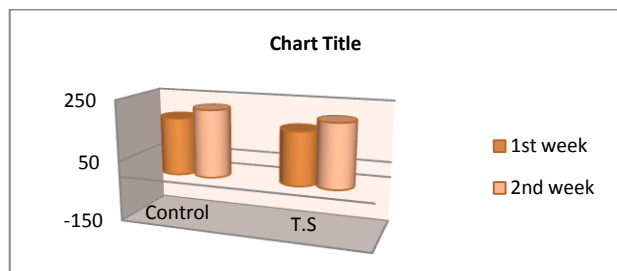
S. No	Groups	Dose /kg, weight	Dose /200 Gms. weight	Volume of administration
1	Vehicle Control	--	--	0.5 ml
2	Therapeutic Dose	22.5mg	4.5mg	0.5 ml

Sacrifice of animals: Animals were sacrificed after 14 days by administering mild anaesthesia. Blood was collected from heart using heparinized syringe and plasma was separated which is stored at 80°C for further use. Liver and kidney were stored in 10% formalin solution for histopathological examination. The tissue samples of liver and kidney were processed for histopathological procedure which was conducted by staining sections in haematoxylin Eosin (Leshner & Poole, 1989, (a)) and TBARS assays as per the prescribed procedures.

OBSERVATIONS AND RESULTS

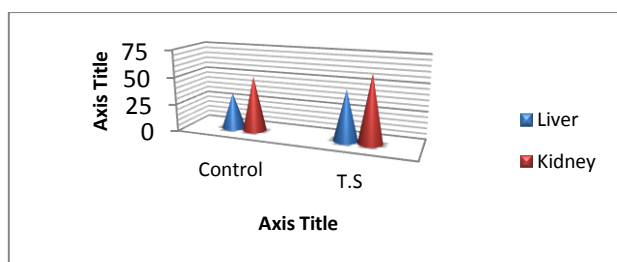
Histopathology reports of the tissue:

Micro section of liver showed normal architecture with hepatic lobules and hepatocytes arranged in sheets and cords with central veins normal for all rats. Micro section of kidney of all rats also showed normal cortex and medulla with normal glomeruli and collecting tubules [Figure-2].



Graph 1: Body weight gain (%) of group I and group II after the completion of the study.

Data express the mean SD for six rats on every week.



