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Protective effects of *Hemidesmus indicus* var. *pubescens* root extract on paracetamol induced hepatic damage

*Hemidesmus indicus* var. *pubescens* kök ekstresinin parasetamolün oluşturduğu hepatik hasar üzerine koruyucu etkileri

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SUMMARY
AIM: This study was conducted to study the hepatoprotective property of *Hemidesmus indicus* (L.) R.Br. roots.

METHODS: Hepatoprotective properties of the methanol extract of the roots of *H. indicus* var. *pubescens* were evaluated on paracetamol induced hepatotoxicity. Hepatotoxicity was induced in albino Wistar rats by the administration of paracetamol (1g/kg), p.o. for 7 days. Methanol extract of *H. indicus* var. *pubescens* roots (MEHI) was administered at the doses 200 and 400 mg/kg/day, p.o. for 7 days. Serum analysis was performed to estimate the levels of SGOT, SGPT, ALP, total proteins, albumin, total bilirubin, total cholesterol, high density lipoprotein and triglycerides. The liver was isolated and homogenized for the estimation of glutathione and malondialdehyde. Histopathology studies were also performed on the liver samples.

RESULTS: The toxic effects of paracetamol were significantly controlled in the extract treated groups which was manifested by the restoration of serum biochemical parameters to near normal levels.

CONCLUSIONS: From the study it was concluded that roots of *H. indicus* var. *pubescens* possess significant hepatoprotective properties.

Keywords: Paracetamol, hepatotoxicity, *Hemidesmus indicus* var. *pubescens*, hepatoprotective activity.

ÖZET
AMAÇ: Bu çalışma *Hemidesmus indicus* (L.) R.Br.’nin hepatoprotektif özelliğini çalışmak için yapıldı.

YÖNTEM: Parasematolun bağlı hepatotoksisite üzerinde *H. indicus* var. *pubescens* kökünün metanol ekstresinin hepatoprotektif özellikleri değerlendirildi. Hepatotoksisite, 7 gün boyunca parasetamolün (1 mg/kg) oral yoldan uygulandı. Albin o Wistar sıçanlarındadır indükledi. *H. indicus* var. *pubescens* kök metanol ekstresi (MEHI), 7 gün boyunca oral olarak 200 ve 400 mg/kg/gün dozlandırarak uygulandı. SGOT, SGPT, ALP, total proteins, albumin, total bilirubin, total kolestrol, yüksek dansiteli lipoprotein ve trigliserit seviyelerini ölçmek için serum analizleri gerçekleştirildi. Glutatyon ve malondialdehit hesapları için karaciğer alındı ve homojenize edildi. Karaciğer numunelerinde histopatoloji çalışmalardan da değerlendirildi.

BULGULAR: Parasematolün toksik etkileri, serum biyokimyasal parametrelerin normal düzeylere gerilemesi ile gösterilen ekstre uygulanan gruplarda belirgin şekilde kontrol altına alındı.

SONUÇ: Bu çalışmadan *H. indicus* var. *pubescens* köklerinin belirgin hepatoprotektif özelliklerine sahip olduğu sonucuna varıldı.


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INTRODUCTION

Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to toxicity from these agents. Chemicals that cause liver injury are called hepatotoxins. Chemicals often cause subclinical injury to liver which manifests as abnormal liver enzyme tests [1]. Paracetamol causes liver disease and acute liver failure when an overdose is consumed [2]. Paracetamol is converted in the liver to its reactive metabolite N-acetyl-p-benzoquinoneimine by cytochrome P-450 enzymes [3].

Hemidesmus indicus (L.) R. Br. is the accepted source of the Ayurvedic drug Sariva. *H. indicus* var. *indicus* is referred to as the Indian Sarsaparilla, an important drug in Ayurveda, endowed with many medicinal properties [4]. The roots of *H. indicus* var. *indicus* are used as antipyretic, anti-diarrheal, astringent, diaphoretic, diuretic, refrigerant, and as a tonic [5] and are also useful in biliousness, blood disorders, dysentery, diarrhea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leucoderma, leucorrhea, itching, bronchitis, asthma, eye diseases, epileptic fits in children and kidney stones [6]. Leaves are used in the treatment of vomiting, wounds and leucoderma, cerebropathy, hepatopathy, nephropathy, syphilis, metropathy, leucoderma, odontalgia, cough and asthma [7]. In addition to the typical variety *H. indicus* var. *indicus*, another taxonomic variety, *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (W. & A.) H k. f. is found in South India. This variety is used in place of *H. indicus* var. *indicus* in various formulations of Ayurveda, in South India [4]. β-sitosterol and tannins have been reported in *H. indicus* var. *pubescens* [8].

