ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF HYGROPHILA SCHULLI LEAVES

A. Sumathi* and S.Vijayakumar

Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore-641028, Tamilnadu, India

ABSTRACT

The objective of this study was to investigate the antioxidant effects of methanolic extract of leaves of Hygrophila schulli against paracetamol induced liver damage. The material was dried in shade, then powdered and extracted with methanol. The antioxidant effects of the methanol extract was assessed in paracetamol induced hepatotoxic rats. Alteration in the levels of antioxidant markers of hepatic damage like LPO, SOD, CAT, GPx and GST were tested in both paracetamol treated and untreated groups. Treatment of methanolic extract of Hygrophila schulli leaves (500 mg/kg) has brought back the altered levels of antioxidant markers to the near normal levels in the dose dependent manner. Our finding suggested that Hygrophila schulli methanol leaf extract possessed Antioxidant activity.

KEY WORDS: Antioxidants, Lipid peroxidation, Hygrophila schulli, Paracetamol

INTRODUCTION

The world health organization has defined traditional medicine as comprising therapeutic practice that has been in existence for hundreds of years. The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drug constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. Liver is the one of the largest organ in human body and the chief site for intense metabolism and excretion. It has a site for intense metabolism and excretion. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Liver disease mainly caused by chemical agents, excess consumption of alcohol, infection and autoimmune disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damage in liver.

Hygrophila schulli (Acanthaceae) is described in ayurvedic literature as Ikshura, Ikshugandha and Kokilasha "having eyes like the Kokila or Indian Cuckoo". The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. The whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for the treatment of Diabetes, atisaram (Dysentery) etc. The plant is known to possess antitumor, hypoglycemic, antibacterial and hepatoprotective activities.

MATERIALS AND METHODS

Plant material

The fresh plant leaves of Hygrophila schulli were collected from Narasipuram, Coimbatore, Tamil Nadu, and India. The plant material was taxonomically identified by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, and India.

Preparation of extract

The plant leaves were collected and dried under shade and powdered leaves (200 gm) were extracted with methanol in a Soxhlet extractor for 36 hr. The extract was concentrated and last trace of solvent was removed by Rotary Vacuum evaporator and used for further investigation.

Animals

Male Wistar rats (150 – 200 g) and were procured from Small Animal Breeding Station, College of Veterinary Animal Science, Mannuthy, and Kerala. They were housed in microloan boxes with standard laboratory diet and water ad libitum.

Experimental design

Rats were divided into five groups, each group consisting of six animals.

Group I: Control received the vehicle viz.,
Group II: Received paracetamol (750 mg/kg P.O.) 13 at every 72h for 21 days
Group III: Received methanol extract of Hygrophila schulli 250 mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.
Group IV: Received methanol extract of Hygrophila schulli 500 mg/kg P.O. for 21 days and simultaneously administered paracetamol 750 mg/kg every 72 h.
Group V: Received Silymarin 50 mg/kg (P.O.) for 21 days and simultaneously administered Paracetamol 750 mg/kg every 72 hrs

ESTIMATION OF LIVER MARKAR ENZYMES

After 21 days animals were sacrificed and the liver was dissected out washed in ice cold saline, and a homogenate was prepared in 0.05M sodium phosphate buffer pH 7.0. The homogenate was centrifuged at 3000rpm for 10 minutes and the supernatant was used for the assay of marker enzymes. Lipid peroxidation (LPO), Superoxide dismutase (SOD) Catalase (CAT) and Glutathione preoxidase (GPx) and Glutathione S-transferase (GST) were tested in both paracetamol treated and untreated groups.

STATISTICAL ANALYSIS

The results were expressed as mean ± SD of six animals from each group. A column means followed by different superscript are significant at 5% DMRT.

RESULT

Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.05) increase in the paracetamol treated rats. Treatment with Hygrophila schulli (250 and 500 mg/kg) significantly (P<0.05) prevented the increase in LPO level which was brought to near normal. The effect of Hygrophila schulli was comparable with that of standard...
drug silymarin (Fig: 1). Paracetamol treatment caused a significant (P<0.05) decrease in the activities of SOD, catalase, GPx and GST in liver tissue when compared with control group (Fig: 2-5). The treatment of Hygrophila schulli at the doses of 250 and 500 mg/kg resulted in a significant increase in the activities of SOD, catalase, GPx and GST when compared to paracetamol treated rats. The liver of silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to paracetamol treated rats.

**DISCUSSION**

Paracetamol, a widely used antipyretic-analgesic drug, produces acute hepatic damage on accidental over dosage. It is established that, a fraction of paracetamol is converted via the cytochrome P450 pathway to a highly toxic metabolite, N-acetyl-p-benzoquinamine (NAPQI) 20, which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQI, mitochondrial dysfunction 21, and development of acute hepatic necrosis. Several P450 enzymes are known to play an important role in N-acetyl-p-aminophenol (APAP) bioactivation to NAPQI. P450 2E1 is establi

REFERENCES


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### Table 1: Effect of Hygrophila schulli on antioxidant activities in paracetamol induced hepatotoxicity rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>LPO</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>10.09±0.71a</td>
<td>1.79±0.11a</td>
<td>28.71±0.96a</td>
<td>91.32±0.55a</td>
<td>110.61±0.93a</td>
</tr>
<tr>
<td>II</td>
<td>Paracetamol Induced 750 mg/kg</td>
<td>30.72±0.98b</td>
<td>0.83±0.07b</td>
<td>15.76±0.23b</td>
<td>61.37±0.99b</td>
<td>60.81±1.59b</td>
</tr>
<tr>
<td>III</td>
<td>Paracetamol + Hygrophila schulli 250 mg/kg (Low dose)</td>
<td>22.00±0.74c</td>
<td>1.01±0.02c</td>
<td>20.01±0.65c</td>
<td>70.13±1.51c</td>
<td>80.53±0.68c</td>
</tr>
<tr>
<td>IV</td>
<td>Paracetamol + Hygrophila schulli 500 mg/kg (high dose)</td>
<td>13.13±0.55d</td>
<td>1.35±0.02d</td>
<td>25.01±0.63d</td>
<td>85.85±1.00d</td>
<td>98.86±0.40d</td>
</tr>
<tr>
<td>V</td>
<td>Paracetamol + Standard Silymarin 50mg/kg</td>
<td>12.27±0.46e</td>
<td>1.65±1.10e</td>
<td>26.04±0.59e</td>
<td>88.53±0.77e</td>
<td>102.72±2.11e</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D (n=6)

A Column means followed by different superscript are significant at 5% (P<0.05) DMRT. Units: LPO= µ moles of MDA/min/mg protein; SOD= Units/min/mg protein; CAT= µ mole of H₂O₂ consumed/min/mg protein; GPx= µ moles of GSH oxidized/min/mg protein; GST= µ moles of choloro-dinitro benzene(CDNB) conjugated formed/min/mg protein.
Figure-3: Effect of methanol extract of Hygrophila schulli on CAT level

Figure-4: Effect of methanol extract of Hygrophila schulli on GPx level

Figure-5: Effect of methanol extract of Hygrophila schulli on GST level

Control= Normal
Induced= Paracetamol 750mg/kg
Lowdose= Paracetamol + Hygrophila schulli 250 mg/kg
Highdose= Paracetamol + Hygrophila schulli 500 mg/kg
Standard= Paracetamol + Silimar 50mg/kg