Evaluation of the Antioxidant and Hepatoprotective Activity of Cryptolepis Buchanani

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ABSTRACT

Cryptolepis buchanani (Berberidaceae) is a climbing tree, leaves is widely used in folk medicine in Southeast Asia. The alcoholic extract of stem of this plant is commonly used for the treatment of inflammatory conditions such as arthritis, and muscle and joint pain. The development of hepatotoxicity induced by acetaminophen is promoted by oxidative stress. The aim of the present study was to investigate the hepatoprotective effect and antioxidant activities of the ethanolic extract of Cryptolepis buchanani on acetaminophen induced hepatotoxicity in rats. We observed that the ethanolic extract of cryptolepis buchanani conferred hepatoprotectivity. Biochemical observations confirmed the beneficial roles of Cryptolepis buchanani and silymarin against acetaminophen induced liver injury in rats.

INTRODUCTION

A naturally derived herbal medicine has a biological activity for use in pharmaceutical drug discovery and drug design. These drugs useful for treating or preventing life style related disorders. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Cryptolepis buchanani (Asclepiadaceae), a climbing tree, is widely used in folk medicine in Southeast Asia. In Thailand, the alcoholic extract of stem of this plant is commonly used for the treatment of inflammatory conditions such as arthritis, and muscle and joint pain (Panthong et al., 1986).

A weak inhibition of eicosanoid generation from rat leukocytes was previously reported (Laupattarakasem et al., 2003). The aqueous plant extract posses broad spectrum of medicinal properties ranging from antibacterial to anti-inflammatory, the root extract is analyzed for its immuno potentiating properties. The plant as a whole is used as cardiotonic. It also plays a great medicinal value in treating arthritis. (Laupattarakasem et al., 2003). Preliminary phytochemical study confirmed the presence of alkaloids form this plant. Alkaloids related compounds has good potential for treating liver diseases. Therefore, we designed experiments to evaluate the hepatoprotective potential of this plant in experimental models.

MATERIALS AND METHODS

Plant Material

The leaves of Cryptolepis buchanani was collected from Nilgiri hills, Ooty, TamilNadu region and authenticated through Government Arts College, Ooty. Voucher specimen (AWC-01/2011-2012) has been retained in the PG Dept of Biochemistry Akilandeswari Womens College, Vandavasi, India.
Extraction

The leaves of Cryptolepis buchanani were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60°C (Chattopadhyay et al., 2003). The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccators.

GC-MS Analysis

The GC-MS analysis of the ethanolic extract of Cryptolepis buchanani was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary coloum (5% phenyl 95% dimethyl polysiloxane) (30mm X 0.25mm ID X 0.25µmids) and mass detector turbomass gold of the company which was operated in El mode. Helium was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 290°C and the oven temperature was programmed as follows; 50°C at 8°C/min to 200°C (5min) at 7°C/min to 290°C (10min).

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the database of Dr.Dukes ethanobotanical databases. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained.

Experimental Animals

Studies were carried out using male Wistar albino rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 20°C) with dark and light cycle (12/12 h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkalinephosphatase (ALP), bilirubin and total protein kits were procured from Span Diagnostics, Surat, India, and the rest of the chemicals utilized were of analytical grade and were obtained from Ranbaxy Research Laboratory, Hyderabad, India.

Determination of Hepatoprotective Effect of Cryptolepis Buchanani

Experimental Treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (AAP) of 750mg/kg body weight was kept as control (Palani et al., 2010). Group III and IV were treated with extract of Cryptolepis buchanani 250 and 500 mg/kg body wt plus AAP and Group V were fed with standard drug silymarin 25mg/kg daily for seven days (Palani et al., 2009). The extract was administered by oral gavages 1 h before AAP administration.

Preparation of Serum from Blood

After 24 h, animals were sacrificed by chloroform anesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600xg for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) (Reitman and Frankel (1957), alkaline phosphatase (ALP) (King et al., 1934), bilirubin (Malloy et al.,1937) and total protein (Gornall et al.1949).

Preparation of Liver Homogenate

Hepatic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000xg for 60 min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical Estimation of Markers of Oxidative Stress

Measurement of MDA content was according to the method of Zhang (1992). SOD activity was analyzed by the method described by Rai et al., (2006). CAT activity was determined from the rate of decomposition of H2O2 by Bergmeyer et al., method (1974). GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H2O2 and NaN3 (Hafemann et al., 1974). Glutathione reductase activity was assayed by the method of (Carlberg and Mannervik 1975) and modified according to Mohandas et al., (1984). Protein content in the tissue was determined (Lowry et al., 1951) using bovine serum albumin (BSA) as the standard.

Statistical Analysis

The obtained results were analysed for statistical significance using one way ANOVA followed by Dunnet t test using the graphpad prism statistical software for comparison with control group and acetaminophen treated group. P < 0.05 was considered as significant.

