

## **ACUTE TOXICITY STUDY OF HAMSA MANDOORA AND VIDANGADI LOHA IN WISTAR ALBINO RATS**

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**Abstract:** Hamsa Mandoora and Vidangadi Loha are rich in iron and are used in regulation of iron disorders in Ayurveda. Safety aspects of these drugs are needed as per government's regulations, so present work was taken up for the same reason. Acute toxicity study was conducted on Wistar albino female rats with objectives to observe histo-pathological, hematological and biochemical changes, if occurs with taken dose 2000 mg/Kg. Except total protein, all biochemical changes revealed significant differences at 0.05 level in Hamsa Mandoora and Vidangadi Loha groups when compared with control, but all observations were under normal range only. No changes in weight in organs or cyto-architecture were noted

**Key words-** Hamsa Mandoora, Vidangadi Loha, Biochemical, Hematological, Histology, Acute Toxicity, Wistar albino rats, mortality, lethal dose.

### **Introduction:**

Based on origin, drugs used in Ayurveda are from animal, herbal, metal and mineral sources<sup>1</sup>. Among the metal and mineral groups, gold and five of lohas viz., copper, silver, tin, lead along with byproducts of lohas, arsenic compounds, silica, calcite, red chalk, salts and gems are referred<sup>2</sup>. These metals and mineral drugs are separately detailed in a branch named Rasashastra. Methods

opted correspond to the alchemy familiar in the Mediterranean and western Europe<sup>3</sup>. Rasashastra specifically mentioned shodhana or purification, murchhana, marana, jarana etc. processes before using the metal or mineral drugs to reduce the toxicity<sup>7</sup>. Specified procedures are noted to make these drugs compatible to human body from absorption and toxicity points of view. Government of India clearly notifies for documentation

of toxicity study of Ayurvedic drugs as per guidelines of OECD (organization for Economic Co-operation and Development) and WHO (World Health Organization), as following the thalidomide catastrophe in forms of severe birth defects, necessities of toxicity study was realized globally.

Toxicity study is carried out to find the toxic effects and safety aspects of drugs. Toxic effect includes at the level of organ, cell and molecular function leading to toxicity either by uptake, distribution, metabolism, mode of action and excretion<sup>4</sup>. Establishing a dose-response curve and mode of action for toxic effects are mainly important of toxicity studies<sup>5</sup>. OECD officially recognizes the need of acute toxicity study to satisfy hazard classification labeling requirements in terms of risk assessment for human health<sup>6</sup>.

Acute toxicity study is produced within 24 hours of single or multiple doses of any drug within limit of 2000mg/kg however exceptionally dose of 5000 mg/kg is allowed<sup>6</sup>. This study is suggested after chemical evaluation of drugs/metals/minerals to

find the minimum dose causing lethality. It becomes mandatory for any medicine to specify the limits of quantity of metals or minerals<sup>8</sup>. So, Reddy et.al. (2017) have detailed with chemical evaluation of Hamsa Mandoora and Vidangadi Loha with XRD and FTIR study. Hamsa Mandoora and Vidngadi Lauha are formulations with two different forms of iron being Mandoora and Loha bhasma as main ingredients<sup>7</sup>. Acute toxicity study of these formulations are not reported, so present study is opted to reveal the safety and efficacy or any hazardous effect of these two Ayurvedic drugs through acute oral toxicity study via single dose application with anticipation to provide information on any major toxic effect.

#### **Materials and Methods:**

**Procurement of Drugs:** Drugs were prepared in pharmacy of *Gopabandhu Ayurveda Mahavidyalaya*, Puri, Odissa. The procedures mentioned in *Yogaratanakara*<sup>9</sup> were followed for preparation of *Hamsmandoora* while the method given in *Chakradatta*<sup>10</sup> was conformed to *Vidangadi Loha*. *Gomutra* was used as medium for purification in the preparation of both

the formulations as laid down in the classical reference stated above.

**Experimental Procedure:** The study was conducted as per OCED (TG)-423 guidelines<sup>11</sup> after approval of the work by the Institutional Animal Ethics Committee (IAEC) of Sugan Life Sciences Pvt. Ltd. and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA Registration number: 982/PO/Rc/S/06).

