HEPATOPROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF
SWERTIA CHIRAYATA ON PARACETAMOL INDUCED LIVER
DAMAGE IN ALBINO RATS

Hari Kumar Cheedella*1, Ramesh Alluri2, G. Krishna Mohan3

1Research Scholar, Research and Development, JNTU-K, Kakinada & Dept. of
Pharmacology, Vasavi Institute of Pharmaceutical sciences, Kapada, A.P. India.
2Dept. of Pharmacology, Vishnu Institute of Pharmaceutical Education and Research,
vishnupur, Narsapur, Medak Dt. A.P
3Centre for Pharmaceutical Sciences, JNTU-H, Hyderabad, A.P. India.

ABSTRACT
Liver disease is one of the common health problems as it is exposed to
many kinds of therapeutic agents and xenobiotics. The present
investigation was aimed at examining the possible hepatoprotective
role of Swertia chirayata against paracetamol-induced hepatotoxicity.
The plant extract (100, 200 and 400 mg/kg, p.o.) showed a remarkable
hepatoprotective activity against paracetamol-induced hepatotoxicity
as judged from the serum marker enzymes in rats. Paracetamol induced
a significant rise in aspartate amino transferase (AST), alanine amino
transferase (ALT), alkaline phosphatase (ALP), total bilirubin.
Treatment of rats with different doses of plant extract (100, 200 and
400 mg/kg) significantly (P<0.001) altered serum marker enzymes
levels to near normal against paracetamol treated rats. The activity of the extract at dose of
400 mg/kg was approximately comparable to the standard drug, silymarin (25 mg/kg, p.o.).
Histopathological changes of liver samples were compared with respective control. The
present study revealed that ethanolic extract of Swertia chirayata has significant
hepatoprotective activity against paracetamol-induced hepatotoxicity in rats and antioxidant
potential.

Key words: Swertia chirayata, Hepatoprotective, Paracetamol.
INTRODUCTION

It is well recognized that liver is a vital organ, involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. Ample supply of blood and the presence of many redox systems (e.g. cytochromes and various enzymes) enable liver to convert these substances into different kinds of inactive, active or even toxic metabolites. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury such as acute or chronic inflammation, toxin-/ drug-induced hepatitis, cirrhosis and hepatitis after viral infection. Liver damage is always 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 AST, ALT, ALP, triglycerides, cholesterol, bilirubin, are elevated [1, 2].

Paracetamol (Acetaminophen; 4-hydroxyacetanilide) is an over-the-counter antipyretic and analgesic drug, which is widely used to cure fever, headache and other pains and, though safe when used at therapeutic doses. When taken in at toxic doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and experimental animals [3, 4].

Paracetamol is metabolized in the liver via glucuronidation, sulfonation and oxidation [5]. The glucuronidation and sulfonation are quantitatively more important metabolic reactions than the oxidation, but the oxidation is the main cause as far as toxicity is concerned [6]. Oxidation of paracetamol is primarily catalyzed by cytochrome P-450 [7] and produces a highly reactive arylating compound called N-acetyl-p-benzoquinoneimine (NAPQI) [8]. In human liver microsome P-4501A2, were shown to be principal catalysts of paracetamol activation [9]. Semiquinone radicals, obtained by one electron reduction of NAPQI is normally rapidly conjugated with GSH and is excreted as the cysteinyl conjugate or in the form of mercapturic acid [10]. As long as the rate of formation of NAPQI is not greater than the maximal rate of synthesis of GSH there will be no deleterious influences to the cell or organ [10]. Hepatic synthesis of GSH, which is directly suppressed within the first few hours following ingestion of hepatotoxic dose of paracetamol, is overwhelmed and manifestations of toxicity appear when GSH level falls below 30% of normal [11]. When more NAPQI is formed than the available GSH for conjugation, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins and DNA [12].
The entire plant is used in traditional medicine; however the stem is mentioned to be the most powerful part. *S. Chirayita* is used in British and American pharmacopoeias as tinctures and infusions. According to Ayurvedic pharmacology, chirata is described as bitter in taste (rasa). Its use has also been mentioned in Unani medicine. Concoction of chirata with cardamom, turmeric, and kutki is given for gastrointestinal infections, and along with ginger it is considered good for fever, but no report is available about the effect of its aqueous extract against Paracetamol induced hepatic damage[13]. In this study, we evaluated the effect of alcoholic extract of PN against Paracetamol induced hepatic damage in rats, by determining the activities of biochemical parameters like SGOT, SGPT, ALP and Bilirubin.

**MATERIAL AND METHODS**

**Plant material**
The stems of *Swertia chirayata* (Gentianaceae), procured from local market of Tirupati, Andhra Pradesh, India, in August 2010, were authenticated by Dr. K. Madhava Chetty, (Assistant professor, Department of Botany) Sri Venkateshwara University, Tirupati, Andhra Pradesh, India. The voucher specimen (002/Hari) was submitted in the Department of Pharmacognosy; Deccan School of Pharmacy, Hyderabad, Andhra Pradesh, India.

**Preparation of ethanol extract**
The *Swertia chirayata* were air dried in shade and were made to coarse size. The coarse sized roots were subjected to extraction by using the soxhlet apparatus. These coarse sized roots were defatted with petroleum ether for 72 hr. on 40-50 °C temperature. Then alcoholic extraction with ethyl alcohol was done 44 to 48 hr. at 40-50 °C temperature. After extraction, solvent was recovered by distillation. The concentrated extract was dried on water bath at 40-50 °C, made in powder form and the yield was 2.66 % w/w.

**Phytochemical analysis**
Phytochemistry of the ethanolic extract was carried out using the method of Khandelwal [14]. The result indicated the presence of glycosides, alkaloids, saponins, flavonoids, and tannins.

