HEPATOPROTECTIVE ACTIVITY OF Morus alba (Linn). LEAVES EXTRACT AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS.

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ABSTRACT

To investigate the hepatoprotective activity of Morus alba Linn. alcoholic leaves extracts against paracetamol-induced hepatitis in rats. The Morus alba leaves extracted with alcoholic (ALE) and water extract (AQE) against Paracetamol induced hepatotoxicity and using Standard drug is Liv-52. Preliminary phytochemical tests were done. The ALE showed presence of alkaloids, flavonoids, carbohydrates, tannins and steroids, did not produce any mortality. Paracetamol produced significant changes in biochemical parameters (increases in serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alanine phosphatase (ALP) and serum bilirubin.), histological (damage to hepatocytes). Using Standard drug Liv-52. Pretreatment with ALE extract significantly prevented the biochemical and histological changes induced by paracetamol in the liver. The present study that shows the ALE extract possessed hepatoprotective activity.

Keywords: Hepatoprotective; Paracetamol; Morus alba Linn.
INTRODUCTION:
The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. Presently only a few hepatoprotective drugs and that too from natural sources are available for the treatment of liver disorders.

*Morus alba* Linn (Moraceae) is also known as Tut in India. The white mulberry has a long history of medicinal use in Chinese medicine; almost all parts of the plant are used in one way or another. Recent research has shown improvements in elephantiasis when treated with leaf extract injections and in tetanus oral doses of the sap mixed with sugar. The fruit has a tonic effect on kidney. It is used in the treatment of urinary incontinence, dizziness, tinnitus, insomnia due to anemia, neurasthenia, hypertension, diabetes, premature graying of the hair and constipation in the elderly. The leaves are showing analgesic and anti-inflammatory activity of hydroalcoholic extract of leaves. Phytochemical review shows the presence of tannins, Vitamin A, flavonoid, thiamine, protein, carbohydrates. It has been used in the indigenous system of medicine for cooling, acrid, purgative, diuretic, laxative, anthelmintic, brain tonic, antibacterial, hepatopathy properties. They are useful in vitiated condition of *vata* and *pitta*, burning sensation.

Hence the present study was aimed to investigate the hepatoprotective activity of alcoholic leaves extracts of *Morus alba* L. against paracetamol-induced hepatitis in rats.

MATERIALS AND METHODS:

Plant Collection and Authentication

The leaves of *Morus alba* Linn. were collected from Ramling mudgad Dist.-Latur (Maharashtra); were authenticated by Dr.Harsha Hegade. Research officer Indian Council of Medical Research, Belgaum. A voucher specimen has been deposited at the herbarium of RMRC-465.

Preparation of Extracts

The plant material (leaves) were dried for several days and powderd with the help of an electric grinder. The course material was extracted in a Soxhlet exctracter with alcohol (90%) and aqueous. The extracts were dried at 50°C in a water bath. The percentage yields of alcoholic extract is 8.231% and aqueous extract is 11.10 %.
Chemicals
Liv-52 obtained from Himalaya drug, Bangalore. All other chemicals used were of analytical grade.

Experimental animals
Swiss albino mice (18-20 g) and Wistar rats (150–200 g) of either sex were procured from Sri Venkateshwara Enterprises, Bangalore and were acclimatized for 10 days under standard housing condition maintained at a room temperature of 24± 1ºC; related humidity 45-55% with 12:12 hrs light/dark cycle. The animals were habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize any nonspecific stress.

LD50 determination
Acute oral toxicity (AOT) of ALE and AQE were determined using nulliparous, non pregnant female mice. The animals were fasted for 3 hrs prior to experiment and were administered with single dose of extracts dissolved in 2 % w/v Tween 80 and observed for mortality for upto 48 hours. Based on the short term toxicity, the dose of next animal was determined as per OECD guidelines 425 (OECD .2001). All the animals were also observed for long term toxicity. The LD50 of the test extracts were calculated using ‘AOT 425’ software provided by Environmental Protection Agency, USA.

Hepatoprotective activity
The method of S. Ramachandra S et al.(S Ramachandra et al.-2008) was used in the study. Animals were divided into seven groups of 6 animals each. The first group received saline 1 ml/kg for one week (control). The group II received saline 1 ml/kg for one week (positive control). The groups III, IV,V,VI and VII received Liv-52 (4 ml/kg p.o.) and 150 mg/kg,300 mg/kg and 175 mg/kg, 350 mg/kg of Morus alba ethanolic extract respectively once a day for seven days. On the fifth day, after the administration of the respective treatments, all the animals of groups II, III, IV,V,VI and VII were administered with paracetamol 2 g/kg orally. On the seventh day after 2 h of respective treatments the blood samples were collected for the estimation of biochemical marker enzymes. Then animals under ether anesthesia were sacrificed. The livers from all the animals were collected, washed and used for the estimation [10-11].

Blood biochemistry
Blood sample were collected in glass tube from retro orbital puncture to obtain haemolysis free clear serum for the analysis of SGOT and SGPT (Reitman and Frankel, 1957), ALP (Walter and Schutt, 1974) and Bilirubin (Malloy and Evelyn, 1937) by standard method [12- 14].
Histopathology

Histopathology of liver was carried out by a modified of Luna. In brief, the autopsied livers were washed in normal saline and fixed in 10% formalin for 2 days followed with bovine solution for 6 hours. Then the livers were paraffin embedded and 5 µ thickness microtome sections made. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin. The slides were studied under a light microscope for any histological damage/protection\textsuperscript{[15-16]}.