**Experimental Animals:** 18 female (nulliparous and non-pregnant) Wistar rats of 10-12 weeks age groups and bodyweight of 185-210 grams were taken. All selected animals were allowed to acclimatize to the experimental room conditions at least for five days prior to the commencement of dosing. The room temperature and relative humidity were maintained at  $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$  and 30-70% respectively. The room was maintained with 12 hours of artificial lighting and 12 hours of darkness phase. During the acclimatization period, the animals were observed daily for any clinical signs. Prior to the commencement of treatment, a detailed physical health examination

was performed on all animals by the veterinarian and animals with any evidence of ill health or poor physical condition were not chosen for the study.

Rats were identified with the animal number marked on the base of the tail using permanent marker pen and the cages were attached with colored cage card showing study number, study code, group number, and number of animals, sex, species, strain, dose, cage number and animal number.

**Dose formulation and administration:** Animals were fasted overnight prior to experiment and water was withheld 4 hours before starting the experiment. The doses of 2000 mg / kg for both Hamsa Mandoora and Vidangadi Loha were mixed with 0.5% Carboxy methyl Cellulose (CMC). For control, 0.5% was used. Initially three rats from these three groups were administered with specified dose of referred drugs. As mortality was not observed, so they were confirmed with three more rats for all three groups. Dose volumes were maintained at 10 ml/kg body weight.

The animals were observed for clinical signs of toxicity, morbidity and mortality twice in a day throughout the 14 days of experimental observation period. Body weights were recorded on day 0 (on the day of dosing) and on day 7 and day 14.

**Necropsy and Gross Pathology:** All surviving animals were euthanized by CO<sub>2</sub> asphyxiation and subjected to necropsy under the supervision of the veterinary pathologist. The animals from control and treatment groups were examined for any external and internal abnormalities of the organs related to drug exposure. The organs were preserved in Bouin solution.

Blood was collected from jugular vein for hematological and biochemical tests. Auto-analyzers like

Diatron Abascus 380 was used for hematological study while ark CKK-24 was for biochemical parameters.

**Statistical Analysis:** The difference in initial weight and final weight (on 14<sup>th</sup> day) was compared statistically. The data were analyzed by ANOVA followed by Dunnett's post hoc test. SPSS, version 20 was used for analysis.

**Result:** Increased bodyweight of rats were observed in all groups. However, difference in bodyweight on 0<sup>th</sup> day (initial) and 14<sup>th</sup> day (final) were more in control group. The change in bodyweight was observed significant at 0.05 level. The details of bodyweight are as shown in table number: 1.

**Table Number: 1**  
**Changes in Bodyweight (in g) in Three Groups**

	Control	Hamsa Mandoora	Vidangadi Loha
Initial bodyweight $\pm$ S.D.	190 $\pm$ 2.10	189.83 $\pm$ 1.83	190 $\pm$ 2.83
Bodyweight (14 <sup>th</sup> Day) $\pm$ S.D.	231.66 $\pm$ 2.58	210.33 $\pm$ 3.27	205.33 $\pm$ 3.14
Increased Bodyweight $\pm$ S.D.	41.67 $\pm$ 2.25	20.50 $\pm$ 2.07	15.33 $\pm$ 5.35

SD: Standard Deviation

**Effects on hematological parameters:** Effects of Hamsa Mandoora and Vidangadi Loha were observed on eight hematological parameters as shown in table number: 2 with mean values and standard deviation. All parameters were observed statistically significant at 0.05 level.

**Table Number: 2**  
**Effects on Hematological Parameters in Three Groups**

	Control	Hamsa Mandoora	Vidangadi Loha
Haemoglobin $\pm$ S.D.	14.04 $\pm$ 0.32	15.15 $\pm$ 0.24	15.59 $\pm$ 0.25
TRBC ( $10^6/\mu$ l) $\pm$ S.D.	8.20 $\pm$ 0.05	8.51 $\pm$ 0.04	8.70 $\pm$ 0.03
TWBC ( $10^3/\mu$ l) $\pm$ S.D.	6.71 $\pm$ 0.07	7.88 $\pm$ 0.29	8.53 $\pm$ 0.10
PCV (%) $\pm$ S.D.	44.83 $\pm$ 1.73	49.88 $\pm$ 1.41	50.50 $\pm$ 2.25
MCV (fl) $\pm$ S.D.	52.40 $\pm$ 0.34	54.11 $\pm$ 0.14	54.84 $\pm$ 0.18
MCH (pg) $\pm$ S.D.	17.39 $\pm$ 0.15	17.55 $\pm$ 0.06	17.62 $\pm$ 0.05
MCHC (g/dl) $\pm$ S.D.	33.00 $\pm$ 0.11	32.71 $\pm$ 0.08	32.64 $\pm$ 0.10
Platelet ( $10^3/\mu$ l)	986.50 $\pm$ 25.95	895.00 $\pm$ 19.75	865.00 $\pm$ 21.68

