HEPATO PROTECTIVE EFFECTS OF SVENSONIA HYDERABADENSIS L. WHOLE PLANT ETHANOLIC EXTRACT AGAINST PARACETAMOL INDUCED LIVER DAMAGE IN RAT

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ABSTRACT
Liver is a vital organ of human body; it has been exposed to various hepatotoxicants (xenobiotics) in our day to day life of modernization especially in the developing countries. Despite of considerable progress in the treatment of liver damage by oral hepatoprotective agents, much attention has been focused on plant derived drugs because synthetic drugs have several limitations. Ethanolic extract of Svensonia hyderobadensis (ESH, 200 and 400 mg/kg, p.o.) was evaluated for prophylactic and therapeutic hepatoprotective activity against Paracetamol induced hepatic damage. Silymarin (100 mg/kg, p.o.) was used as standard drug. Hepatoprotective activity was assessed by biochemical markers viz, AST, ALT, ALP, total bilirubin, direct bilirubin, and histopathological examination of liver sections. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP, bilirubin were studied in both treated and untreated groups. Paracetamol (2 g/kg, bw. p.o.) has enhanced the SGPT, SGOT, ALP, bilirubin. Treatment with ethanolic extract of Svensonia hyderobadensis 200&400 mg/kg, bw. p.o.) brought back the altered levels of biochemical markers to the near normal levels in the dose dependent manner. Histological examination of the liver tissues confirmed the hepatoprotective effect of Svensonia hyderobadensis .And therefore, it was concluded
that the ethanolic extract of Svensonia hyderobadensis root possesses commendable hepatoprotective activity.

**Key words:** Hepatoprotective, Svensonia hyderobadensis, paracetamol, AST, ALT, ALP, bilirubin.

**INTRODUCTION**

The liver is one of the vital organs chiefly responsible for drug metabolism a sensitive target site for substances modulating biotransformation. The duration and intensity of the pharmacological response to drugs is influenced by their metabolic rate; hence substances capable of modifying drug metabolism are able to alter the outcome of drug therapy. Paracetamol (Acetaminophen), the most commonly used analgesic, which effectively reduces fever and responses mildly to moderate pain, is considered to be safe at therapeutic doses. However, an overdose of paracetamol results in severe hepatotoxicity that leads to health hazards such as liver failure in both humans and under treatment animal’s experiment. Most of the experiments that aim to elucidate the mechanism of paracetamol toxicity are performed on animal models both in vivo and in vitro. It proved that when taken at supratherapeutic doses, paracetamol does cause centrilobular hepatocyte degeneration and necrosis in rodents and humans. In response to injury with Paracetamol and other centrilobular hepatotoxicants, there is a recovery phase in which hepatocytes are stimulated to repopulate the liver lobule.

Similarly, Resistance to a different toxicant (heteroprotection) has also been observed. But it is a scientific surprise that the mechanism(s) underlying the resilience of proliferating hepatocytes to further toxicity is not completely established. A small amount of paracetamol can metabolize together with cytochrome P450. As a result, N-acetyl-p-benzoquinone imine (NAPQI) or Nacetyl-p-benzoquinone imine (NAPSQI) is traced in the human system. Both these compounds are very active chemically and their chemical structures indicate that they are capable of taking part in free radical reactions.

Consequently, an overdose of paracetamol can trigger a number of unfavourable consequences especially those affecting the liver.

Silymarin is in use for over 20 years in clinical practice for treatment of toxic liver diseases. It is an extracted from the seeds of the plant Silybum marianum also called “milk thistle”.
It is an antioxidant and exhibits anti-carcinogenic, anti-inflammatory, hepatoprotective and growth modulatory effects. In this study, Silymarin was used as a positive control against paracetamol-induced acute hepatic damage in rats.

Svensonia hyderobadensis is a shrub or perennial herb, often bushy, about 1 m tall. It belongs to the family of verbanaceae. The youngest parts are purplish with short and scattered hairs. Leaves decussate, opposite, elliptic-ovate to obovate, coarsely serrate, acute, and base rounded to decurrent. Petioles slender, 0.7-2.9 cm long, convex beneath, flattened and canaliculate above. Young leaves ovate or lanceolate, 2.7-10.7 cm long, 1-6.5 cm wide, acute at apex. Flowers pink-purple, in terminal spikes; bracts linear-lanceolate, scarious. It is widely used by the folklore for hepatoprotective activity.

The present study is aiming at studying the effects of extract Svensonia hyderobadensis on the function of hepatocytes using an in vivo model of paracetamol induced hepatocyte injury and finding out whether it can be potentially used as a medicine for liver damage in rats. The hepatoprotective effect of the were determined by assessing the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, HDL, Protein and total bilirubin. The effects of the extract of Svensonia hyderobadensis the whole plant. In addition, histopathological studies had been given an ardent attention to prove their relevance in the preventive and curative role against paracetamol induced hepatotoxicity in vivo.

