Atropa acuminata (AC) is a traditional medicinal plant that is commonly used for disorders like Central nervous system abnormalities, and in ayurvedic medicine, it is used for the treatment of fevers, cough, colds, colitis, conjunctivitis (inflamed eyes) and diarrhea. This medicinal plant also proved to be effective in homeopathic treatment for skin reactions during radiotherapy treatment for breast cancer1.

The aerial parts are used to treat abnormal menstrual periods, acute infections, acute inflammation, anesthetic, antispasmodic, anxiety, arthritis, asthma, bedwetting, bowel disorders, chicken pox, colds, colitis, conjunctivitis (inflamed eyes), dental conditions, diarrhoea, and urinary tract disorders (difficulty passing urine), warts, whooping cough14,15. However, no data are available on the hepatoprotective and antioxidant properties of this plant. Therefore, this study was designed to investigate the protective effects of the ethanol extract of Atropa acuminata against acetaminophen (APAP)-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Material

Aerial part of Atropa acuminata (Solanaceae) was collected from Tirunelveli district, Tamil Nadu, India in the month of March. The plant material was taxonomically identified and authenticated by V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India.

Extraction

The aerial part of Atropa acuminata was 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. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze dried and stored in a vacuum desiccator.

GC–MS analysis of ethanol extract of Atropa acuminata

The identification of chemical composition of ethanol extract of Atropa acuminata was performed using a GC–MS spectograph (Agilent 6890/Hewlett-Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0 mL) of Atropa acuminata was injected with a Hamilton syringe to the GC–MS manually for total ion current (TIC) mode. The identification of chemical composition

Keywords: Hepatoprotective, Atropa acuminata, Acetaminophen, Silymarin, Antioxidant.
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 (APAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of Atropa acuminata at two different doses of 250 and 500 mg/kg body weight plus APAP. Group V were fed with standard drug silymarin 25mg/kg daily for seven days. The extract was administered by oral gavages 1 h before APAP administration 17.

Preparation of serum from blood

After 24 h, animals were sacrificed by chloroform anaesthesia. 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 600 × g for 15 min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), 18 alkaline phosphatase (ALP) 19, bilirubin 20 and total protein 21.

Preparation of liver homogenate

Heaptic tissues were homogenized in KCl [10 mM] phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000 × g 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

MDA content was Measured according to the earlier method reported 22. SOD activity was determined according to previous report 23. CAT activity was determined from the rate of decomposition of H2O2 by the reported method 24. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and protein content in the tissue was determined by earlier method reported 25, using bovine serum albumin (BSA) as the standard.

Histopathological study

On completion of closing regimen animals were sacrificed and the liver dissected out. Paraaffin sections were prepared for histological examination and following standard procedure 29. Hematoxylin & eosin stained sections were observed.

Statistical analysis

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

RESULTS

Phytochemical analysis

The ethanol extract of Atropa acuminata was a complex mixture of many constituents and 20 compounds were identified in this plant by GC-MS. Phytoconstituents such as Glycerin, Thymine, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 2-Coumaranone, 1,2,3-Benzotriazol, Succrose, 1,2,3,4-Cyclohexanetetrol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Phenol, 2,4,6-trimethyl-5-isopropyl-6-methyl-hepta-3,5-dien-2-ol, Dibutyl phthalate, Phytol, Hexanedioic acid, bis(2-ethylhexyl) ester, 1,2-Benzenedicarboxylic acid, dioctoeyl ester, Tetracol-5-amine, N-(3,4-dimethoxybenzyl)-4,13,20-Tri-O-propylphorbol 12-acetate, Bufe-20,22-dienolide, 14,15-epoxy-16-dihydroxy-., (3a,5a,15a,16a)- [Synonyms: Cineobufagin, deacetyl-1,2-Allyl-3,6-dimethoxybenzyl alcohol, 1,2-Dimethoxy-4-n-propylbenzen]- were identified in the ethanol extract of Atropa acuminata by relating to the 185 corresponding peak area through coupled GC-MS [Table 1; Figure 1].