The pharmacological studies reported on *H. indicus* var. *pubescens* are toxicity [9-11], antiulcer activity [12-14] and antioxidant activity [15, 16] studies on the leaf of *H. indicus* var. *pubescens* and in vitro antioxidant activity studies on the root of *H. indicus* var. *pubescens* [17]. Hepatoprotective properties of the typical variety, *H. indicus* var. *indicus*, have been reported [18-21]. Since no such studies have been reported on *H. indicus* var. *pubescens* [22], the present work has been undertaken to evaluate the hepatoprotective potential of this variety of *H. indicus*.

MATERIALS AND METHODS

Collection and authentication of the plant material

The roots of *H. indicus* var. *pubescens* were collected from the vicinity of Thuthukudi District, Tamil Nadu, India, during November 2008. The plant material was identified and authenticated by Dr. S.N. Yoganarasimhan, Taxonomist and Research Coordinator at M S Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The taxonomic identification was carried out following local flora [23] and the herbarium specimen (*Anita Murali No.034*) along with crude drug sample has been deposited at the herbarium and crude drug museum of P.G. Department of Pharmacognosy, MSRPC. This research protocol was approved by the Institutional Animal Ethics Committee of M S Ramaiah College of Pharmacy (Protocol No. MSRPC/P-04/2008 Dt. 22.11.08).

Preparation of extract

The roots of *H. indicus* var. *pubescens* were cleaned, dried at room temperature and coarse powdered. The powdered drug was successively extracted in a soxhlet apparatus with solvents of increasing polarity using petroleum ether, chloroform and methanol. Methanol extract of *H. indicus* var. *pubescens* roots (MEHI) was concentrated under reduced pressure. The extract was subjected to preliminary organic analysis and HPTLC studies [24, 25]. The methanol extract was suspended in distilled water containing 2% w/v gum acacia and used for the pharmacological studies.

Animals

Albino Wistar rats (170-200 g) of either sex were used for the study. The animals were bred and maintained in the animal house of M S Ramaiah College of Pharmacy. Animal maintenance was in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Acute toxicity

Acute toxicity studies were performed on methanol extract of *H. indicus* var. *pubescens* roots (MEHI) following OECD guidelines [26]. Female Wistar rats of 8-12 weeks weighing around 170-200 g were used. Methanol extract (MEHI) at doses 300 and 2000 mg/kg were orally administered to separate groups of animals. The animals were observed continuously for 2 h for any symptoms of toxicity and/or death. They were under observation for further 2 weeks.
Paracetamol induced hepatotoxicity in rats

Animals were randomized and divided into 5 groups with 6 rats in each group. Animals of group 1 received only vehicle (1% acacia in distilled water). Animals of group 2 received (positive control) received paracetamol 1 g/kg for 7 days. Animals of group 3 received paracetamol 1 g/kg and the standard drug silymarin 100 mg/kg. Animals of groups 4 and 5 received MEHI 200 and 400 mg/kg and paracetamol 1 g/kg. The treatments were continued for 7 consecutive days and on the 8th day, all animals were anesthetized by anaesthetic ether and blood samples were collected from retro-orbital plexus, serum separated by centrifugation at 8000 rpm for 10 min in a micro centrifuge (Remi Motors Ltd, Mumbai, India). The serum was analysed for SGOT, SGPT, ALP, total proteins, albumin, total bilirubin, total cholesterol, triglycerides and HDL using assay kits. The estimations were carried out in a biochemical analyser (CA 2005, B4B Ranbaxy Diagnostic Division). The animals were further sacrificed with excess of anaesthetic agent and liver was rapidly excised, washed in ice cold saline, immediately blotted dry by gently pressing between two filter papers and weighed on digital balance. The weight of liver per 100 gram body weight of animal was calculated. The isolated liver was divided into 2 parts for the preparation of homogenates. A 10% w/v homogenate in potassium chloride (0.15 M) was used for the estimation of malondialdehyde (MDA) and 10%w/v homogenate with sucrose in phosphate buffer (pH 7.4) was used for the estimation of GSH [27].

Biochemical estimations

Evaluation of effect of paracetamol treatment on the serum levels of SGPT

Assay kits from Biosystems SA, Costa Brava 30, Barcelona, Spain was used and estimation was done at the wavelength of 340 nm. The concentration of SGPT was determined from the rate of decrease of NADH, measured at 340 nm by means of lactate dehydrogenase (LDH) coupled reaction [28].

