Hepatoprotective activity of Mercury Containing Lokanatha Rasa with special reference to Hepatocellular carcinoma

Coordinated by the Ministry of Environment, Sri Lanka
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Research team

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Introduction and theoretical framework

• Rasa shastra is a major branch in Auyurveda pharmaceutics
• Mercury is the major element of Rasashastra in Ayurveda medicine.
• The mercury preparations are called Rasaushadies.
• Hepatocellular carcinoma (HCC) is one of common carcinoma in the world. Due to lacks of effective therapeutics, and is one of the leading causes of death.
• Non-alcoholic fatty liver disease (NAFLD) is the acommonest predisposing factor for HCC.
• Lokanatha rasa (LKN) is a mercurial preparation mentioned in the classical Rasa shastra text Rasendrasara Sangraha for the effective management of liver and spleen disorders. Despite the doubts regarding the toxicity of mercurial preparations, there is no scientific research conducted regarding the efficacy of LKN for liver disorders and it is a timely need to scientifically evaluate and establish the safety of such preparations.
• This study was designed to standardize and evaluate the physicochemical parameters, antioxidant, anti-inflammatory properties of LKN, and its effect on HCC and along with its acute and chronic toxicity analyses.
Ayurveda and Rasaushadhis

- Ayurveda is a science of life that has been around for about 5000 years. Plants, minerals, animals, and metals are the most common ingredients in Ayurvedic medicines.

- Mercury, many metals, minerals, gems, calcium-containing materials, and several hazardous compounds are dealt with in Rasa shastra, an Ayurvedic area of medicine.

- All of these materials are subjected to unique processing techniques such as Shodana, marana, jarana, amritikarana according to Rasa shastra.

- As a result, these materials lose their undesired or harmful effects on the human body.

- All these mercurial formulations are known as Rasaushadhis, and many of them are utilized to treat a variety of chronic and incurable ailments, as well as for rejuvenation.

- Rasaushadhis provide several advantages, including fast action, minimal doses, tastelessness, longer storage life, and improved palatability.
Research problem

Mercurial medicines have been used for thousands of years to treat a variety of chronic and debilitating diseases.

However, concerns about the toxicity of mercurial, mineral, and herbomineral medicines have been raised recently.

The potential of Rasaushadies needs to be explored where contemporary medicine has limitations.

As a result, a scientific evaluation and determination of the safety of such preparations is required.
Objectives

Main objective
• To Evaluate the efficacy of LKN on HCC.

Specific objectives
• To standardize and analyze the physicochemical parameters of LKN.
• To evaluate the *in vitro* anti-inflammatory activity and antioxidant activity of LKN.
• To evaluate the anticarcinogenic activity of LKN on HepG2 cell line.
• To determine acute and chronic toxicity studies of Lokanatharasa.
Hepatocellular carcinoma

Prevalence in Sri Lanka = 78.9% of liver cancers account for HCC
Non Alcoholic Fatty Liver Disease

Prevalence of NAFLD in Sri Lanka = 32.6%

Global epidemiology of NAFLD related HCC (Huang et al., 2020)
Lokanatha Rasa

Ingredients

- Shuddha Parada (Purified mercury)
- Shuddha Gandhaka (Purified sulphur)
- Kajjali / mercury sulphide (1 part)
- Abhraka Bhasma / incinerated Mica (1 part)
- Lauha Bhasma / incinerated Fe (2 parts)
- Thamra Bhasma / incinerated Cu (2 parts)
- Varatika bhasma / incinerated Cowrie (6 parts)
The mixture is crushed for 7 days.

Subject to sharava samputa (800°C – 1000 °C) in muffle furnace.

Using the juice of Nagavalli (*Piper betel*)

Recommended dose - Two to three rakthika (250mg-375mg) (Rasendra Sarasangraha Pleeharogadeekara 2-5th stanza, Ayurveda Pharmacopoeia, Volume 1, Part 1, Pg 183)
Significance of the proposed research

• To prove the safety of mercurial preparations and promote to use of safe, efficacious mercurial preparation for hepatocellular carcinoma and hyperlipidemia.
Research design

- Ethical approval
- Standardization and physicochemical analysis of LKN
- Acute and chronic toxicity studies (using Wistar rats)
- Anti-inflammatory activity of LKN (using the Human RBC stabilization method)

Anti-oxidant effect of LKN (using the reducing power method, superoxide radical scavenging by DMSO and NO scavenging activity)

- Anticarcinogenic activity of LKN on HCC (MTT assay)
- Genotoxicity study (comet assay and micronucleus assay)
Preparation and standardization of LKN

Organoleptic characteristics of LKN

- Color – Ash
- Odor – None
- Taste – None
- Consistency – very fine and soft

- Total Ash value = 82.96%
- Acid insoluble Ash value = 3.29%
X-ray diffraction

X-ray fluorescence
• Atomic absorption spectroscopy

<table>
<thead>
<tr>
<th>Name</th>
<th>Quantity in the sample (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>1.2191</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.0442</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.5491</td>
</tr>
<tr>
<td>Antimony</td>
<td>0.4056</td>
</tr>
<tr>
<td>Lead</td>
<td>0.0896</td>
</tr>
</tbody>
</table>