**Fig.1. Photograph of Svensonia hyderobadensis**

**MATERIALS AND METHODS**

**Collection of drug:** Whole plant of Svensonia hyderobadensis were collected from Tirupati Hills, A.P. It is commonly known as adivi chiki. Botanist of Sri. Venkateshwara University,
Tirupathi, authenticated the plant. The whole plant was carefully dried in shade for 15 days, to ensure complete dryness; it was kept in hot air oven at 45°C for 5 minutes and then subjected to size reduction to make powder. The crushed mass was then ready for extraction.

**Extraction**

The dried and powder whole plant was subjected to hot extraction in Soxhlet apparatus with petroleum ether, chloroform, methanol and ethanol successively. The residue in the R.B flask was transferred into a beaker and was concentrated under reduced vacuum pressure to give an average yield of 70% (w/w). Solutions of the Svensonia hyderobadensis extract (ESH) were prepared freshly for the pharmacological studies.

**Drugs and chemicals**

Paracetamol, Tween 80, Silymarin and all solvents used were of analytical grade and were obtained from Sd. Fine Chemicals, Mumbai, India.

**Animals**

The study was carried out on Swiss albino mice weighing 25-30gm of either sex, which were procured from Sai Animal Distributors, Musheerabad. The animals were acclimatized for 1 week. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12 h light–12 h dark cycle. They were fed with commercial pelleted chow and were given free access to water ad libitum throughout the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output. All animal experiments strictly complied with the approval of institutional animal ethical committee.

**Experimental Design:** Twenty five mice were used and were classified into 5 groups (5 animals/group; n=5) as follows

**Group I:** Served as normal control and received 5% Tween 80 (1 ml/kg body weight).

**Group II:** Paracetamol control and received Paracetamol (2 g/kg body weight).

**Group III:** Received Silymarin (100 mg/kg body weight) as standard drug.

**Group IV:** Received ESH 200 mg/Kg body weight.

**Group V:** Received ESH 400 mg/Kg body weight.

Out of twenty five mice, five were retained as normal group i.e., Group I served as the normal control and received the vehicle 5% v/v Tween 80 at a dose of 1 ml/kg B.W., p.o. for
7 days. and remaining 20 animals were induced with hepatotoxicity, where in Group II served as Paracetamol control and received 5% v/v Tween 80 at a dose of 1ml/kg.B.W., p.o. for 5 days and received Paracetamol at a dose of 2 g/kg. b.w. for 6th and 7th days. Group III served as the standard and received the Silymarin at a dose of 100 mg/kg. b.w., p.o. for 5 days and received Paracetamol at a dose of 2 g/kg. b.w for 6th and 7th days. Group IV received the plant extract at a dose of 200 mg/kg.b.w., p.o. for 5 days and treated with paracetamol at a dose of 2 g/kg. b.w. for 6th and 7th days. Group V received the plant extract at a dose of 400 mg/kg.b.w., p.o. for 5 days and treated with paracetamol at a dose of 2 g/kg b.w. for 6th and 7th days.

BIOCHEMICAL ESTIMATION FOR ASSESSMENT OF LIVER FUNCTION

Mice of all groups were anaesthetized by diethyl ether, 24 h after the last administration of hepatotoxin paracetamol. The blood was obtained from all groups of mice by puncturing Retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and analyzed for various biochemical parameters: Serum transaminases viz. glutamic pyruvic transaminase (SGPT), serum glutamic oxalacetic transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin according to the reported methods.

**Determination of SGOT levels**

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood stream SGOT converts L-Aspartate and $\alpha$ - Ketoglutarate to Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with 2, 4, Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a brown coloured complex whose intensity is measured.

\[
L-\text{Aspartate} + \alpha \text{Ketoglutarate} \xrightarrow{\text{SGOT}} \xrightarrow{\text{pH 7.4}} \text{Oxaloacetate} + L-\text{Glutamate}
\]

\[
\text{Oxaloacetate} + 2,4,\text{DNPH} \xrightarrow{\text{Alkaline Medium}} \xrightarrow{\text{Brown coloured complex}} 2,4,\text{Dinitrophenyl Hydrazone}
\]

**Determination of SGPT levels**

SGPT is found in variety of tissues but is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. SGPT converts L-
Alanine and α-Ketoglutarate to Pyruvate and Glutamate. The pyruvate formed reacts with 2, 4, Dinitrophenyl hydrazine to produce a hydrazone derivative, which in an alkaline medium produces a colored complex whose intensity is measured.

\[
L - \text{Alanine} + \alpha \text{Ketoglutarate} \xrightarrow{\text{SGPT} \atop \text{pH 7.4}} \text{Pyruvate} + L - \text{Glutamate}
\]

\[
\text{Pyruvate} + 2,4,\text{DNPH} \xrightarrow{\text{Alkaline Medium}} \text{2,4, Dinitrophenyl Hydrazone} \quad (\text{Brown coloured complex})
\]

**Determination of ALP levels** \(^{16}\)

Alkaline Phosphatase (ALP) is an enzyme of the Hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver biliary tract epithelium and in the bones. Increased levels are associated mainly with liver and bone disease.

ALP at an alkaline pH hydrolyses di sodium Phenylphosphate to form phenol. The phenol formed reacts with 4-Aminooantipyrine in the presence of Potassium ferricyanide, as an oxidising agent, to form a red coloured complex. The intensity of the colour formed is directly proportional to the activity of ALP present in the sample.

\[
di \text{Na Phenylphosphate} + \text{Water} \xrightarrow{\text{Alkaline Medium} \atop \text{pH 10}} \text{Phenol} + di \text{Na Hydrogen Phosphate}
\]

\[
\text{Phenol} + 4 - \text{Aminoantipyrine} \xrightarrow{K_3 \text{Fe(CN)}_6} \text{Red coloured complex}
\]

**Determination of BILIRUBIN levels** \(^{17}\)

Bilirubin is mainly formed from the heme portion of aged or damaged RBC’s. It then combines with albumin to form a complex which is not water soluble. This is referred to as indirect or unconjugated Bilirubin. In the liver this Bilirubin complex is combined with glucuronic acid into a water soluble conjugate. This is referred to as conjugated or direct Bilirubin. Elevated levels of Bilirubin are found liver diseases (Hepatitis, cirrhosis), excessive hemolysis/destruction of RBC (Hemolytic jaundice) obstruction of the biliary tract (obstructive jaundice) and in drug induced reactions.

Bilirubin reacts with diazotised sulphanilic acid to form a coloured azobilirubin compound. The unconjugated Bilirubin couples with the sulphanilic acid in the presence of a caffien-
benzoate acclerator. The intensity of the colour formed is directly proportional to the amount of Bilirubin present in the sample.

\[ \text{Bilirubin} + \text{Diazotized Sulphanilic acid} \rightarrow \text{Azobilirubin compound} \]

**Histopathological studies**

The animals were then sacrificed by cervical dislocation. The tissues (liver) were removed and cleared off blood, rinsed in ice-cold physiological saline. The ratio of wet liver weight was calculated. The livers were examined grossly, were fixed in 10\% buffered neutral formalin for 48 hour and then with bovine solution for 6 hour. Paraffin sections were taken at 5 \( \mu \)m thickness processed in alcohol-xyline series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes, later the microscopic slides of the liver cells were photographed at a magnification of 100x.

**Statistical analysis**

All values were expressed as mean ± SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett’s ‘t’ test. P values < 0.01 were considered to be statistically significant when compared to standard (silymarin) group.

**RESULTS**

Hepatoprotective activity of Ethanolic extract of Svensonia hyderobadensis (ESH) was studied. For the acute oral toxicity studies, the extract treated animals were observed for mortality upto 48 h (short term toxicity). Based on the results the extract did not produce any mortality upto 2000 mg/kg body weight.

The results of biochemical parameters revealed the elevation of biochemical markers like SGPT, SGOT, ALP, Bilirubin in toxicant treated group indicating that Paracetamol induces damage to the liver. Pretreatment with ESH (200 mg/kg and 400 mg/kg) significantly reduced (P<0.01) the elevated levels of all the mentioned biochemical indicators. The enzyme levels were almost restored to the normal.

It was observed that the size of the liver was enlarged in paracetamol intoxicated mice but it was normal in ESH treated groups. A significance (P<0.01) in liver weight variation supports the findings. Histopathological examination of the liver section of the mice treated with paracetamol showed an intense centrilobular necrosis and vacuolization. The mice treated...
with siymarin and ESH showed a good sign of protection against the toxicant to considerable extent as it was evident from the formation of normal hepatic cords and absence of necrosis and vacuoles.

