Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

# COMPARATIVE STUDY OF HEPATO-PROTECTIVE ACTIVITY OF LOHA-PARPATI PREPARED BY FOUR DIFFERENT METHODS

Kotabagi J. D., Vaidya S. S., B. Santhosh*, Jadar P. G.
K. L. E. U's Shri B. M. K. Ayurveda Mahavidyalaya,, Shahapur, Belgaum-03, Karnataka, India

Received on: 29/12/2010 Revised on: 01/02/2011 Accepted on: 11/02/2011

**ABSTRACT**
Loha parpati is one of the mineral based preparations which belongs to Parpati Kalpana and is said to be having properties like Lekhaniya (scraping), Rasayana (rejuvenation), Deepana (appetizer), Pachana (digestive), Raktavardhaka (haematinic) and Amahara (detoxifier). It is assumed that to have such properties it has action on Liver, which plays a major role in detoxification and excretion of endogenous and exogenous compounds. Hence Loha parpati was prepared by using four different methods and hepatoprotective activity was compared. The results showed that Dvigunajarita Loha parpati prepared by using Kantaloha bhasma shows significantly higher hepatoprotective activity compared to other Loha parpati.
**KEYWORDS:** Liver, Dvigunajarita, Loha parpati, hepatoprotective.

***Corresponding Author**
Dr. Santhosh B., PG Scholar Dept. of Rasashastra, K. L. E. U's Shri B. M. K. Ayurveda Mahavidyalaya,, Shahapur, Belgaum-03, Karnataka, India. Email: santtt@gmail.com

## INTRODUCTION

There is tremendous scientific advancement in the field of hepatology. But still the management of liver diseases is a challenge to the modern science. Liver, which plays a major role in detoxification and excretion of endogenous and exogenous compounds, has been threatened today by the indiscriminate use of systemic agents like tetracycline, paracetamol, anti-tubercular drugs, oral contraceptive pills, chemicals used as food preservatives, agro chemicals, etc. Liver is not only expected to perform physiological function, but it has to protect itself against the hazards of harmful medicines and chemicals. Hence, need of the hour is effective and safe hepatoprotective drugs. In this regard, the present study has been aimed to prepare Loha parpati by four different methods and to compare the hepatoprotective activity of the prepared Loha parpati on Albino rats exposed to $CCl_4$ hepatotoxicity.

## MATERIALS AND METHODS

### Preparation of loha parpati

Raw materials, Parada (Mercury), Gandhaka (Sulphur) and Kantaloha (Magnetite) were procured from Department of rasashastra, KLE University Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belagavi (Karnataka)-03. Shodhana (purification) of Parada and Shodhana of Gandhaka were done as per the text Rasatarangini. Shodhana of Kantaloha was done as per Rasaratna samuchchay and the Marana (incineration) was done as per Rasatarangini. Nirutthikarana (de-reviving) of Kantaloha bhasma was done as per Rasatarangini and its Amrutikarana was done as per Ayurveda Prakasha.

### Procedure

Kajjali (black sulphide of mercury) was prepared initially by triturating Shuddha Parada and Shuddha Gandhaka continuously in a clean khalva yantra (mortar and pestle). Equal amount of Parada and Gandhaka were taken for Samagunajarita (equal) and for Dwigunajarita Kajjali (double), Gandhaka was taken double the quantity of Parada. Kantaloha bhasma was then added and triturated for one hour continuously. Obtained mixture was taken in a Ghrtalipta lohadarvi (ghee smeared iron laddle) and heated on mandagni (moderate fire). Then the mixture, after melting completely, was poured on a Ghrtalipta (ghee smeared) Kadali patra (leaf of Musa paradisiaca), which was kept on Gomaya (cow dung) bed. Immediately it was pressed by another Ghrtalipta Kadali patra for 1 minute. After Swangasheeta (self cooling), thin flake (parpati) was obtained. The same procedure was adopted to prepare Loha parpati by using

Dwigunajarita kajjali, Kantaloha bhasma and Amrutikarana kantaloha bhasma.

**Observations**

Paka kalina (while heat treatment), the Kajjali started to melt in 5 minutes and at 160 $^{o}$ C – 180$^{o}$ C it looked like Tailaba (oily) and attained Pindibhuta (confection) consistency. Paka paschat (after heat treatment), venation marks of Kadali patra were observed on the Parpati (flake) and when it was broken "kut" like sound was produced.