Results

GC-MS chromatogram of the ethanolic leaf extract of Cryptolepis buchanani (Fig. 1) showed 20 peaks indicating the presence of twenty compounds. The chemical compounds identified in the ethanolic extract of the Cryptolepis buchanani...
are presented in Table 1. GC-MS analysis revealed that the presence of 2-Octenoic acid, 4,5,7-trihydoxy, 1-Amino-2,6-dimethylpiperidine, α-Methyl-D-mannopyranoside, Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl- [Synonyms: α-Ionene] 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydoxy-6-methyl- D-Galactose, 6-deoxy- [Synonyms: D-Fucose], 1,6-Anhydro-α-D-glucopyranose (Synonyms: levoglucosan, Dodecanaic acid, Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester, (1R,3R,4R,5R)-()-Quinic acid, Tetradecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z)- Oleic Acid, Octadecanoic acid, 4,8,12,16- Tetramethylheptadecan –4- olide, 1,2-Benzenedicarboxylic acid, di isooyl ester. The GC-MS analyses revealed that the alcoholic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These phtochemicals are responsible for various pharmacological actions like antimicrobial, anti-oxidant, hepatoprotective and antiinflammation activities.

This study is only a preliminary study of the occurrence of certain properties of Cryptolepis buchanani extract an in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above.

The acetaminophen induction resulted in a marked increase in the specific activities, serum SGOT, SGPT, SALP and total bilirubin levels (Fig. 2&3). Maximum hepatoprotective activity was observed at 500 mg/kg dose level of Cryptolepis buchanani which was comparable to that of silymarin. Activities of SOD, CAT, LP, GSH and GPX activities were significantly (p<0.05) enhanced only in the high dose of Cryptolepis buchanani plus acetaminophen treated group. However the levels of hepatic GSH and GST were found to be not significant compared to control group (Fig. 4 & 5). Hepatic LP levels was significantly (p<0.01) elevated in the control group (Fig.6). Moreover, the activity of CAT in the 500mg/kg Cryptolepis Buchanani and acetaminophen treated group were significantly increased from the control group (p<0.05) (Fig.6).  

![Fig 1: The chromatogram showing Quinic acid (12.14), n-Hexadecanoic acid (16.22), Oleic Acid (18.87) and Octadecanoic acid (19.17).](image)

![Fig 2: Effect of ethanol extract of Cryptolepis buchanani and silymarin on serum enzymes (SGPT, SGOT and ALP), on acetaminophen induced hepatotoxicity in rats.](image)

![Fig 3: Effect of ethanol extract of Cryptolepis buchanani and silymarin on serum enzymes total bilirubin and total protein on acetaminophen induced hepatotoxicity in rats.](image)
Effect of ethanol extract of *Cryptolepis buchanani* & silymarin on antioxidants levels (SOD, & GST) in acetaminophen induced hepatoxicity in rats.

**Fig 4.** Effect of ethanol extract of Cryptolepis buchanani & silymarin on antioxidants levels (SOD, & GST) in acetaminophen induced hepatoxicity in rats.

**Fig 5.** Effect of ethanol extract of *Cryptolepis buchanani* & silymarin on antioxidants levels (GSH, GPX) in acetaminophen induced hepatoxicity in rats.

**Fig 6.** Effect of ethanol extract of *Cryptolepis buchanani* and silymarin on antioxidants levels (CAT, MDA) in acetaminophen induced hepatoxicity in rats.