Statistical Analysis

The data obtained were analyzed by One Way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test using computerized program. \(P\)-value <0.05 or was taken as the criterion of significance.

RESULTS & DISCUSSION:

Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, steroids in ALE. While alkaloids, carbohydrates, flavonoids and tannins were noticed in AQE. The ALE was found to be nontoxic up to a dose of 3000 mg/kg and LD\textsubscript{50} of AQE was found to be 3500 mg/kg.

Treatment of rats with paracetamol produced an increase in the weight and volume of wet liver. Rats were pretreated with Liv-52, ALE and AQE. ALE and AQE showed significant effect.

Paracetamol administration resulted in significant elevation of SGOT, SGPT, ALP and serum Bilirubin. Biochemical parameters were found to be decreased compared to normal control group due to pretreatment with Liv-52, ALE and AQE which significantly prevented the biochemical changes induced by paracetamol. The hepatoprotective effect offered by ALE was found to be significantly greater than AQE treatment. (Table -1).

Hepatocytes of the normal control group showed normal lobular architecture of the liver. In the paracetamol treated group the liver showed microvascular fatty changes and the hepatocytes were surrounded by large No. of fat droplets, Liv-52, ALE and AQE pretreated groups showed minimal fatty changes (fig.1) and their lobular architecture was normal indicating the hepatoprotective effect of these extracts.

However, ALE showed more microvascular fatty changes (fig.1) than AQE. The hepatoprotective activity of the extracts were in the order of Liv 52 > ALE > AQE.

The liver can be injured by many chemicals and drugs. In the present study, paracetamol was selected as a hepatotoxicant to induce liver damage, since it is clinically
relevant. Paracetamol produces a constellation of dose related deleterious effects in the liver \[^{17}\].

During hepatic damage, cellular enzyme like SGOT, SGPT, ALP and serum Bilirubin present in the liver cell, leak into the serum, resulting in increase concentration \[^{18}\].

Histological changes such as steatosis (fatty changes in hepatocytes) and perivenular fibrosis were observed in paracetamol control group. Both the extracts prevented these histological changes, further indicating their hepatoprotective activity. All the histological changes observed were in correlation with the biochemical and functional parameters of the liver.

It can be concluded that Morus alba leaves extracts viz. ALE and AQE possess a protective effect against paracetamol induced hepatotoxicity in rats but ALE shows more significant effect as evidenced by the biochemical, functional and histological parameters.

Fig 1: Histology of liver showing normal hepatocyte (a), Paracetamol induced microvascular fatty changes surrounding by large number of small fatty droplets (b), hepatocytes in groups treated with Liv-52 (c), ALE (150 & 300mg/kg) (d &e ) and AQE (175 & 350 mg/kg) (f & g) prior to administration of Paracetamol showing minimal fatty changes.
Fig. 2: Effect of PEE, CHE, ALE and AQE on serum biochemical parameters against Paracetamol (2 g/kg, s.c) induced liver damage. (A) Representation of alanine aminotransferase. (B) Representation of aspartate aminotrasferase. (C) Representation of alkaline phosphatase. (D) Representation of serum bilirubin.
Tab.1: Effect of *Morus alba* L. leaves extracts on paracetamol-induced hepatotoxicity
in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (ml/kg)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>Serum bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>31.42 ± 0.7574</td>
<td>88.50 ± 2.766</td>
<td>91.17 ± 5.498</td>
<td>0.2733 ± 0.1258</td>
</tr>
<tr>
<td>Liv-52</td>
<td>4</td>
<td>47.17 ± 1.851</td>
<td>100.2 ± 3.260</td>
<td>107.5 ± 3.631</td>
<td>0.2367 ± 0.015</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>2 g/kg s.c</td>
<td>400.25 ± 1.302</td>
<td>282.3 ± 2.290</td>
<td>431.3 ± 1.116</td>
<td>0.7150 ± 0.0217</td>
</tr>
<tr>
<td>ALE</td>
<td>150</td>
<td>114.7 ± 1.926*</td>
<td>287.7 ± 3.809*</td>
<td>213.3 ± 4.410*</td>
<td>1.882 ± 0.0452*</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>52.33±1.909**</td>
<td>255.2 ± 4.175**</td>
<td>184.5 ± 1.727**</td>
<td>1.015 ± 0.0506**</td>
</tr>
<tr>
<td>AQE</td>
<td>175</td>
<td>195.0 ± 1.880</td>
<td>361.8 ± 3.208</td>
<td>385.5 ± 8.884</td>
<td>2.175 ± 0.0495</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>108.0 ± 3.152*</td>
<td>309.5 ± 3.160*</td>
<td>307.5 ± 3.263*</td>
<td>1.740 ± 0.1145*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM, n= 6, (P**< 0.01) Vs Paracetamol treated group using one –way ANOVA followed by Tukey Kramer’s post hoc test.

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REFERENCES: