Research Article

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A TOXICOLOGICAL EVALUATION OF SCHEDULE "E" IRRITANT POISON HARATAL (ORPIMENT/AS_2S_3) IN WISTER ALBINO RATS: AN EXPERIMENTAL STUDY

Sourav Ballav [1]*, Ashwini Kumar S Bharati [2], Sudhakar Bhat [3]

[1] Associate Professor, Department of Agada Tantra, Dhanvantari Ayurvedic Medical College and Hospital, Bareilly, Uttar Pradesh, India

[2] Professor, Department of Agada Tantra, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

[3] Department of Toxicology, S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India

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***Corresponding author**
E-mail: drsouravballav@gmail.com

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ABSTRACT

Background: Haratal (orpiment) is a schedule "E" of poisonous drug under THE DRUGS and COSMETICS ACT and RULES, 1945. Haratal (orpiment) is an allotropic α-form of arsenic, containing 61% arsenic and 39% sulphur. Humans are commonly exposed to haratal (orpiment) mainly through oral, inhalation, or per vagina route. In this study, the median lethal dose of haratal (orpiment) poisoning was calculated. Aim and Objectives: To evaluate LD_{50} of haratal (orpiment) poisoning by different routes. Material and Method: In this study, the median lethal dose of haratal (orpiment) poisoning was estimated in albino rats using OECD 425 guidelines with AOT software; Wistar-strain rats were taken from the Animal House of S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. Experimental study design: Albino rats aged 8-12 weeks, either sex, with an average weight of 170-300 grams were used. They will be housed under standard laboratory conditions and ambient temperature with a 12-hour natural light/dark cycle. All animals will be acclimatised in the laboratory for about a week and will be fed with free access to water before commencement of the study. AOT and OECD -425 guidelines were followed throughout the experiment. Food will be withdrawn one day before the experiment starts, but water will be provided. Conclusion: Estimated LD_{50} is 1098mg/kg-body weight.

Keywords: Arsenic Poisoning, LD_{50}, OECD, Orpiment, Fetal Dose, Arsenic Poisoning

INTRODUCTION

Ayurveda most commonly uses heavy metal and mineral compounds in therapeutic management. Among those compounds, the use of Parad (mercury / Hg), Haratal (orpiment / AS_2S_3), Manashila (realgar / AS_2S_2) and Gouripasana (white arsenic/AS_2O_3), etc., is common. However, the pioneer Ayurvedic text Charaka Samhita mentions the therapeutic use of metals, but Acharya Nagarjuna developed the authentication of metal compounds and their medico-legal use. However, in the present scenario, Haratal (orpiment) has several medicinal and industrial preparations[1]. Haratal (orpiment) is considered as the nucleus of rasaaushadhi (herbo-mineral formulations). "The father of Indian surgery" Sushruta first described Haratal as a "dhatu visha" (metallic poison) [2]. Haratal (orpiment) is an inorganic arsenic compound that contains 61% arsenic and 39% sulphur[3]. Suicidal poisoning is common by taking a large amount of Haratal (orpiment) [4,5]. Haratal (orpiment) has been used for suicidal purposes because it is inexpensive, easily available in the market, has a sweet taste and has high toxicity. Dr J. B. Mukherjee and Dr R.N. Karmakar[19] in the year 2000 cited a case where a lady from West Bengal, India, took a sweetmeat of butter, flour and yellow sulphide of arsenic at 6 p.m.; toxic signs and symptoms appeared 6 hours later, and she died after 39 hours. Her stomach contained coarsely powdered yellow arsenic[6]. Haratal's (orpiment) accidental poisoning is not uncommon when the orpiment is widely used as the main ingredient of herbal-mineral pharmaceutical industries and as a pigment agent in the art industry for painting dolls, toys, and wooden poles [7] and as a moderate cytotoxic agent in chemotherapy [8], aphrodisiacs, and external dermatological applications. Injudicious use of haratal (orpiment) as a cytotoxic agent, overdosing of haratal-containing medication, improper manufacturing process and therapeutic modification, prolonged use of haratal (orpiment) containing ointment over skin or open wounds, and applications into the uterus for abortion can lead to accidental poisoning. Workers in different herbal-mineral pharmaceutics and art industries have suffered from accidental haratal (orpiment) poisoning due to exposure to haratal (orpiment) dust and aerosol. Medicinal and industrial deposition of haratal (orpiment) may also be a cause of elevated arsenic levels in groundwater in India[9]. Bengal Chemical Examiner's 1945 annual reports have cited a case where a woman inserted a cotton wool swab smeared with haratal (orpiment) into her vagina to produce abortion; on having failed to get the desired results, she took it orally also and died from its effect. Arsenic was detected in her viscera as well as the cotton wool swab[10]. Previously orpiment was the poison of choice for assassinations creeds. Haratal (orpiment), when used for homicidal purposes, is commonly given in curries and cooked lentil curry (dal), to mask their colour[11]

The Government of India (Ministry of Health and Family Welfare) declared haratal (orpiment) as a deadly metallic irritant poisonous substance and enlisted it in schedule "E" of a poisonous drug under The Drugs and Cosmetics Act and Rules, 1945, [12] but the fatal dose, fatal period of haratal (orpiment) and even a specific antidote have not been discovered yet. To benefit human beings and protect them from haratal (orpiment) poisoning, it's time to conduct an experimental study to

scientifically validate and document the LD 50 (Median lethal dose) of haratal (orpiment) poisoning.

