HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF GOSSYPIUM HERBACIUM ON PARACETAMOL INDUCED LIVER DAMAGE IN ALBINO RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of alcoholic extract of Gossypium herbacium seeds against paracetamol induced liver damage in Albino rats. The alcoholic extract of seeds of Gossypium herbacium (200 & 400mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the alcoholic extract of G. herbacium seeds possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: Gossypium herbacium, Paracetamol, hepatoprotective and hepatotoxicity

INTRODUCTION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects1. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders2. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Cotton is used to make a number of textile products. The cottonseed which remains after the cotton is ginned is used to produce cottonseed oil, which, after refining, can be consumed by humans like any other vegetable oil. The cotton seed meal that is left generally is fed to ruminant livestock; the gossypol remaining in the meal is toxic to mono gastric animals but no report is available about the effect of its aqueous extract against Paracetamol induced hepatic damage. In this study, we evaluated the effect of alcoholic extract of seeds of Gossypium herbacium, against Paracetamol induced hepatic damage in rats, by determining the activities of Biochemical parameters like SGOT, SGPT, ALP and Bilirubin.

MATERIALS AND METHODS

Animals

Wister albino rats of either sex were used for the study of the crude extracts. Institution Animal Ethics Committee has approved the project (786/ac/11/PCSEA). The animals were kept at 27±2°C, relative humidity 44–56% and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with standard diet (Lipton, India) and the food was withdrawn 18 h before the start of the experiment and water ad libitum. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals14.

Plant resources and preparation of crude drug extract

The seeds of G. herbacium were collected from Tirupati, Andhra Pradesh, India. And authentication was done by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. Seeds were shade dried and defatted with petroleum ether. The defatted material was extracted with 95% ethanol using soxhlet apparatus and then vacuum dried.
PHYTOCHEMICAL STUDIES
All the extracts were subjected for phytochemical study.\textsuperscript{15}

Acute toxicity studies
The acute toxicity study for ethanolic extract of seeds of G. herbacium was performed using albino rats. The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the extracts were administrated orally in increasing dose and found safe up to dose of 2000 mg/kg for all extracts.

Experimental animal and design
The experiment was conducted according to the modified procedures described previously\textsuperscript{16}. PCT was dissolved in 0.5 % CMC for oral administration. Rats were randomly divided into six groups, each consisting of six rats. Group1 served as normal control and was orally given pure water for seven days, and then intraperitoneally injected with 10 ml/kg body weight isotonie 0.9% NaCl. Group 2 served as hepatotoxicity control and was orally given pure water for seven days and then orally intoxicated with 3 g/kg PCT. Group 3 served as standard, and received Standard drug Silymarin 25gm/kg, orally. Group 4 and 5 were treated with the ethanol extract of G. herbacium (each at two concentrations of 200 and 400 mg/kg respectively) for seven days. After 24 h of PCT intoxication, the rats were euthanized by ether and then sacrificed. The blood was collected by cardiac puncture in heparinized tubes. The liver was immediately taken out and washed with ice-cold saline. The blood and liver samples were assessed for their biochemical, as well as histological observation.

Biochemical determinations
The biochemical parameters like serum enzymes: aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (ALT)\textsuperscript{17}, serum alkaline phosphatase (ALP)\textsuperscript{18} and total bilirubin\textsuperscript{19} were assayed using assay kits (Span Diagnostic, Surat).

Histopathological studies
The liver tissue was dissected out and fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H–E) dye for photomicroscopic observation, including cell necrosis, fatty change, hyaline regeneration, ballooning degeneration.

Statistical analysis
The data are expressed as mean±S.E.M. The difference among means has been analyzed by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant.

RESULTS
Phytochemical study:
All extracts subjected for phytochemical study showed the presence of alkaloids, proteins, amino acids, phenolic compounds, glycosides and flavonoids.

Acute toxicity studies
Ethanolic and aqueous extracts did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Effects of extracts on AST, ALT, ALP and total bilirubin
The results of hepatoprotective effect of extracts on PCT-intoxicated rats are shown in Table 1. The elevated levels of serum AST, ALT, ALP, and total bilirubin were significantly reduced in the animals groups treated with various extracts. Treatment with ethanolic extract showed highly significant activity ($P < 0.001$) with maximum inhibition. So, the ethanol extract treated group was superior to the other extracts but not as effective as the silymarin.