SD: Standard Deviation, g: gram, dl: deciliter,  $\mu$ l: microliter, fl: femtoliter, pg: pictogram, TRBC: Total Red Blood Cell, TWBC: Total White Blood cell, PCV: Packed Cell Volume, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHV: Mean Corpuscular Haemoglobin Volume

**Effects on Biochemical Parameters:** Ten parameters were observed for biochemical comparisons among the groups. All parameters except total protein exhibited significant differences at 0.05 level. Total protein revealed the significance difference of 0.26. Observed mean values and standard deviation of different biochemical parameters are shown in table number:3.

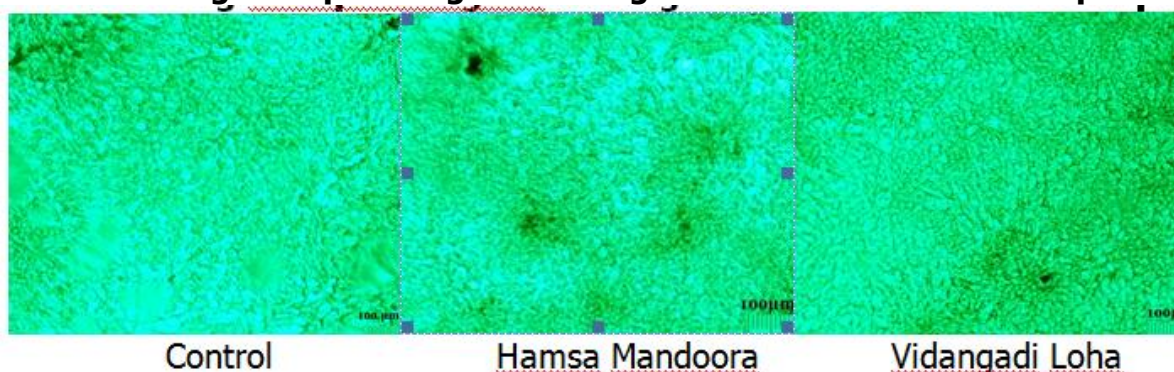
**Table Number: 3**  
**Effects on Biochemical Parameters in Three Groups**

	Control	Hamsa Mandoora	Vidangadi Loha
Blood Sugar (mg/dl) $\pm$ S.D.	84.50 $\pm$ 1.04	92.33 $\pm$ 1.21	94.00 $\pm$ 1.41
Cholesterol (mg/dl) $\pm$ S.D.	55.33 $\pm$ 1.75	51.33 $\pm$ 1.37	48.50 $\pm$ 1.87
Triglycerides (mg/dl) $\pm$ S.D.	191.16 $\pm$ 1.83	181.66 $\pm$ 2.07	179.16 $\pm$ 1.47
Total Protein (g/dl) $\pm$ S.D.	7.02 $\pm$ 0.10	7.12 $\pm$ 0.12	7.13 $\pm$ 0.16
Urea(mg/dl) $\pm$ S.D.	39.50 $\pm$ 1.64	36.16 $\pm$ 1.47	35.67 $\pm$ 1.03
Creatinine (mg/dl) $\pm$ S.D.	0.96 $\pm$ 0.01	0.90 $\pm$ 0.02	0.85 $\pm$ 0.01
Total Bilirubin(mg/dl)	0.54 $\pm$ 0.02	0.72 $\pm$ 0.02	0.78 $\pm$ 0.02
AST/SGOT (IU/L)	150.83 $\pm$ 3.76	183.67 $\pm$ 4.50	205.83 $\pm$ 5.77
ALT/SGPT (IU/L)	109.83 $\pm$ 3.43	104.50 $\pm$ 3.01	99.67 $\pm$ 2.16
Alkaline Phosphatase (IU/L)	148.84 $\pm$ 4.96	194.50 $\pm$ 6.72	206.17 $\pm$ 6.73

SD: Standard Deviation, g: gram, mg: milligram, dl: deciliter, IU: international unit, L: Liter, AST: Aspartate Aminotransferase, SGOT: Serum Glutamic-oxaloacetic Transaminase, ALT: Alanine Aminotransferase, SGPT: Serum Glutamic Pyruvic Transaminase

Cytoarchitecture of the spleen, thymus, lung, stomach, lymph node, trachea, adrenal, prostate, seminal vesicle, testis, ovary and uterus exhibited normal structure. Only slight fatty changes were observed with liver (figure number:1).