Aim and objective: LD_{50} of haratal calculation.

MATERIALS AND METHODS

250 g Haratal (orpiment) was purchased from the local market in West Bengal and authenticated at Sri Dharmasthala Manjunatheshwara College of Ayurveda Pharmacy, Hassan. After authentication, the toxicant haratal (orpiment) was ground into a fine powder using a mortar and pestle under aseptic conditions to reduce particle size, increase bioavailability, and achieve the desired plasma toxin concentration. The toxicant Haratal (orpiment) was then preserved in an airtight container. The final product weight was 210 g. Wistar strain rats were taken from the Animal House of S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. The toxicant dose was calculated based on data generated during AOT analysis using OECD 425 guidelines and AOT Software. Experimental study IAEC approval number: CPCSEA/IAEC/SDMCH/-AT-19.

Inclusion Criteria

1. Healthy Albino rats of either sex were considered.
2. Weight: about 170-300 gm.

Exclusion Criteria

Rats that did not fulfil the above criteria, pregnant rats, diseased rats, and rats under other experimental trials were excluded.

Study design: An experimental Randomised Trial.

OBSERVATION AND RESULT

Table 1: Oral Haratal (Arsenic trisulphide) dose fixation (According to the AOT software)

Rat. No.	Identification the animal	Desired dose (mg)	Body weight (grams)	Calculated dose (ml)
R1	Head	175	253	2.53
R2	Neck	550	254	2.54
R3	Middle of the back	2000	2.10	2.10
R4	Tail	2000	2.25	2.25
R5	Fore limb	2000	278	2.78

Mortality: All animals were observed at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 24 hours, and 48 hours after dosing, and then daily for mortality throughout the study. The animals were normal. There was no mortality in higher doses and no signs of acute oral toxicity. After 10 days, sub-acute toxicity signs and symptoms, especially haematuria, salivation and alopecia, were observed.

Table 2: Intra-peritoneal Haratal (Arsenic trisulphide) dose fixation (According to the AOT software)

Rat. No.	Identification the animal	Desired dose (mg)	Body weight (grams)	Calculated dose (ml) (1ml/100g body weight)
R1	Head	175	253	2.5
R2	Neck	550	254	2.5
R3	Middle of the back	2000	250	2.5
R4	Tail	550	225	2.2
R5	Fore limb	2000	275	2.7
R6	No mark	2000	247	2.47
R7	Head and tail	2000	248	2.48

Table 3: Intra-peritoneal Haratal (Arsenic trisulphide) result

Rat no	Dose (ml)	Observation	Mortality	Sign and symptom
R1	2.5	The animal looked normal	No	No sign of any toxicity
R2	2.5	The increased motor activity and auditory response were observed after 1 hour of drug administration. Straub's reaction was present even after 24 hours of drug administration	No	The animal looked normal with no sign of any toxicity
R3	2.5	Convulsions, increase in respiratory rate, decrease in motor activity, ataxia movement, and nasal secretion were observed.	Yes	Rat died after 50 minutes after the drug administration
R4	2.2	Irritability, rearing and jerk movements were present within 6 hours of drug administration	No	Salivation, diarrhoea, hematuria, decreased motor activity
R5	2.7	Increase in respiratory rate, decreased motor activity and nasal bleeding were observed.	Yes	Rat died after 30 minutes due to respiratory failure.
R6	2.47	The animal looked normal	No	No toxic signs were present even after 24 hours of drug administration.
R7	2.48	Convulsion was observed	Yes	Rat died after 1 minute of drug administration

LD_{50} (Median lethal dose) calculation: The above data were entered into the AOT software to obtain the LD_{50} value and confidence limits. Software Graph pad -3 was used. Estimated LD_{50} = 1098 (Based on an assumed sigma of 0.5). The approximate 95% confidence interval is 550 to 2000 mg/kg.