Evaluation of effect of paracetamol treatment on the serum levels of SGOT

Assay kit from Biosystems SA, Costa Brava 30, Barcelona, Spain, was used and the wavelength of 340 nm was chosen for the estimation. The concentration of SGOT was determined from the rate of decrease of NADH, measured at 340 nm by means of malate dehydrogenase (MDH) coupled reaction [28].

Evaluation of effect of paracetamol treatment on the serum levels of ALP

Assay kit from Biosystems SA, Costa Brava 30, Barcelona, Spain, was used and estimation was done at the wavelength of 405 nm. The ALP concentration was determined from the rate of 4- nitro phenol formation, measured at 405 nm [29].

Evaluation of effect of paracetamol treatment on the serum levels of total proteins

The estimation was done using assay kits from Agappe Diagnostics, Kerala, India. Colorimetric estimation of total proteins was based on the principle of the Biuret reaction (copper salt in an alkaline medium). Protein in serum forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration [30].

Evaluation of effect of paracetamol treatment on the serum levels of albumin

The reaction between albumin from serum or plasma and the dye bromocresol–green produces a change in color that is proportional to the albumin concentration [31].

Evaluation of effect of paracetamol treatment on the serum levels of total bilirubin

The estimation was done using assay kits from Agappe Diagnostics, Kerala, India. Bilirubin in the sample reacts with diazotized sulfanilic acid forming a colored complex which was measured at 540 nm [32].

Evaluation of effect of paracetamol treatment on the serum levels of total cholesterol

The estimation was done using assay kits from Agappe Diagnostics, Kerala, India. The formation of red quinine, proportional to the amount of total cholesterol in the sample was estimated at 640 nm [33].

Evaluation of effect of paracetamol treatment on the serum levels of triglycerides

The estimation was done using assay kits from Agappe Diagnostics, Kerala, India. The formation of red quinoneimine, proportional to the amount of total TG in the sample was estimated at 630 nm [34].

Evaluation of effect of paracetamol treatment on the serum levels of HDL

The estimation was done using assay kits from Agappe Diagnostics, Kerala, India. Estimation of
Hepatoprotective effect of *Hemidesmus indicus*

HDL was done at 630 nm, after precipitation of cylomicrons, VLDL and LDL in serum by phophotungstic acid [35].

**Evaluation of effect of paracetamol treatment on the hepatic levels of MDA and GSH**

MDA is the main product of lipid peroxidation. It reacts with thiobarbituric acid to form a pink colored chromogenic adduct with two molecule of TBA. The intensity of the color was measured at 532nm. The MDA content was calculated as thiobarbituric acid reacting substance (TBARS) and expressed in terms of nmol/100 mg tissue, using the molar extinction co-efficient, 1.56x10^5 M-1cm-1[36,37].

Glutathione (GSH) was estimated using Ellman reagent i. e., 5,5’dithiobis (2- nitrobenzoic acid), which reacts with sulphhydryl compounds to give a relatively stable yellow color. The water soluble compound formed is proportional to the amount of GSH present in the sample. The amount of GSH was determined using its molar extinction coefficient of 13,600 M-lcm-1 and expressed in terms of nmol/100 mg of tissue [38].

**Histopathological studies**

One animal from each group was utilized for the purpose. The liver specimens were fixed with 10% neutral formalin and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed according to standard procedures [39]. The histological evaluation of the extent of liver injury was carried out microscopically using an image microscope.

**Statistical analysis**

The results were expressed as mean ± SEM and statistically analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey Kramer multiple comparison test.

**RESULTS AND DISCUSSION**

**Phytochemical analysis**

The presence of glycosides, phenolic compounds, tannins and coumarins were detected in MEHI when the extract was subjected to preliminary phytochemical analysis. HPTLC fingerprint of the above phytoconstituents have been earlier reported by us [17]. In addition to these constituents, the presence of steroidal sapogenin- sarsapogenin was confirmed by HPTLC fingerprint analysis (Figure 1 and Figure 2). The RF values obtained were compared with that available in the literature and presence of these phytoconstituents in the extract was confirmed.

![Figure 1. Polyphenols and tannins at 254 nm](image1)

![Figure 2. Smilagenin and sarsapogenin at 366 nm](image2)

**Acute toxicity studies**

The extract MEHI was found to be nontoxic and the lethal dose was higher than 2000 mg/kg b.w. when tested on female albino Wistar rats.