**Figure Number:1**  
**Showing Histopathological Findings in Liver of Different Groups**



**Discussion:** Increased bodyweight observed in all three shows no deleterious effects in any of these groups after drugs administration. Even so, comparatively greater differences in initial and final bodyweight in control group are observed. All the same, microscopic studies follow the same trend with slight changes in fatty acid deposition in histological revelation of liver under control and Hamsa Mandoora groups. Histological studies of all other organs marked normal status.

Hematological parameters are marked under normal range in all three groups. Haemoglobin (Hb) is iron containing metalloprotein in red blood cells (RBCs) works as oxygen carriers. It corresponds to total RBC, packed cell volume (PCV) percentage, mean cell volume (MCV) of red cells,

mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). All three groups revealed normal range of Hb, TRBC, TWBC, PCV, MCV, MCH and MCHC. Significant changes noted between the groups are based on intake of Hamsa Mandoora and Vidangadi Loha. Vidangadi Loha shows slightly higher reading than Hamsa Mandoora. Platelet count is also under normal range for all three groups however, decline in count was observed in Vidangadi Loha from Hamsa Mandoora and Control group.

Hamsa Madoora and Vidangadi Loha are suggested for regulation of iron in body<sup>9,10</sup>. As iron deficiency is associated with increased hepatic lipogenesis and lipemia<sup>14</sup> which contributes to increasing bodyweight by increased lipid level. Cholesterol and

triglycerides are types of lipids which circulate in blood. Regulated iron maintains hyperlipidemia<sup>15</sup> as shown in significant differences between groups at 0.05 level, being least cholesterol and triglycerides in Vidangadi loha group.

Blood sugar level was marked maximum in Vidangadi loha and minimum in control group as iron depletion is beneficial in insulin secretion, increasing glycolysis and in controlling glucose level<sup>12,13</sup>. Both Hansa Mandoora and Vidangadi Loha are rich in iron<sup>7</sup>. Iron increases formation of reduced NADH by citric acid cycle and increases mitochondrial consumption and ATP formation through oxidative phosphorylation<sup>19</sup> which downregulates glucose. Still all groups have shown sugar level under normal range only.

Total protein comparison was not significant between and within groups. The end product of protein metabolism comes in forms of urea. This is formed by condensation of ammonia and carbon dioxide with phosphate<sup>16, 18</sup>. Similarly creatinine is byproduct of important muscle components viz., creatine and phosphocreatine<sup>17</sup>. Still

urea and creatinine are in normal range.

Bilirubin is an endogenous anion resulted from haemoglobin destruction and reading beyond the normal range suggests liver problems<sup>20</sup>. Bilirubin noted in all three groups are under normal range only. Aspartate aminotransferase (AST) and Alanine aminotransferase catalyze the transfer of amino acids of aspartate and alanine to ketoglutaric acid. Higher AST indicates liver necrosis while that of ALT marks problems with liver along with heart, skeletal muscle and brain<sup>21</sup>. Present study exhibited normal range of AST and ALT in all three groups which reveals normal functioning of related organs. Even alkaline phosphatase is also under normal range with significant differences between the groups. Alkaline phosphatase originates from liver or bone and its level increases with intrahepatic or extrahepatic obstruction of bile flow<sup>22</sup>. Observations of all parameters shows no toxicity with given dose in any of these three groups. Only detailed study with greater doses are required to evaluate further.

**Conclusion:** At the oral dose level of 2000 mg/kg neither Hamsa Mandoora nor Vidangadi Loha produced observable toxic effects for hematological, biochemical or histological parameters. Even mortality is not caused at this dose. Lethal dose may be higher than the taken dose.

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**Source of Support: NIL**

**Conflict of Interest : None declared**