ANTIHEPATOTOXIC EFFECTS OF METHANOLIC EXTRACT OF LAGENARIA SICERARIA LEAVES IN RATS

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Summary

Treatment of rats with paracetamol turned out in significant elevation in the levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin. Rats treated with methanolic extract of leaves of Lagenaria siceraria (MELS) in dose of 250 mg/kg and 500 mg/kg body weight, reported significant (p<0.05) collapse in the biochemical enzymes. The protective effect of MELS extract was compared with the standard drug silymarin.
Various biochemical parameters such as glutathione (GSH), lipid peroxide (LPO), superoxide dismutase (SOD) and catalase (CAT) were also evaluated. The antioxidant parameters GSH, SOD and catalase levels were increased considerably in a dose dependent manner compared to their levels in paracetamol control groups. The results revealed that MELS possesses a potential antihepatotoxic activity.

**Keywords:** Hepatoprotective, Lagenaria siceraria, Biochemical parameters, Paracetamol

**Introduction**

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, etc). Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated.

Management of liver disease is still a challenge to the modern medicine [1]. In spite of remarkable strides in allopathic medicine, no effective hepatoprotective medicine is available that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Some of these plants have already been reported to possess strong antioxidant activity [2-3].

*Lagenaria siceraria* (Molina) Standley syn. *L. leucantha* Rusby; *L. vulgaris* Ser. (Family: Cucurbitaceae) commonly known as Bottle gourd, is a reputed vegetable plant used in traditional system of medicine for the treatment of various oxidative stress conditions. Its fruit has a composition of all the essential constituents that are required for normal and good health of humans [4].
Since time immemorial the fruit is used as immunosuppressant [5], diuretic [6], cardio-tonic, cardio-protective [7] and nutritive agent [8]. The fruit is also reported to have good source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene [9]. It is also reported to contain Cucurbitacins, fibres, and polyphenol [10]. Two sterols namely campesterol and sitosterol have been identified and isolated from the petroleum ether fraction of methanol extract of *Lagenaria siceraria* fruits, which is reported to possess antihepatotoxic activity [11]. The fruit has been reported to possess antioxidant activity [12], hypolipidemic and triton-induced hyperlipidemic rats [13]. Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of *lagenaria siceraria* possesses immunoprotective, antitumor, anti HIV and antiproliferative properties [14]. The present study was aimed to explore the prospect for hepatoprotective activity of methanolic extract of *L.siceraria* (MELS) leaves in the experimental rodents.

**Materials and methods**

**Plant material**

The leaves of *L. siceraria* were procured locally from Moradabad district of Uttar Pradesh in India and were authentified by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India) as *Lagenaria Siceraria* (Molina) standl. (Cucurbitaceae) leaves. Voucher specimens are kept in the herbarium (HC.MBD/HAP/BK/2010/7/167) of the Institute for further references. Leaves were washed with tap water and dried in shade. Dried leaves were ground to coarse powder and stored in an airtight container. The dried leaves were defatted with petroleum ether (60-80˚) by hot percolation in soxhlet apparatus and then extracted (250 g) with methanol in a soxhlet extractor for 18–20 h. The extract was concentrated to dryness under reduced pressure and controlled temperature (40–50 °C). Preliminary phytochemical screening [15] was carried out on the MELS to assess the presence of terpenoids, steroids, flavonoids and tannins in the extract.
Animals
Studies were carried out using male Wistar albino rats (120–170g). They were obtained from the animal house of the Teerthanker Mahaveer College of Pharmacy, Moradabad, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 22 ± 2 °C) with light and dark cycles of 12 and 12 h, respectively. They were allowed free access to the standard dry pellet diet and water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Paracetamol induced hepatotoxicity
Animals were divided into five groups of six animals each. Group I served as vehicle control and received 1% gum acacia suspension only. All other Groups received paracetamol once (500 mg/kg, p.o., aqueous solution) with Group II serving as paracetamol treated control. 48 h after paracetamol administration, Groups III, IV & V received MELS extract 250 mg/kg, 500 mg/kg and silymarin 100 mg/kg b.w., p.o. respectively, once daily for 5 consecutive days. 16 h after administration of last dose of drugs, the blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. ALP, SGOT, SGPT, and Bilirubin levels were estimated from the serum by using standard kits. The rats were sacrificed by ether anaesthesia on day 6 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for estimation of catalase and lipid peroxidation. Further the homogenate was centrifuged at 1000 g for 20 min at 4°C and the supernatant was used for the estimation of SOD and glutathione.

Biochemical analyses
The SGOT and SGPT levels in the serum were estimated using commercially available kits. The reduced glutathione (GSH) level in the liver was determined according to the method of Ellman [16]; hepatic superoxide dismutase (SOD) activity by the method of Kakkar et al. [17] and catalase by the method of Aebi [18]. The hepatic TBARS level, an index of malonyldialdehyde (MDA) production, was determined by the method of Ohkawa et al. [19].
Histopathological analysis of liver
For histological studies, liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50–100%) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Statistical analysis
The results are expressed as mean±SE and the difference between the groups were analyzed by one-way ANOVA. P<0.05 was considered significant. The post hoc analysis was carried out by Dunnet’s multiple comparison test.

Results and discussion
The preliminary phytochemical screening of MELS indicated the presence of terpenoids, steroids, flavonoids and tannins. The extract was found to contain 98.986 ± 0.1789 µg/mg total polyphenolics expressed as GAE (micrograms per milligram of GAE). There was a significant increase in the SGPT, SGOT and ALP levels in rats treated with paracetamol compared to normal control. In groups treated with 250 and 500 mg/kg of MELS, above activities of enzymes were found to be significantly (P<0.05) decreased. Maximum protection against paracetamol generated hepatic damage was offered at dose of 500 mg/kg of MELS.