**Paracetamol induced hepatotoxicity**

Acetaminophen (paracetamol) is a commonly used analgesic/antipyretic that produces necrosis of the centrilobular cells of the liver when consumed in overdose [40]. Liver injuries induced by paracetamol are commonly used for the screening of hepatoprotective agents [41].The effect of methanol extract of *H. indicus var. pubescens* roots (MEHI) was evaluated on paracetamol induced hepatic damage. The serum levels of SGOT, SGPT, ALP, total bilirubin, albumin, total cholesterol, triglycerides, HDL and hepatic levels of MDA and GSH were estimated to study the protective effect of *H. indicus var. pubescens* roots.

In the present investigation it was observed that the positive control animals treated with paracetamol...
alone resulted in significant hepatic damage as shown by the elevated levels of serum markers. Treatment with root extract of *H. indicus* var. *pubescens* significantly attenuated elevation in the levels of serum markers, thus exhibiting its protective effects on the liver. SGOT levels decreased significantly (*p* < 0.05) on treatment with 200 and 400 mg/kg of the extract. This was also accompanied by a significant (*p* < 0.001) decrease in the SGPT levels. The decrease in the levels of serum markers by the extract is an indication that they are able to protect cell membrane integrity of hepatocytes against acetaminophen induced leakage of marker enzymes into the circulation. The above changes can be considered as evidence of the functional improvement of hepatocytes.

Levels of ALP and total bilirubin were significantly increased in the positive control group when compared with the normal control. Increase in serum levels of ALP is due to its increased synthesis in presence of increasing biliary pressure [42]. The ALP levels were significantly (*p* < 0.01) decreased by MEHI 400 mg/kg. Effective control of bilirubin and ALP levels points towards an improvement in the hepatic secretory mechanisms.

Paracetamol is metabolically activated by cytochrome P-450 enzymes (2E1, 1A2, 3A4, and 2A6) to form NAPQI, which binds covalently to proteins [3, 43]. At therapeutic doses, NAPQI is efficiently detoxified by GSH, which protects hepatic cells by combining with the reactive metabolite of paracetamol to form adducts, thereby preventing its covalent binding to liver proteins [44]. In the positive control animals the GSH levels were significantly low and treatment with MEHI significantly (*p* < 0.001) increased the levels of GSH in the respective groups.

Lipid peroxidation has been postulated to be involved in liver injury due to paracetamol. N-acetyl-p-benzoquinone imine acts as a free radical to initiate lipid peroxidation [45]. In the present study, elevation in the levels of MDA in the liver of positive control rats was observed. Treatment with MEHI significantly reversed these changes.

It has been postulated that proteins are most commonly affected especially those involved in cellular ion control as well as the mitochondrial proteins, with resultant loss of energy production [46]. Covalent binding of NAPQI to these proteins is the major cause of acetaminophen toxicity [47]. There was a significant increase in the serum levels of proteins with MEHI 400 mg/kg (*p* < 0.001). Albumin levels were maintained to near normal levels with the extract (*p* < 0.001 for MEHI 200 mg/kg and *p* < 0.01 for MEHI 400 mg/kg).

The levels of total cholesterol, triglycerides and HDL also were estimated. Serum levels of TC and TG were increased and that of HDL were decreased in the paracetamol treated group. This biochemical aberration was corrected in animals that were administered MEHI along with paracetamol. Both doses of MEHI reduced the elevated TC levels and the effect of MEHI 200 mg/kg (*p* < 0.01) was significant. The extract also significantly (*p* < 0.01) reduced the elevated TG levels. The depleted levels of serum HDL in paracetamol treated rats may be due to hypertriglyceridemia, in the positive control group induced by the reactive metabolite formed during biotransformation. The HDL levels improved on treatment with extract. The effect of MEHI 200 mg/kg (*p* < 0.01) was significant. The results are presented in Table 1.

Paracetamol toxicity causes decrease in liver weight which was the case in the positive control group. Treatment with the extracts significantly increased (*p* < 0.001) the weight of liver in respective groups (Figure 3).