Histopathological observations
Histology of the liver sections of the
Group 1. Normal = Normal architecture of liver tissue with mild congestion and sensitivity (Fig.1).
Group 2. Paracetamol (3g/kg) = Ballooning degeneration of hepatocytes with fatty liver tissue areas, indicating acute liver damage. (Fig.2).
Group 3. Standard treated (25 mg/Kg Silymarin) = mild peripheral necrosis, less percentage of liver damage in comparison with other groups. (Fig.3).
Group 4. Ethanolic seed extract (100 mg) = Mild congestion in sinusoids and central vein. Ballooning degeneration in mid and peripheral zones, mild peripheral necrosis while no evidence of Cirrhosis. This indicates the mild liver damage. (Fig.4).
Group 5. Ethanolic seed extract (200mg) = less peripheral necrosis, very less percentage of liver damage in comparison with all other groups. (Fig.5).
Group 6. Ethanolic seed extract (400mg) = less peripheral necrosis, very less percentage of liver damage in comparison with all other groups. (Fig.6).
The hepatotoxin is associated with changes at cellular levels that may lead to deterioration of organ functions. Therefore, any improvement in the treatment of hepatic function could be of potentially a great importance. The possible mechanism of herbal preparation as hepatoprotective agent against paracetamol could be by substantially decreasing lipid per-oxidation through the elevation of MDA level in liver homogenate. Generally it is known that most of the paracetamol is metabolized by cytochrome P-450 system to produce a highly toxic N acetyl-p- benzoquinone-imine (NAPQI) which is readily detoxified by enzymatic conjugation with hepatic glutathione (GSH). But, when the detoxification process is disturbed, an active agent NAPQI is produced which in turn binds covalently to tissue macromolecules thereby causing severe hepatic damage.

Disregarding the defensive nature of the liver, a pure hepatotoxin administered, strongly points out the prominent significance of *Gossypium herbacium* in hepatoprotection against paracetamol induced hepatotoxicity.

### DISCUSSION AND CONCLUSION:

Table 1: Effect of extracts of *G. herbacium* on biochemical parameters of liver in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen (Dose)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NORMAL</td>
<td>88.67 ± 1.085</td>
<td>64.83 ± 0.600</td>
<td>70.50 ± 0.763</td>
<td>0.246 ± 0.006</td>
</tr>
<tr>
<td>II</td>
<td>PARACETAMOL (25mg/kg)</td>
<td>242.5 ± 2.349</td>
<td>290.5 ± 0.763</td>
<td>209.5 ± 0.75</td>
<td>0.958 ± 0.007</td>
</tr>
<tr>
<td>III</td>
<td>STANDARD (25 mg/kg)</td>
<td>105.5 ± 0.763**</td>
<td>85 ± 0.577**</td>
<td>84.50 ± 0.763**</td>
<td>0.295 ± 0.007**</td>
</tr>
<tr>
<td>IV</td>
<td>GHS Extract (100 mg/kg)</td>
<td>213 ± 0.577</td>
<td>259.5 ± 0.763</td>
<td>184.8 ± 0.763</td>
<td>0.691 ± 0.007</td>
</tr>
<tr>
<td>V</td>
<td>GHS Extract (200 mg/kg)</td>
<td>195.2 ± 0.703**</td>
<td>209.5 ± 0.600**</td>
<td>156.8 ± 0.703**</td>
<td>0.566 ± 0.004**</td>
</tr>
<tr>
<td>VI</td>
<td>GHS Extract (400 mg/kg)</td>
<td>159.5 ± 0.763**</td>
<td>130.3 ± 0.881**</td>
<td>110.8 ± 0.792**</td>
<td>0.460 ± 0.005**</td>
</tr>
</tbody>
</table>

Values are the mean±S.E.M. of six rats. Symbols represent statistical significance.

** **P < 0.01, Ns: not significant, as compared to Paracetamol-intoxicated group.

### REFERENCES:


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