Table 1: Effect of ESH & Paracetamol on Liver Enzymes

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>SGPT U/l</th>
<th>SGOT U/l</th>
<th>ALP U/l</th>
<th>Total Bilirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>40.31 ±1.18</td>
<td>90.31 ±0.61</td>
<td>106.06 ±0.59</td>
<td>0.43 ±0.017</td>
</tr>
<tr>
<td>Paracetamol control</td>
<td>81.76 ±0.73</td>
<td>143.28 ±0.96</td>
<td>135.47 ±0.77</td>
<td>1.29 ±0.049</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg/day)</td>
<td>53.93 ±0.94**</td>
<td>114.25 ±1.605**</td>
<td>116.24 ±0.98**</td>
<td>0.61 ±0.038**</td>
</tr>
<tr>
<td>ESH (200 mg/kg/day)</td>
<td>61.88 ±0.89**</td>
<td>126.36 ±1.404**</td>
<td>122.18 ±0.46**</td>
<td>0.74 ±0.028**</td>
</tr>
<tr>
<td>ESH (400 mg/kg/day)</td>
<td>71.02 ±0.43**</td>
<td>137.27 ±1.468*</td>
<td>129.46 ±0.64**</td>
<td>0.92 ±0.011**</td>
</tr>
</tbody>
</table>

Each value is the mean ±S.E.M. of five mice.

*p < 0.05 vs. control, One way ANOVA followed by Dunnet’s t-test.

**p < 0.01 vs. control, One way ANOVA followed by Dunnet’s t-test

Graphical representation

Graph 1. Shows effect of Svensonia hyderobadensis on ALP Levels
Graph 2. Shows effect of Svensonia hyderobadensis on Bilirubin Levels
Graph 3. Shows effect of Svensonia hyderobadensis on SGOT Levels

Graph 4. Shows effect of Svensonia hyderobadensis on SGPT Levels

Histopathology

Histological studies of liver give a visual assessment of hepatic architecture. The comparison of normal architecture with hepatotoxicity caused by hepatotoxin (paracetamol) can be clearly distinguished by degeneration of hepatocytes, necrotic areas and non-visible portal tract. Fig. (2) Shows the representative photomicrographs of liver section of mice.

Fig. 2A: Normal control
Fig. 2B: Paracetamol control
Fig. 2C: Silymarin std.
Fig. 2D: ESH 400 mg/kg.B.W.

Fig. 2. Photomicrographs of liver section taken from the mice of Normal control, paracetamol control, silymarin and ESH 400mg/kg.
The mice treated with a vehicle control (group-I) (Fig. 2A) show a normal hepatic architecture and visible portal tract. Treatment of animals with paracetamol (2g/kg) (group-II) results in acute hepatotoxicity as can be observed from necrotic patches and degenerative hepatocytes with mild inflammation and unremarkable portal tract (Fig.2B). Pre-treatment of animals with Silymarin(100 mg/kg B.W., group-III) resulted in hepatoprotection, as can be observed by absence of necrotic areas, degenerated hepatocytes and visible portal tract which indicate normal hepatic architecture (Fig. 2C). The similar results were observed on pre-treatment of animals with Svensonia hyderobadensis complex at 400 mg/kg B.W. (group-V) (Fig. 2D)

**DISCUSSION**

The present study reports the potential hepatoprotective activity of ESH against hepatic injury produced by paracetamol in mice. Paracetamol is a well-known antipyretic and analgesic agent, which is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. It is employed as an experimental hepatotoxic agent. It is metabolized in the liver to excretable glucuronide and sulphide conjugates. An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes. When liver cell plasma is damaged; a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. In the present investigation, the dose of paracetamol used (2g/kg), caused liver injury in mice. The mice treated with an overdose of paracetamol developed significant hepatic damage, which was observed through a substantial increase in the concentration of serum parameters. Pre-treatment of the mice with ESH extract at 200 and 400 mg/kg, p.o., for 7 days before paracetamol administration resulted in a significant protection of paracetamol induced elevation of serum marker enzymes. The silymarin and the ethanolic extract of Svensonia hyderobadensis significantly decreased the paracetamol induced elevated levels of the enzymes in the treatment group, indicating the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin after treatment with ESH indicated the effectiveness of the extract in the normal functional status of the liver.

ESH appears to be effective in reducing the injurious effect of paracetamol, observed in the study. This was an indication of stabilization of plasma membrane, as well as repair of
hepatic tissue damage, caused by paracetamol. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. The hepatoprotective effect of ESH was further confirmed by histopathological examination of the liver. The histological observation basically supported the results from the serum assays as ESH administration reversed to a large extent, hepatic lesions produced by paracetamol.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology, which has been disturbed by hepatotoxins.

Preleminary phytochemical studies and literature review revealed the presence of flavonoids, phenolic compounds and tannis in ESH.. Therefore, there is a possibility that the whole plant of Svensonia hyderobadensis may posses hepatoprotective activity.

CONCLUSION

In conclusion the present study demonstrated that the whole plant of Svensonia hyderobadensis possess significant hepatoprotective activity. In addition, the hepatoprotective property may be attributed to the active principles of the plant namely, flavonoids, tannis and the other polyphonlic compounds.

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