Histopathology studies showed the liver section of vehicle treated hepatotoxic control with periportal inflammation, hepatic degeneration, necrosis, and feathery degeneration of hepatocytes around the central vein. Treatment with the root extract of *H. indicus* var. *pubescens* helped in regaining the normal hepatic parenchymal architecture. MEHI 200 mg/kg treated liver section showed very minimal ballooning degeneration and that of 400 mg/kg showed only some sinusoidal dilation and congestion, degeneration was absent (Fig.4).
Hepatoprotective effect of *Hemidesmus indicus*

Table 1. Effect of methanol extract of *H. indicus* var. *pubescens* roots on serum biochemical parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Positive control</th>
<th>Silymarin 100mg/kg</th>
<th>MEHI 200mg/kg</th>
<th>MEHI 400mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT IU/L</td>
<td>140.2±2.7</td>
<td>320.8±49.1^*</td>
<td>153.3±9.2***</td>
<td>210.7±32.4^*</td>
<td>208.7±13.7^*</td>
</tr>
<tr>
<td>SGPT IU/L</td>
<td>41±2.3</td>
<td>168±11.3^*</td>
<td>81.33±7.1***</td>
<td>73.7±8.5***</td>
<td>65.7±3.6***</td>
</tr>
<tr>
<td>ALP IU/L</td>
<td>214.9±36</td>
<td>779.8±98.6^*</td>
<td>499.7±34.3^*</td>
<td>682±29.8</td>
<td>413±9.5**</td>
</tr>
<tr>
<td>Total protein g/dl</td>
<td>6.7±0.1</td>
<td>5.5±0.2^*</td>
<td>6.2±0.1</td>
<td>6.3±0.1^*</td>
<td>7.1±0.2***</td>
</tr>
<tr>
<td>Total Bilirubin mg/dl</td>
<td>0.4±0.1</td>
<td>0.9±0.1^*</td>
<td>0.4±0.1***</td>
<td>0.6±0.1^*</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.8±0.1</td>
<td>2.7±0.4^*</td>
<td>3.6±0.1**</td>
<td>3.8±0.1***</td>
<td>3.6±0.1**</td>
</tr>
<tr>
<td>TC mg/dl</td>
<td>54.2±4.4</td>
<td>75±7.2</td>
<td>64±4.1</td>
<td>43.6±3.4**</td>
<td>61.1±4.8</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>46.2±2.1</td>
<td>125.8±13.6^*</td>
<td>64.8±9.4**</td>
<td>66±6.8***</td>
<td>59.7±6.6**</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>28.4±0.8</td>
<td>15.66±3.6^*</td>
<td>21.33±0.6</td>
<td>27.33±1.5**</td>
<td>19.33±0.8</td>
</tr>
<tr>
<td>GSH nmol/100mg tissue</td>
<td>53.7±0.3</td>
<td>46.8±0.9^*</td>
<td>51.3±0.4***</td>
<td>54.5±0.4***</td>
<td>54.3±0.2***</td>
</tr>
<tr>
<td>MDA nmol/100mg tissue</td>
<td>0.3±0.2</td>
<td>0.4±0.1</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
</tr>
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</table>

Values expressed as Mean ± SEM; Tukey Kramer Multiple Comparison Test.

*P<0.05, **P<0.01, ***P<0.001 in comparison with the positive control; ^P<0.001, in comparison with the normal control.

Fig. 4. Histopathological sections of liver samples. (A)-Normal control; (B)- Positive control; (C)- MEHI 200 mg/kg; (D)- MEHI 400 mg/kg; (E)- Silymarin 100 mg/kg. HP – Normal hepatic parenchyma; CV – Central vein; CG – Congestion; BD – Ballooning degeneration; SD–Sinusoidal dilatation
Silymarin treated liver sample showed ballooning degeneration, with otherwise normal hepatic parenchyma (Fig.4.). Histopathological grading of the sections were also performed (Table 2.).

Table 2. Histopathological grading of the liver sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.0</td>
</tr>
<tr>
<td>Positive Control</td>
<td>3.5</td>
</tr>
<tr>
<td>Standard (Silymarin 100 mg/kg)</td>
<td>2.5</td>
</tr>
<tr>
<td>MEHI 200 mg/kg</td>
<td>3.5</td>
</tr>
<tr>
<td>MEHI 400 mg/kg</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The presence of vital phytoconstituents such as glycosides, phenolic compounds, tannins and coumarins could have contributed to the hepatoprotective effect of this plant. Further work may be undertaken to isolate the phytoconstituent(s) responsible for this effect and the exact mechanism of action may be elucidated.

CONCLUSION

From this study it is concluded that roots of *H. indicus* var. *pubescens* possess significant hepatoprotective properties. This study may thus provide a platform for further research in isolating the active principles responsible for the hepatoprotective effect and elucidating the exact mechanism of action.

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Hepatoprotective effect of *Hemidesmus indicus*