• Particle size analysis

- XRD Image shows the major peaks of Calcium Carbonate (Calcite), sulphur, and strontium are also found.
- No mercury was detected in the formulation.
- No heavy metal content was detected in AAS
- Particle size analysis shows that the mean diameter of the particle was 30.49μm.
• Anti-oxidant activity of LKR
  • Reducing power method

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Standard (gallic acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.119±0.002</td>
<td>0.117±0.021</td>
<td>0.0118±0.013</td>
<td>0.068±0.000</td>
</tr>
<tr>
<td>100</td>
<td>0.135±0.001</td>
<td>0.136±0.013</td>
<td>0.138±0.008</td>
<td>0.087±0.001</td>
</tr>
<tr>
<td>250</td>
<td>0.151±0.034</td>
<td>0.149±0.007</td>
<td>0.152±0.012</td>
<td>0.098±0.001</td>
</tr>
<tr>
<td>500</td>
<td>0.164±0.010</td>
<td>0.161±0.019</td>
<td>0.165±0.013</td>
<td>0.112±0.001</td>
</tr>
<tr>
<td>1000</td>
<td>0.178±0.007</td>
<td>0.176±0.012</td>
<td>0.177±0.010</td>
<td>0.123±0.000</td>
</tr>
</tbody>
</table>

• Superoxide radical scavenging using DMSO

<table>
<thead>
<tr>
<th>Lokanatha rasa sample No</th>
<th>IC 50 Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>48.07</td>
</tr>
<tr>
<td>Sample-2</td>
<td>90.21</td>
</tr>
<tr>
<td>Sample 3</td>
<td>105.13</td>
</tr>
<tr>
<td>Standard gallic acid</td>
<td>176.80</td>
</tr>
</tbody>
</table>
• **NO scavenging activity**

<table>
<thead>
<tr>
<th>Antioxidant activity</th>
<th>IC$_{50}$ of Lokanatha rasa µg/mL</th>
<th>IC$_{50}$ of Standard ascorbic acid µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide scavenging activity (%) inhibition</td>
<td>1256.52</td>
<td>868.44</td>
</tr>
<tr>
<td>DPPH scavenging activity(%) inhibition</td>
<td>793.80</td>
<td>511.61</td>
</tr>
</tbody>
</table>
## ANTI INFLAMMATORY ACTIVITY - By-HRBC stabilization assay

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentrations(mg/ml)</th>
<th>Hydro alcoholic extraction(% protection)</th>
<th>Diclofenac sodium(% protection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>50</td>
<td>95.39</td>
<td>96.73</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>96.27</td>
<td>96.45</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>96.54</td>
<td>96.63</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>98.37</td>
<td>99.33</td>
</tr>
<tr>
<td>Sample 2</td>
<td>50</td>
<td>93.74</td>
<td>96.37</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>94.39</td>
<td>96.27</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>93.28</td>
<td>96.35</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>.</td>
<td>95.67</td>
</tr>
<tr>
<td>Sample 3</td>
<td>50</td>
<td>94.31</td>
<td>96.31</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>92.67</td>
<td>96.18</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>93.79</td>
<td>96.24</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>95.57</td>
<td>99.07</td>
</tr>
</tbody>
</table>
• Acute and chronic toxicity

✓ No significant difference between the LKN-treated test groups and control groups (p>0.05) with respect to behavioral changes, body weight, organ weights, biochemical parameters (glucose, urea, creatinine, total protein, albumin, globulin, ALP, SGOT, SGPT, Uric acid, D. Bilirubin, T. Bilirubin), and lipid profile (total cholesterol, HDL-cholesterol, Triglycerides, VLDL cholesterol, LDL cholesterol).

✓ No symptoms of toxicity and mortality were observed in different doses treated groups.

✓ Test drug at all dose levels did not affect the RBC-related parameters.

✓ Furthermore, histopathological analysis of the brain, liver, heart, spleen, kidneys, lungs and stomach revealed no gross lesions and/or ponderal changes of major organs.
• Genotoxicity
  • Micronucleus assay
  Micronuclei were not observed in the cells treated with LKN

• Comet assay
  Comets were not observed in the LKN treated cells
• Cell line study - By using Hep G2 cell line
  • MTT

Inhibition percentage with the treated concentration of Ayurvedic preparation.

Calculated IC\textsubscript{50} values for the ayurvedic preparations and for Cisplatin.
CONCLUSION

• Lokanatha Rasa is a mercurial preparation mentioned in Ayurveda Rasashastra texts and especially recommended for liver diseases.

• In this study Lokanatha rasa prepared by following standard operating procedures. This preparation showed remarkable antioxidant and anti inflammatory actions. Being a mercurial product it not shows any acute, chronic or geno toxicities.

• Lokanatha rasa can used for hepato cellular carcinoma patients effectively. Further clinical studies need for confirmed the effectiveness of this preparation.
Acknowledgements

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• THANK YOU