**Table 1:** Phyto-Components identified in the *Cryptolepis buchanani* [GC MS study].

<table>
<thead>
<tr>
<th>No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.31</td>
<td>2-Octenoic acid, 4,5,7-trihydroxy</td>
<td>C_{12}H_{16}O_{2}</td>
<td>128</td>
<td>1.52</td>
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<tr>
<td>2</td>
<td>4.65</td>
<td>1-Amino-2,6-dimethylpiperidine</td>
<td>C_{9}H_{14}O_{5}</td>
<td>190</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>5.14</td>
<td>à-Methyl-D-mannopyranoside</td>
<td>C_{7}H_{14}O_{6}</td>
<td>194</td>
<td>2.12</td>
</tr>
<tr>
<td>4</td>
<td>5.75</td>
<td>Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-[Synonyms: à-lonone]</td>
<td>C_{6}H_{8}O_{4}</td>
<td>144</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>8.92</td>
<td>4H-Pyan-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-</td>
<td>C_{13}H_{18}</td>
<td>174</td>
<td>2.94</td>
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<tr>
<td>6</td>
<td>9.21</td>
<td>D-Galactose, 6-deoxy-[Synonyms: D-Fucose]</td>
<td>C_{6}H_{12}O_{5}</td>
<td>164</td>
<td>6.06</td>
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<tr>
<td>7</td>
<td>10.26</td>
<td>1.6-Anhydro-á-d-glucopyranose ([Synonyms: levoglucosan])</td>
<td>C_{6}H_{10}O_{5}</td>
<td>162</td>
<td>5.85</td>
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<tr>
<td>8</td>
<td>10.91</td>
<td>Dodecanoic acid</td>
<td>C_{12}H_{24}O_{2}</td>
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<td>0.83</td>
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<tr>
<td>9</td>
<td>11.02</td>
<td>Phosphonofluoridic acid, (1-methylthyl)-, cyclohexyl ester</td>
<td>C_{9}H_{18}FO_{2}P</td>
<td>208</td>
<td>0.50</td>
</tr>
<tr>
<td>10</td>
<td>12.14</td>
<td>(1R,3R,4R,5R)-(++)-Quinic acid</td>
<td>C_{7}H_{2}O_{6}</td>
<td>192</td>
<td>26.04</td>
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<tr>
<td>11</td>
<td>13.38</td>
<td>Tetradecanoic acid</td>
<td>C_{14}H_{28}O_{2}</td>
<td>228</td>
<td>15.69</td>
</tr>
<tr>
<td>12</td>
<td>14.45</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>C_{20}H_{40}O</td>
<td>296</td>
<td>2.55</td>
</tr>
<tr>
<td>13</td>
<td>16.22</td>
<td>n-Hexadecanoic acid</td>
<td>C_{16}H_{32}O_{2}</td>
<td>256</td>
<td>11.44</td>
</tr>
<tr>
<td>14</td>
<td>16.49</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>C_{18}H_{36}O_{2}</td>
<td>284</td>
<td>0.88</td>
</tr>
<tr>
<td>15</td>
<td>18.44</td>
<td>Phytol</td>
<td>C_{20}H_{40}O</td>
<td>296</td>
<td>0.32</td>
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<tr>
<td>16</td>
<td>18.77</td>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>C_{18}H_{32}O_{2}</td>
<td>280</td>
<td>1.85</td>
</tr>
<tr>
<td>17</td>
<td>18.87</td>
<td>Oleic Acid</td>
<td>C_{18}H_{34}O_{2}</td>
<td>282</td>
<td>7.70</td>
</tr>
<tr>
<td>18</td>
<td>19.17</td>
<td>Octadecanoic acid</td>
<td>C_{18}H_{36}O_{2}</td>
<td>284</td>
<td>1.89</td>
</tr>
<tr>
<td>19</td>
<td>21.96</td>
<td>4,8,12,16-Tetramethyleptadecan-4-olide</td>
<td>C_{21}H_{40}O_{2}</td>
<td>324</td>
<td>0.65</td>
</tr>
<tr>
<td>20</td>
<td>24.68</td>
<td>1,2-Benzenedicarboxylic acid, diisooctyl ester</td>
<td>C_{24}H_{38}O_{4}</td>
<td>390</td>
<td>0.93</td>
</tr>
</tbody>
</table>

**Source:** Dr. Duke’s Phytochemical and Ethnobotanical Databases
DISCUSSION

In this study, we show that ethanolic extracts of Cryptolepis Buchananii protected against acetaminophen induced hepatotoxicity. This suggests that a fat-soluble metabolite or metabolites constitutes the active ingredient. Candidate molecules include alkaloids, flavonoids, etc. In the Preliminary phytochemical studies reveal the presence of alkaloids and flavonoids in ethanolic extract of Cryptolepis Buchananii, these molecules are reported to have hepatoprotective activity (Seevela et al., 1984; Wegner and Fintelmann, 1999). The hepatic damage is established that, a fraction of acetaminophen is converted via the cytochrome P450 pathway to a highly toxic metabolite; N-acetyl-p-benzoquinamine (NAPQI) (Dahlin et al., 1984) which is normally conjugated with glutathione and excreted in urine. In overdose situations, however, glutathione levels are exhausted and NAPQI can directly modify susceptible protein residues in what is widely believed to be the first step in a cascade of biochemical events leading to hepatic cell death. (Bessems et al., 2001; Nelson and Bruschi, 2002; Hinson et al., 1981; Nelson and Pearson 1990; Potter et al., 1973; Jollow et al., 1973; Pierce et al., 2002; and Adams et al., 2001).

In this study, rat treated with single dose of AAP treated animals developed a significant hepatic damage and oxidative stress, resulted in a marked increase in serum SGOT, SGPT SALP and total bilirubin levels. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). However the total protein level was decreased.

The significant (P<0.01) restoration of these enzyme levels on administration of the leaf extract also by silimarin 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. This is the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987; Maiti et al., 2005).

Effective control of ALP, bilirubin and total protein levels points towards an early Improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by acetaminophen.

During hepatic injury, superoxide radicals generate at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages liver. Decreased CAT activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by Acetaminophen. The SOD and CAT activities were brought to near normal after pretreatment with extract in APA-treated rats evidently shows the antioxidant property of the extract against oxygen free radicals.

Both reductions of GPX &GSH activity in Acetaminophen treated rats as observed in this study indicate the damage to the hepatic cells. Administration of Cryptolepis Buchanani extract promoted the reactivation of hepatic glutathione reductase enzyme in acetaminophen treated rats. The restoration of GSH level after the administration of plant extract to such acetaminophen treated rats due to the protective effect of the extract.

CONCLUSION

Ethanolic leaf extract of Cryptolepis Buchananii significantly protects against liver injuries as well as oxidative stress, resulting in increased serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of SOD, CAT, GSH, GPX, and GST in acetaminophen treated rats were significantly increased by treatment with the extract. Further studies to characterize the active chemical compounds and to elucidate the mechanism are in progress.

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