ABSTRACT
The objective of this study was to investigate the hepatoprotective activity of ethanolic extract of leaves of Holopetlea integrifolia (Roxb). against paracetamol induced hepatic damage. There are lack of reliable hepatoprotective drugs in modern medicine to prevent and treat paracetamol induce liver damage. The ethanolic extract showed presence of flavonoid and steroids. The plant material was dried in shade, powdered and extracted with ethanol. The hepatoprotective activity of the ethanolic extract was assessed in paracetamol induced hepatic toxic rats. Biochemical parameters like SGOT, SGPT, ALP and Bilirubin were tested in drug induced and untreated groups of rats. Treatment of ethanolic extract of Holopetlea integrifolia (Roxb). leaves has brought back the altered level of biochemical parameter to the normal level in the dose dependent manner and also compared with silymarin used as standard drug.

KEYWORDS : Hepatoprotective, ethanol, leaves, paracetamol, silymarin.

INTRODUCTION
The liver is one of the most important vital organs in the human body. Liver plays a significant role not only in metabolism and detoxification of exogenous toxins and therapeutic agents, but also in the bio regulation of fats, carbohydrates, amino acids, proteins, blood coagulation and immunomodulation. Many toxins target the liver and cause hepatotoxic effects that can be observed through some biochemical parameters. Impairment of the liver generally occurs from excessive exposure to xenobiotics, alcohol, chemotherapeutic agents, virus and protozoan infections. Depending upon the severity of toxicant insult, hepatic cell injury can lead from acute to chronic hepatitis, which if left untreated can result in cirrhosis or malignant lesions. 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. 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). Plant drugs are known to play a vital role in the management of liver diseases\[2,3\]. *Holoptelea integrifolia*, the versatile medicinal plant is the unique source of various types of compounds having diverse chemical structure a very little work has been done on the biological activity and plausible medicinal application of the compounds and hence extensive investigation is needed to exploit their therapeutic utilities to combat diseases. Phytochemical investigation shows the presence of chemical constituent such as terpenoids, alkaloids, glycoside, carbohydrates, steroids, sterols, saponins, tannins, protein, and flavonoids\[5\]. The isolated principle are Beta amyrin, Beta sitosterols, octacosanol, holopettelin-A, holopettelin-B, hederagenin, hexacosanol, Beta-D-glucose, fridelin, epifriedelin, 2-amino napthaqunone, 1,4-napthalenedione are considered as responsible for various activity\[7\]. The traditional uses, reported biological/pharmacological activity, isolated compounds and therapeutic application of holoptelea integifolia which might be useful for scientific and researchers to find out new entities responsible for therapeutic activity.

**MATERIAL AND METHOD:**

**Drugs and Chemicals:**
All the chemicals were analytical grade. Paracetamol was obtained from Amol Pharma Jaipur (Rajasthan). and silymarin from kyptron pharma.

**Plant collection and authentication**
The leaves of *Holoptelea integrifolia* was collected from Jhansi (U.P) and Identified and authenticated by National vrkshayurveda research institute Jhansi. The accession no. is NVRI/05551/2011. The leaves was dried in shade, and finally grounded in powdered form in and electronic grinder and stored in cellophane bags at 4°C until use.

**Preparation of Extract:**
The air dried and coarsely powdered leaves (350 g) were first extracted with 1ltr petroleum ether about 40-80°C to remove all fatty acids and again it is extracted with ethanol (95%) in a soxhlet apparatus for 70 hr .The extract were concentrated to dryness under reduced pressure and controlled temperature (30-50°C). The yield value of both the leaves extract is recorded.

**Animals:**
Healthy Wistar-albino rats weighing about (180-250gm) of either sex were obtained from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi. The animals were housed in specific standard laboratory conditions. The conditions were kept in a temperature-controlled environment (25±1°C) and with a regular 12h light/12h dark cycle. All animals were fed with commercial diet & water *ad libitum*, during the experiment. All protocols of the study was approved by the Institutional Animal Ethical Committee with reference no. BU/Pharm/IAEC/11/022. The IAEC is approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration no. 716/02/a/CPCSEA

**Dose and Treatments:**
Rats were divided into different groups (n=6). Silymarin syrup (0.5ml/100gm), Paracetamol (2gm/kg) & HIEE (200 and 400 mg/kg) were administered orally. The control group received 1.5% Tween 80 in distilled water as vehicle (10ml/kg B.W.).

**Experimental Design:**
The general principle involved in the evaluation of Hepatoprotective activity is to induce liver toxicity or infection with the help of hepatotoxin in the liver of experimental animals. The magnitude of the protective activity is measured *in-vivo* by estimating the following parameters:

**Biochemical Parameters:** These are the most reliable parameter in the *in-vivo* study & include the estimation of different enzyme like SGOT or Aspartate transferase & Alanine transferase or SGPT & Serum alkaline phosphate (SALP). It also includes estimation serum bilirubin (SBLN) & estimation of Hydroxyproline fat & protein content of Livers.

**Groups of Animals:** The animals were divided into five groups of six animals each.
• **Group I:** served as control & received 1.5% Tween 80 in distilled water as vehicle (10ml/kg B.W.) for 7 days.