**Experimental study**

To assess the hepatoprotective activity of Lohaparpati hepatotoxicity was induced by $CCl_4$ in Albino rats and parameters like enzyme study (SGOT and SGPT) and histo-pathological studies were carried out and the extent of regenerative changes were observed and compared.

**Determination of $LD_{50}$ and $ED_{50}$**

$LD_{50}$ was determined by adopting Miller and Tainter (1954) method and the $LD_{50}$ (mg/kg) of Samagunajarita Lohaparpati using kantaloha bhasma was 2499.76±52. Then the $ED_{50}$ (mg/kg) of the same was evaluated as $1/10^{th}$ of $LD_{50}$ i.e. 249.9±5.2 by using Paget G. E. and Barnes J. M. Conversion table.

**Screening of hepatoprotective activity**

Handa S. S. and Anupama Sharma (1990) method was followed. Healthy Albino Rats of either sex weighing between 150gm – 200gm and 8 – 10 weeks old were used for the screening. Three groups consisting of 6 rats in each group were made. CPCSEA guidelines were followed for their housing. Drug suspension was prepared by mixing the powder of parpati with 2% gum acacia and administered.

Group A served as control and received single daily dose of 1ml/kg i.p. (intra peritoneal) of sucrose solution for 4 days along with 1ml/kg s.c. (subcutaneous) of olive oil on $2^{nd}$ and $3^{rd}$ days. Group B received single daily dose of 1ml/kg i.p. (intra peritoneal) of sucrose solution for 4 days along with 2ml/kg of Carbon tetra chloride ($CCl_4$) s.c. (subcutaneous) dissolved in equal volume of olive oil on $2^{nd}$ and $3^{rd}$ days. Group C, D, E and F received single daily dose of 250mg/kg of formulation by oral route for 4 days respectively and 2ml/kg of $CCl_4$ by i.p. route on $2^{nd}$ and $3^{rd}$ days.

**Note**: A - control, B - $CCl_4$ treated, C-Samgunajarita lohaparpati (using kantaloha bhasma), D - Dvigunajarita lohaparpati (using kantaloha bhasma), E - Samgunajarita lohaparpati (using Amruthikarana kantaloha bhasma) and F - Dvigunajarita lohaparpati (using Amruthikarana kantaloha bhasma).

All the rats in all the groups were sacrificed on $5^{th}$ day under light anesthetic ether. Blood from each rat was collected through retro-orbital plexus under ether anesthesia for bio-chemical investigation i.e. SGOT and SGPT estimation. Blood was allowed to coagulate at 37$^{o}$ C for 30 minutes and the serum was separated by centrifugation at 2500rpm for 10 minutes. The liver of all the experimental animals were removed and processed immediately for histological investigation.

**Enzyme level observation and results**

The degree of hepatotoxicity developed can be known by elevated levels of SGOT and SGPT activity which is attributed to generation of $CCl_3$ free radical during metabolism by hepatic microsomes which in turn causes peroxidation of lipids of cellular membrane. The results indicated that the D and C test groups showed significantly reduced the elevated levels of SGOT and SGPT when compared to other test groups. F group has reduced the elevated levels of SGOT and SGPT to lesser extent compared to D and C groups. E group has not reduced elevated levels of SGOT and SGPT, thus was insignificant. The enzymatic levels of SGOT and SGPT are indicated in table 3 and table 4 respectively.

**Histopathological observation and results**

Group A showed liver tissue within normal limits, normal hepatocytes, sinusoids, kupffer cells and architecture within normal limits. Group B showed hepato-cellular necrosis. Group C showed normal hepatocytes and normal architecture and hence proved to be a good hepatoprotective formulation. Group D showed normal hepatocytes and normal architecture hence showing good hepatoprotective activity. Group E showed marked hepatocytic degeneration which denotes poor hepatoprotective activity. Group F showed moderate hepatocytic degeneration denoting moderate hepatoprotective activity.

The above histo-pathological observations revealed that D and C test groups treated livers were found to have normal hepatocytes and normal architecture which signifies better hepatoprotective activity as compared to other groups. Results of biochemical estimation were reported as Mean ± S.D. for determination of significant inter group difference. Each parameter was analyzed separately and one way analysis of variance (ANOVA) was carried out. Dunnet's test was used for individual comparison.