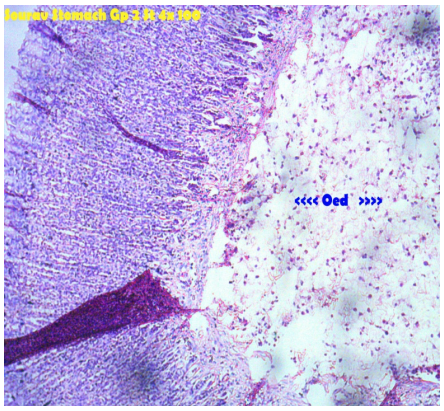

Figure 1: Stomach sub-mucosal oedema

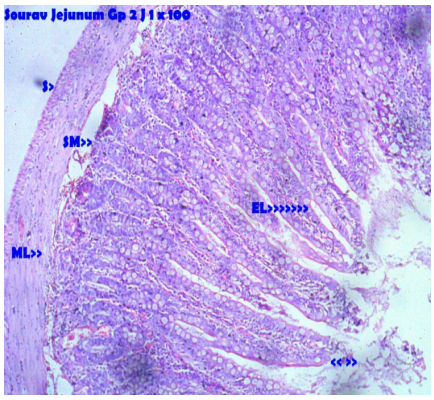

Figure 2: Jejunum epithelial layer erosion

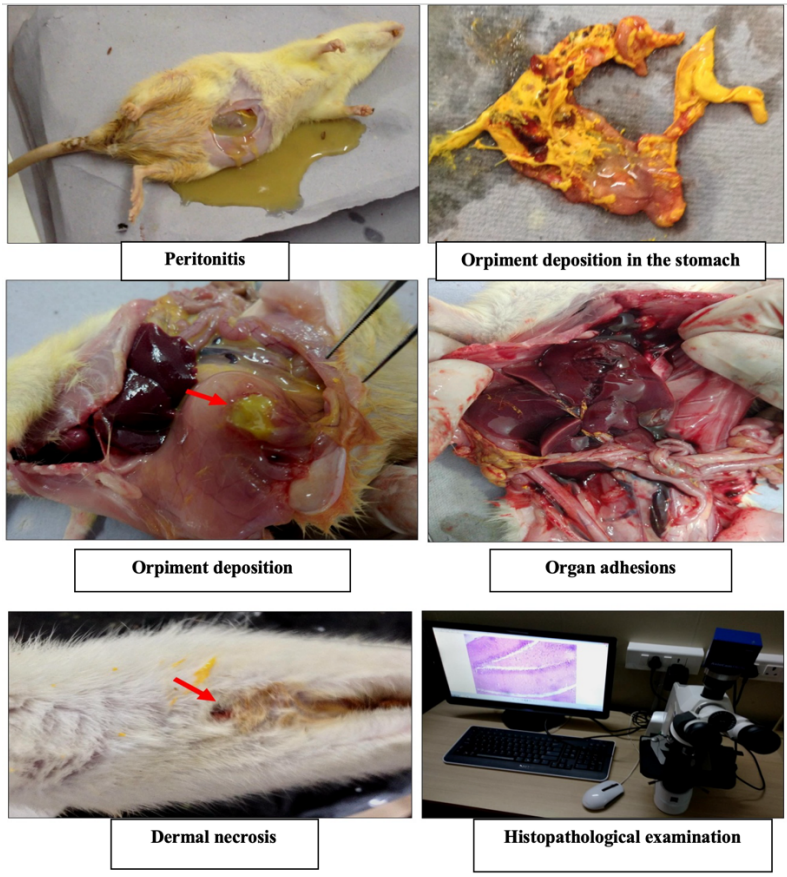

Figure 3: Toxicological changes due to Haratal (orpiment)

DISCUSSION

Haratal (Orpiment/Arsenic trisulphide) has low solubility in water and is poorly absorbed in the gastrointestinal tract of Wistar albino rats.[13] Haratal (Orpiment/Arsenic trisulphide) is expelled through urine and faecal matter due to the fate of the poison [14, 15]. Thus, only a small portion of arsenic trisulphide was absorbed and reached the bloodstream, which was insufficient to produce acute toxicity. It's only produced sub-acute toxicity in albino rats. For further study, an intraperitoneal Haratal (Arsenic trisulphide) injection procedure was used[16]. However, from inorganic arsenic compounds like Haratal (Arsenic trisulphide), arsenic is secreted into the stomach and intestine after absorption, even when given by routes other than the mouth [17]. After intraperitoneal injection, haratal (orpiment) is absorbed into the circulatory system and secreted into the stomach and intestines; reabsorption then occurs from the stomach and intestines, producing submucosal haemorrhages [18]. Haratal (orpiment) induced stomach submucosal oedema (Figure 1) and Jejunum epithelial layer erosion (Figure 2) were found in this study.

CONCLUSION

LD_{50} (lethal dose) of haratal (orpiment) was obtained by administering the toxicant through the intraperitoneal route in Wistar albino rats. Estimated LD_{50} =1098mg / kg-body weight of Wistar albino rats (Based on an assumed sigma of 0.5). The approximate 95% confidence interval is 550 to 2000 mg/kg. However, in this study, we found that oral administration of haratal (orpiment) produced only sub-acute toxicity in albino rats. The aforesaid needs further evaluation.

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