• **Group II:** received vehicle for 7 days followed by Hepatotoxin (2gm/kg B.W.).

• **Group III:** received silymarin (0.5ml/100gm B.W. per day) simultaneously 7 days followed by Hepatotoxin.

**Table 1:** Effect of *Holopetlea integrifolia.* Extract on Biochemical Parameters in Rats Subjected to Paracetamol Induced Hepatotoxicity.

<table>
<thead>
<tr>
<th>Enzyme group</th>
<th>SGOT</th>
<th>SGPT</th>
<th>SALP</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>25.30±0.089</td>
<td>27.5524±0.085</td>
<td>89.217±1.5</td>
<td>0.78±0.06</td>
</tr>
<tr>
<td>PCM treated group</td>
<td>67.28±1.25</td>
<td>42.350±0.150</td>
<td>2414.25±26.03</td>
<td>1.875±0.006</td>
</tr>
<tr>
<td>Standard group</td>
<td>56.20±1.03</td>
<td>34.383±0.160</td>
<td>1456.20±0.839</td>
<td>1.317±0.006</td>
</tr>
<tr>
<td>HIEE(200mg/kg B.W.)</td>
<td>44.28±1.22</td>
<td>30.28±0.054</td>
<td>424.100±0.052</td>
<td>1.11±1.696</td>
</tr>
<tr>
<td>HIEE(400mg/kg B.W.)</td>
<td>29.183±0.060</td>
<td>21.383±0.145</td>
<td>116.217±0.122</td>
<td>1.157±0.011</td>
</tr>
</tbody>
</table>

**Statistical Analysis** The results were expressed as mean ± SEM of six animals from each group. The statistical analysis were carried out by one way analysis of variance (ANOVA) P values < 0.05 were considered significant.

**Histopathological Examination:** Small pieces of liver tissue were collected in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax sections were made about 4-6μm in thickness. They were stained with hematoxylin and photographed.

![Photograph: 1, 2 Liver of rat treated with vehicle, Liver of rat treated with paracetamol.](image)

![Photograph: 3 Liver of rat treated with syrup silymarin.](image)

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RESULT AND DISCUSSION:
The present work was to carried out with the objective of ethanolic extract of leaves of Holopetlea integrifolia (Roxb.). Phytochemical investigation, evaluation of the Hepatoprotective activity (in-vivo) against PCM induced Hepatotoxicity model. Liver is a versatile organ in the body concerned with regulation of internal chemical environment. Therefore damage to the liver inflicted by a hepatotoxic agent is of grave consequence. In the present study, the ethanolic extract of Holopetlea integrifolia (Roxb.) was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in SGOT, SGPT and SALP concentration, and preventing liver histopathological changes in rats induced with PCM hepatotoxicity. Paracetamol is a well known antipyretic & analgesic agent, which is safe in therapeutic doses but can produce fatal necrosis in experimental animals & humans & is employed as an experimental hepatotoxic agent. A sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbances caused in the transport functions of hepatocytes. The estimation of enzymes in the serum is a useful quantitative marker of the extent & type of hepatocellular damage[6]. The mode of action of paracetamol on the liver is by covalent binding of its metabolite, n-acetyl-p-benzoquinone-amine to sulphydryl group of protein resulting in cell necrosis and lipid peroxidation[9]. Due to liver injury caused by PCM overdose, the transport function of the hepatocytes gets disturbed resulting in the leakage of plasma membrane[8] thus causing an increase in serum enzyme levels. Assay of the activities of these serum marker enzymes and HIEE helps to assess the liver function[9].

Herbal Silymarin is a unique, all natural, complex multi-ingredient formula. It helps in protecting the liver from harmful toxins & regulates levels of enzymes and optimizes assimilation. Herbal Silymarin has been found to be associated with an increase in serum albumin & restores the functional efficiency of the liver by promoting the hepatocellular regeneration. Silymarin is believed to be the first multi-herb remedy granted regulatory approval as a drug, Silymarin, the well known hepatoprotective product. Silymarin is a favoured drug for different liver diseases because...
of its oral effectiveness, good safety profile, availability in India and importantly affordable[10]. In this experiment, it is observed that the level of hepatic biochemical markers i.e. SGOT, SGPT, SALP & Bilirubin is increased due to PCM in comparison to the control group. This clearly indicates that there is significant hepatic damage due to the paracetamol. The toxic effect of PCM was controlled in animals treated with ethanolic extract of Holopetlea integrifolia. 400 mg/kg/day by way of restoration of the markers levels in the liver with comparison to positive control Silymarin. Hepatoprotective effect of HIEE was further confirmed by histopathological studies of the liver, which basically supported the results from the serum assay. HIEE administration resulted in bringing about an almost normal histological architecture of the liver. The preliminary phytochemical screening of Holopetlea integrifolia leaves shows the presence of flavonoids and Steroids compounds as major active principle in ethanolic extract of H.integrifolia. leaves. Many of flavonoids and steroids(Beta- steroid) compounds have been reported for hepatoprotective activity.

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