**DISCUSSION AND CONCLUSION**

The Lohaparpati showed decrease in enzyme activity of both SGOT and SGPT which has been shown to be inducer of the microsomal enzymes. Thus hepatoprotective action of this drug is likely to be due to its ability to induce microsomal enzymes; there by accelerating the excretion of $CCl_4$. Also the

histopathological report showed normal hepatocytes and normal architecture. Hence hepatoprotective activity of Lohaparpati can be proved and it may be due to the combine effect of Lekhaniya, Rasayana, Deepana, Pachana, Raktavardhaka and Amahara properties of it. Dvigunajarita loha parpati prepared by using Kantaloha bhasma showed significant results as compared to other formulations.

**AKNOWLEDGEMENT**

Authors are thankful to **Dr B. S. Prasad,** Principal of KLE University's Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belgaum, Karnataka-03 for his guidance during the study.

## BIBLIOGRAPHY

1. Sharma Sadananda: Rasa Tarangini : 11th edition : Motilal Banarasi Das Publication : Jawahar nagar, Delhi.
2. Rasa Vagbhata: Rasa Ratna Samuchchaya: commentary- Dr. Indra dev Tripathi : Chaukhamba Sanskrit Bhavan, Varanasi.
3. Mishra Guluraj Sharma: Ayurved Prakash : 1999 : Chaukhamba Bharati Academy, Varanasi.
4. Anonyms: Bhaishajya Ratnavali: translated by – Ambikadatta Shastri : 1st edition : Shri Krishna das Prakashana, Mumbai.
5. Mishra Siddhinandan : Ayurvediya Rasashastra : 7th edition: 1997: Chaukhamba Orientalia, Varanasi.
6. Handa S.S. and Anupama Sharma, 1990, "Antihepatotoxic activity of Andrographolide paniculata", Indian journal oj Medicinal Research (B), vol-92(8), 276-283.

**Table 1: Showing the details of Loha parpati preparation**

| Sl no | Names | Weight in gm (before preparation) | Weight in gm (after preparation) | Time (minutes) | Melting point ($^oC$) |
|---|---|---|---|---|---|
| 1 | Samgunajarita lohaparpati (using kantaloha bhasma) | 15 | 13 | 5 | 180 |
| 2 | Dvigunajarita lohaparpati (using kantaloha bhasma) | 20 | 18 | 4 | 175 |
| 3 | Samgunajarita lohaparpati (using Amruthikarana kantaloha bhasma) | 15 | 14 | 4 | 170 |
| 4 | Dvigunajarita lohaparpati (using Amruthikarana kantaloha bhasma) | 20 | 18 | 3 | 160 |

Special Preacautions: Moderate heating to be done.

**Table 2: Method of screening of Hepatoprotective activity**

| Sl no | Group | Days | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1 | A | SS | SS,OO | SS,OO | SS | Animals were sacrificed |
| 2 | B | SS | SS, $CCl_4$ | SS, $CCl_4$ | SS | |
| 3 | C | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 4 | D | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 5 | E | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |
| 6 | F | TS | TS, $CCl_4$ | TS, $CCl_4$ | TS | |

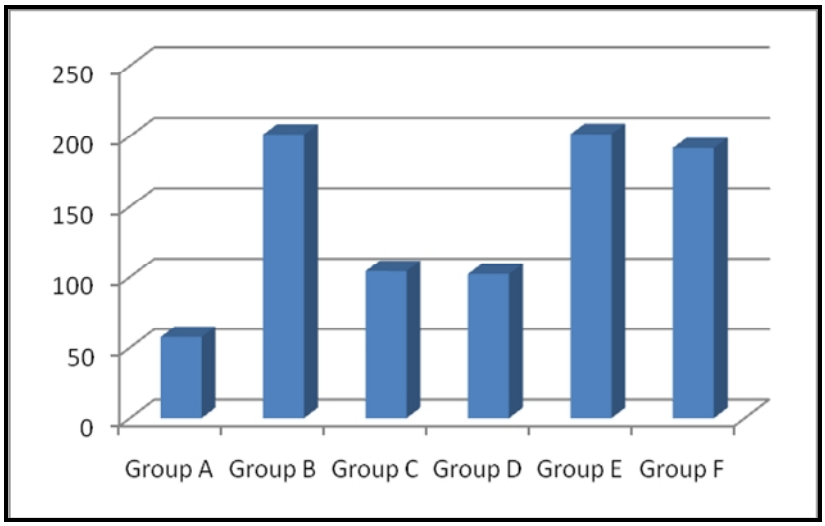
Note: SS – sucrose solution, OO – olive oil, $CCl_4$ - carbon tetra chloride in olive oil (1:1), TS – Test solution

**Table 3: SGOT levels**

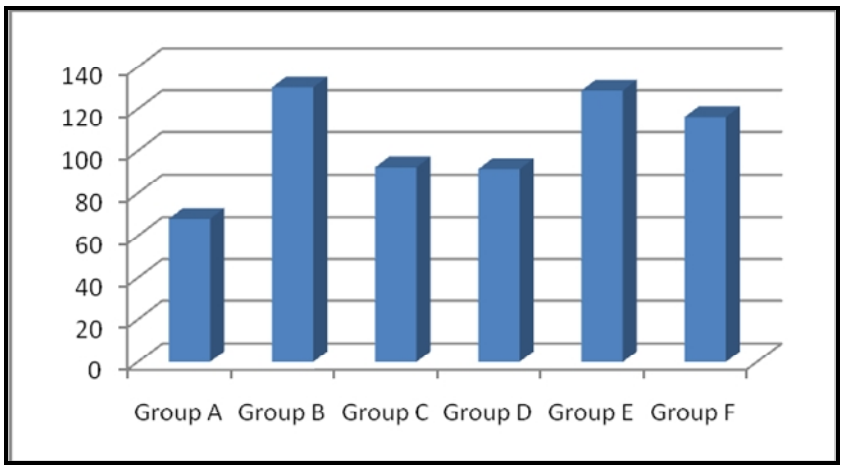
| Sl no | Group A IU/L | Group B IU/L | Group C IU/L | Group D IU/L | Group E IU/L | Group F IU/L |
|---|---|---|---|---|---|---|
| 1 | 58 | 210 | 100 | 110 | 210 | 200 |
| 2 | 54 | 198 | 98 | 98 | 198 | 188 |
| 3 | 60 | 205 | 110 | 98 | 198 | 188 |
| 4 | 56 | 198 | 107 | 100 | 207 | 197 |
| 5 | 61 | 205 | 100 | 107 | 196 | 186 |
| 6 | 56 | 201 | 110 | 100 | 200 | 190 |
| Mean | 57.5 | 201.2 | 104.2 | 102.2 | 201.5 | 191.5 |
| SD | 2.665 | 3.189 | 5.456 | 5.076 | 5.648 | 5.648 |
| SE | 1.088 | 1.302 | 2.227 | 2.072 | 2.306 | 2.306 |
| F ratio | 1035 | | | | | |
| P value | | | P<0.01 | P<0.01 | P<0.05 | P<0.01 |
| significance | | | Significant | Significant | Non Significant | Significant |

**Table 4: SGPT levels**

| Sl no | Group A IU/L | Group B IU/L | Group C IU/L | Group D IU/L | Group E IU/L | Group F IU/L |
|---|---|---|---|---|---|---|
| 1 | 64 | 130 | 96 | 88 | 129 | 110 |
| 2 | 67 | 127 | 91 | 91 | 121 | 107 |
| 3 | 71 | 129 | 98 | 94 | 134 | 120 |
| 4 | 66 | 131 | 89 | 91 | 127 | 124 |
| 5 | 72 | 134 | 88 | 89 | 129 | 110 |
| 6 | 68 | 130 | 92 | 96 | 132 | 126 |
| Mean | 68 | 130.2 | 92.33 | 91.5 | 128.7 | 116.2 |
| SD | 3.033 | 2.317 | 3.933 | 3.017 | 4.502 | 8.159 |
| SE | 1.238 | 0.9548 | 1.606 | 1.232 | 1.838 | 3.331 |
| F ratio | 687 | | | | | |
| P value | | | P<0.01 | P<0.01 | P<0.05 | P<0.01 |
| significance | | | Significant | Significant | Non Significant | Significant |




**Graph 1: Enzymatic level of SGOT**




**Graph 2: Enzymatic level of SGPT**

Source of support: Nil, Conflict of interest: None Declared