Silymarin, which was used for comparative evaluation, produced a highly significant fall in the enzyme levels at 100 mg/kg dose level. Alleviated bilirubin levels were also reduced in MELS treated animals from 11.66 mg/dl to 3.42 mg/dl, when compared to paracetamol intoxicated animals.
Table 1. Effect of MELS on various biochemical parameters in rats with paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>32.71±1.56</td>
<td>41.14±2.07</td>
<td>51.03±2.39</td>
<td>2.1±0.42</td>
</tr>
<tr>
<td>PCM control</td>
<td>84.32±1.71</td>
<td>118.02±2.60</td>
<td>127.89±3.76</td>
<td>11.66±0.82</td>
</tr>
<tr>
<td>MELS (250 mg/kg)</td>
<td>58.83±2.74</td>
<td>79.26±3.28</td>
<td>77.01±3.62</td>
<td>4.59±0.73</td>
</tr>
<tr>
<td>MELS (500 mg/kg)</td>
<td>43.01±1.81</td>
<td>68.95±4.81</td>
<td>61.49±4.08</td>
<td>3.42±0.61</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>35.69±2.10</td>
<td>54.06±3.03</td>
<td>54.32±3.91</td>
<td>2.52±0.53</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group. P<0.05

N-acetyl-p-benzoquinoneimine (NAPQI) is the highly reactive metabolite of paracetamol, capable of binding covalently to cellular macromolecules (proteins, DNA) to produce protein adducts. Higher doses of paracetamol and N-acetyl-p-benzoquinoneimine can alkylate and oxidise intracellular GSH, which results in the depletion of liver GSH pool subsequently leads to increased lipid peroxidation and liver damage [20]. In our experiments it is observed that the lipid peroxidation levels in the paracetamol group is increased. This clearly indicates that there is a significant hepatic damage due to paracetamol and this is further evident from the fact that there is elevation in the levels of various markers of hepatic damage like SGOT, SGPT, ALP and total bilirubin. Treatment with MELS has decreased the levels of lipid peroxidation and the elevated levels of above mentioned biochemical markers to the near normal levels.
The data obtained in the present study (Table 2) clearly shows an increase in the MDA levels of rats treated with paracetamol suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. The increase in TBARS levels was significantly reduced by the treatment with 250 mg/kg and 500 mg/kg MELS (4.02 nmol/mg and 3.42 nmol/mg respectively) and by the treatment with silymarin (3.23 nmol/mg).

SOD has been reported as one of the most important enzymes in the antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage. The decreased SOD level in paracetamol treated rats was also observed when compared to control. The level of SOD was increased again by the administration of MELS and Silymarin. MELS causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver.

### Table 2. Effect of MELS on various parameters of oxidative stress in rats with paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO (nM/mg)</th>
<th>GSH (mM/gm)</th>
<th>Catalase (U/mg)</th>
<th>SOD (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>2.99±0.76</td>
<td>33.78±1.69</td>
<td>18.74±0.9</td>
<td>68.79±1.77</td>
</tr>
<tr>
<td>PCM control</td>
<td>7.27±0.74</td>
<td>9.51±0.802</td>
<td>7.37±0.93</td>
<td>22.67±2.03</td>
</tr>
<tr>
<td>MELS (250 mg/kg)</td>
<td>4.02±0.98</td>
<td>26.79±1.84</td>
<td>13.83±0.85</td>
<td>55.57±3.14</td>
</tr>
<tr>
<td>MELS (500 mg/kg)</td>
<td>3.42±0.67</td>
<td>28.14±2.68</td>
<td>14.14±1.12</td>
<td>61.33±2.58</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg)</td>
<td>3.23±0.83</td>
<td>30.85±1.51</td>
<td>16.66±1.18</td>
<td>64.19±2.01</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group. P<0.05
Catalase (CAT); an enzymatic antioxidant, decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [21]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. Administration of MELS increased the CAT level in paracetamol treated rats with induced liver damage, thus preventing accumulation of excessive free radicals and protecting the liver from paracetamol intoxication.

Fig. 1. Section of the liver of (A) rat treated with PCM, (B) rat treated with MELS (250 mg/kg) (C) rat treated with MELS (500 mg/kg) (D) rat treated with silymarin (100 mg/kg).

Histological observations basically supported the results obtained from serum enzyme assays. The liver of paracetamol-intoxicated rats showed massive fatty changes, gross necrosis and broad infiltration of lymphocytes and loss of cellular boundaries. Histopathological observations of the liver of rats treated with MELS showed a more or less normal architecture of the liver, having reversed to a large extent the hepatic lesions produced by the toxin, almost comparable to the normal control and the silymarin group (Fig. 1a-d).
Conclusion

Based on the results obtained, it may be concluded that the *Lagenaria siceraria* leaves extract has a significant effect on liver injuries as well as on oxidative stress, resulting in reduced lipid peroxidation and improved serum biochemical parameters such as AST and ALT. The reduced levels of parameters of SOD, CAT, and GSH in paracetamol-treated rats were significantly increased by treatment with *Lagenaria siceraria* leaves extract. However, the exact hepatoprotective mechanism of MELS is unknown, the extract may either inhibit the formation of the toxic paracetamol metabolite or stimulate the hepatic regeneration. This type of stimulation is known to cause the liver to become more resistant to damage by toxins. Apart from all these antioxidant effect of MELS can be the root cause for its hepatoprotective activity.

Acknowledgements

Authors are very thankful to Shri Suresh Jain, Hon’ble chancellor of the Teerthanker Mahaveer University for his invariable encouragement and endowing us with facilities necessitated for successful completion of the study.

References