Graph 2: Estimation of lipid peroxidation

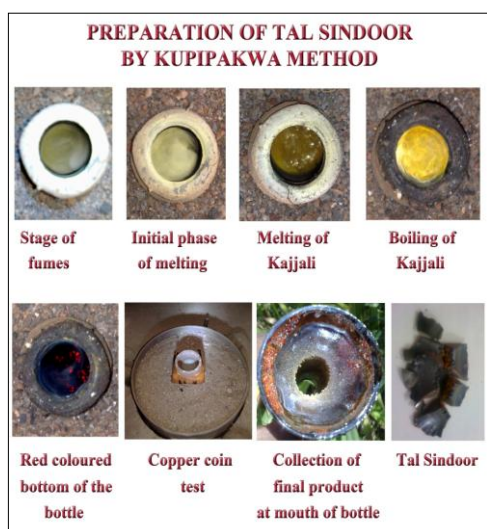


Figure 1: Preparation of Tal Sindoor

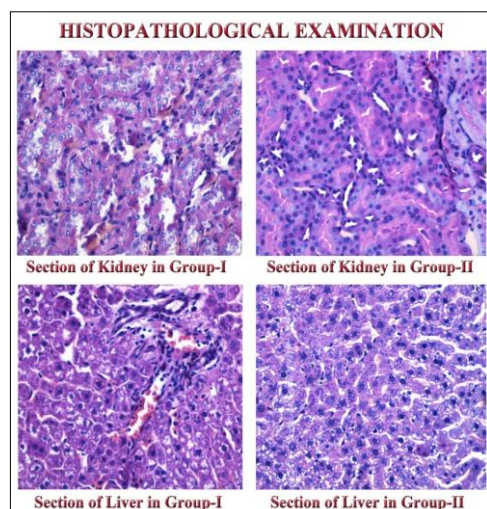


Figure 2: Liver and kidney section photomicrograph

DISCUSSION

Kupipakwa Rasayana are the most popular and advance preparations of Parada which involves the Jarana process. Tal Sindoor is final product of equal quantity by weight of mercury, sulphur and arsenic tri sulphide as ingredients processed by Kupipakwa method. Mercury and arsenic are metalloid and sulphur is non-metal. Arsenic is highly toxic in oxide form and their sulphate/sulphide compounds are less toxic comparatively. Mercury can give away 1 or 2 electrons to form a bond, sulphur can share by taking 2 to 6 electrons forming S^{-2} to S^{-6} valence and arsenic can share by giving away 3 electrons forming As^{+3} . Both arsenic and mercury have more affinity towards sulphur to form sulphide compound. Thus in this process of Kupipakwa, Parada is heated steadily along with Gandhak and haratal resulting in a profound bonding that may help to exhibit surpassing qualities compared to other formulations with same ingredients.

According to Ayurveda and modern science mercury and arsenic compound are considered toxic. But Ayurveda also describes usage of poison and toxic materials after stringent processing at judicial dose that can act as ambrosia. Thus to understand these aspects in different horizon this study was taken up. Healthy male albino rats of 140 g – 160 g, were selected for the study, for 14 days to evaluate toxicity and drug's effect on metabolism of these animals before clinical trials. Fixation of dose, preparation of medicine to administrable form and mode of administration to both groups were as per CCRAS SOP of administering Rasoushadhis. As drug was in powder form, for administration of Tal Sindoor a solvent media was required, so honey and de-ionized water was used. Honey is used as an Anupana (adjuvant) for administration of Tal Sindoor according to Ayurvedic classic too. Regular examination of rats was performed outside the cage to evaluate their general physical health and activity. General physical changes like colour, consistency of fur, colour of eye, nature and consistency of stool, secretions from eyes, ears, nostrils and, body weight of each animal was observed to elicit if any gross systemic toxicity existed. Intake of food and water was monitored. Around 15-20 g of pellet diet was consumed. Food and water intake monitoring would help to assess the physical health of animal and to ensure non-injury of oral cavity or oropharynx during the force feeding.

There was no morbidity or mortality in between or after the study. After 14 days of study animal was sacrificed to evaluate the toxicity on various systems. The data is expressed as mean standard deviation of the mean (SD). Data was analyzed using Student's t-test. Differences at $p < 0.05$ were considered to be significant. There was no significant change in the parameters like weight gain or food and water intake of the rats posted in Tal Sindoor group with comparison to the rats of vehicle control group. The weight gain of all animals of both groups was similar i.e. statistically not significant. The haematological values of group-II when compared with group-I indicated a marginal decrease of WBC, MCHC, LYM%, LYM, PDW, MPV and P-LCR parameters, and an increase in RBC, Hb, HCT, MCV, PLT and RDW parameters, which are non-significant statistically. Biochemical values are mean \pm SD of 6 rats. Values bearing different superscripts in the same column are significantly different if $p < 0.05$. There was no significant difference in SGOT, SGPT, Creatinine, urea, uric acid, total bilirubin and direct bilirubin in both the groups.

The TBRAS Essay was conducted to Liver and kidney tissues of rats of both the groups. Optic density is the marker value of this test. The value of optic density is specific to the type of organ. For example, optic density of brain tissue is 1.9-2.1, that of liver is 1.7-1.9. The presentation of normal limits in final optic density is suggestive of non-lysis of tissue and no remarkable pathological changes or transitional metal concentrations in cell due administration of Tal Sindoor for 14 days. The increased metal concentration can bring about changes in lipid peroxidation values. Since the statistical data was not significant,

it may be inferred that Tal Sindoor did not induce any metal concentration or depositions in liver and kidney tissues. Micro-section of liver and kidney suggested normal architecture. All the above tests conducted are to evaluate the liver and kidney function since liver is the defence organ that engulfs toxins/metals etc., to protect the body. Renal system is one of the vital systems excreting metabolic wastes and protects body by eliminating the toxins. Further evaluation in other animals is warranted before conducting human trials.

CONCLUSION

After peruse of data obtained from body weight gain, haematological, biochemical, TBARS assay and histopathological parameters it may be inferred that the test drug Tal Sindoor at treatment dose indicated no statistical significant variations. Tal Sindoor administered group showed absolutely good tolerance in all parameters studied when compared with vehicle control group evincing no toxicity of drug. Hence the mineral preparation Tal Sindoor can be considered safe for administration at treatment dose as described in classics at 2 ratti (250mg) dose.

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Conflict-of-Interest: There is no conflict of interest

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