**Animals**
Healthy adults Albino Wistar rats (100-150 g each) aged 60-90 days were used for the study. The rats were housed in polypropylene cage and maintained under standard conditions (12 h light/ 12 h dark cycle; 25±3 °C; 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. The study was approved by the Institutional Animal Ethical
Committee of Annamacharya College of Pharmacy, Rajampet, Andhra Pradesh, India, registered under CPCSEA, India.

**Acute toxicity study**
The acute toxicity study of ethanolic extract of *Swertia chirayata stems* was studied as per to the OECD (organization for economic co-operation and development) – 420 in Albino Wistar rat maintained under standard condition. The animals were fasted at overnight prior to experiment. The initial oral dose was administered 2000 mg/kg to five female rats and rats were observed for behavioral changes and were under observation up to fourteen days. There was no mortality occurred.

**Experimental design for Paracetamol-induced hepatotoxicity**
The experiment was conducted according to the modified procedures described previously. 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 isotonic 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 3, 4 and 5 were treated with the ethanol extract of *S. chirayata* (with concentrations of 100, 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[15].

**Blood biochemistry**
Rats were sacrificed by cervical dislocation. Blood samples were withdrawn by cardiac puncture in heparinized tubes and were centrifuge at 3000 X g at 4 °C for 10 min to obtain serum. The liver function markers such as AST, ALT, ALP and total bilirubin were measured according to the standard procedures given along with the kits purchased.

**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 of biochemical estimations were reported as mean ± SEM. The statistical significance was determined by using one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison tests. P< 0.001 was used to determine statistical significance.

**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 no: 1
Group 2. Paracetamol (3g/kg) = Ballooning degeneration of hepatocytes with fatty liver tissue areas, indicating acute liver damage. Fig no:2
Group 3. Standard treated (25 mg/Kg Silymarin) = mild peripheral necrosis, less percentage of liver damage in comparison with other groups. Fig no: 3
Group 4. Alcoholic extract of *Swertia chirayata* (100 mg) = Ballooning degeneration in mid and peripheral zones, Cross linkage is observed. Fig no: 4
Group 5. Alcoholic extract of *Swertia chirayata* (200mg) = Two parenchyma are coming close. Fig no: 5

Group 6. Alcoholic extract of *Swertia chirayata* (400mg) = very less percentage of liver damage in comparison with all other groups. Fig no: 6

<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 (25mg/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>SCS Extract (100mg/kg)</td>
<td>202.8 ± 0.600</td>
<td>265.2 ± 0.600</td>
<td>180.5 ± 0.763</td>
<td>0.676 ± 0.004</td>
</tr>
<tr>
<td>V</td>
<td>SCS Extract (200mg/kg)</td>
<td>197.3 ± 0.494**</td>
<td>212.5 ± 0.703**</td>
<td>160.3 ± 0.666**</td>
<td>0.573 ± 0.008**</td>
</tr>
<tr>
<td>VI</td>
<td>SCS Extract (400mg/kg)</td>
<td>170.2 ± 0.600**</td>
<td>133.5 ± 0.763**</td>
<td>115.3 ± 0.666**</td>
<td>0.475 ± 0.004**</td>
</tr>
</tbody>
</table>

N= 6 animals in each group. **P < 0.001 when compared with Paracetamol. Values are expressed as mean ± SEM.
DISCUSSION

Paracetamol-induced acute liver damage as an experimental model of drug-induced acute hepatic necrosis is well-established [16, 17, 18]. The mechanism by which, paracetamol-induced hepatocellular injury and death involves its conversion to a toxic highly reactive and cytotoxic intermediate metabolite, N-acetyl-para-benzoquinonimine (NAPQI). Normally, paracetamol is primarily metabolized via cytochrome P-450 to form the highly electrophilic NAPQI [16] which is eliminated by conjugation with glutathione (GSH) and further metabolized to a mercapturic acid which is excreted in the urine [19, 20].

However, during paracetamol poisoning, NAPQI can rapidly react with glutathione (GSH) and lead to a 90% total hepatic GSH depletion in cells and mitochondria, which can result in hepatocellular death and mitochondrial dysfunction [21]. NAPQI can also induce DNA strand breaks and promote apoptosis and necrosis in paracetamol induced hepatotoxicity [22, 23].

The assessment of paracetamol-induced hepatocellular injury is mainly done by determining the enzyme levels such as AST, ALT, ALP and bilirubin. Necrosis or membrane damage releases the enzyme into circulation and hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [24]. Serum ALP and bilirubin levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure [25].

In the present investigation it was observed that the administration of paracetamol increased the levels of serum marker enzymes significantly (P<0.001) which is an evidence of existence of liver toxicity, (Table: 1). There was a significant (P<0.001) restoration of these enzyme levels on administration of the S. chirayata stems extract in a dose dependent manner and also by silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in acetaminophen induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. The possible mechanism by which ethanolic extract of S. chirayata stems exhibited significant protection against paracetamol induced hepatotoxicity may be due to the active constituents present in
various ingredients like flavonoids, alkaloids, sterols etc and its free radical scavenging activity.

CONCLUSION
Our aim was to develop Hepatoprotective preparation which could be safe with no interactions and beneficial in hepatoprotection, biochemical studies revealed a dose dependent significant fall in the levels of SGOT, SGPT, ALP, Bilirubin , an increase in the weight of liver in case of stem extract treated animals against paracetamol induced hepatotoxicity. Histopathological studies supplemented the findings by showing mild hepatic degeneration with absence of necrosis in comparison with the model control. Thus indicating the prominent significance of *Swertia chirayata* in hepatoprotection against paracetamol induced hepatotoxicity.

REFERENCES