Biochemical analysis

The effect of Atropa acuminata on serum marker enzymes is presented in Figure 2, 3 & 5. The serum levels of GOT, GPT, ALP and total bilirubin were markedly significantly (p< 0.01) elevated and that of protein levels were significantly (p< 0.01) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of Atropa acuminata at the doses of 250 and 500 mg/kg remarkably significantly (p< 0.01; p< 0.05) prevented hepatotoxicity induced by acetaminophen.

Acetaminophen treatment caused a significant (P<0.01) decrease in the level of SOD, catalase, GPX and GST when compared to control group. The treatment of Atropa acuminata at the doses of 250 and 500 mg/kg resulted in a significant (P<0.01; P<0.05) increase of SOD, catalase, GPX and GST when compared to Group II. The standard drug, silymarin treated animals also showed a significant (P<0.01) increase in antioxidant enzymes levels compared to Group II.

Analysis of MDA levels by thiobarbituric acid reaction showed a significant (P<0.01) increase in the acetaminophen treated rats. Treatment with Atropa acuminata at the doses of 250 and 500 mg/kg remarkably significantly (P<0.01; P<0.001) prevented the increase in MDA level presented in Figure 2, 3 & 5. The serum levels of GOT, GPT, ALP and total bilirubin were markedly significantly (p< 0.01) elevated and that of protein levels were significantly (p< 0.01) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of Atropa acuminata at the doses of 250 and 500 mg/kg remarkably significantly (p< 0.01; p< 0.05) prevented hepatotoxicity induced by acetaminophen.

Histopathological studies

Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also Atropa acuminata at the two different doses tested groups. Histopathological profile of the normal animal showed normal hepatocytes with well-preserved cytoplasm and there was no sign of inflammation, which has been illustrated in Figure 6 a. The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration [Figure 6 b]. Treatment with different doses of ethanol extract of Atropa acuminata and silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control [Figure 6 c, d, & e]. All these results indicate a hepatoprotective potential by the Atropa acuminata.
Table 1: Identification of Phyto components in the ethanol extract of *Atropa acuminata* by GC MS analysis

<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>
<th>Compounds Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.34</td>
<td>Glycerin</td>
<td>C3H8O3</td>
<td>92</td>
<td>2.29</td>
<td>Alcohol</td>
</tr>
<tr>
<td>2.</td>
<td>3.22</td>
<td>Thymine</td>
<td>C5H6N2O2</td>
<td>126</td>
<td>0.97</td>
<td>DNA fraction</td>
</tr>
<tr>
<td>3.</td>
<td>3.92</td>
<td>2H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-</td>
<td>C6H8O4</td>
<td>144</td>
<td>0.64</td>
<td>Flavonoid fraction</td>
</tr>
<tr>
<td>4.</td>
<td>4.79</td>
<td>2-Furancarboxaldehyde, 5-{(hydroxymethyl)-</td>
<td>C8H6O3</td>
<td>126</td>
<td>1.95</td>
<td>Aldehyde compound</td>
</tr>
<tr>
<td>5.</td>
<td>4.99</td>
<td>2-Coumaranone</td>
<td>C8H6O2</td>
<td>134</td>
<td>0.79</td>
<td>Coumaranone compound</td>
</tr>
<tr>
<td>6.</td>
<td>6.65</td>
<td>1,2,3-Benzemlietiol</td>
<td>C6H6O3</td>
<td>126</td>
<td>6.76</td>
<td>Pyrogallol</td>
</tr>
<tr>
<td>7.</td>
<td>7.81</td>
<td>Sucrose</td>
<td>C12H20N2O2</td>
<td>342</td>
<td>2.73</td>
<td>Sugar moiety</td>
</tr>
<tr>
<td>8.</td>
<td>8.34</td>
<td>1,2,3,4-Cyclohexanetetrol</td>
<td>C6H12O4</td>
<td>148</td>
<td>3.33</td>
<td>Poly Hydroxy benzene</td>
</tr>
<tr>
<td>9.</td>
<td>10.07</td>
<td>Phenol, 2,4,6-trimethyl-</td>
<td>C9H12O2</td>
<td>136</td>
<td>0.48</td>
<td>Phenolic compound</td>
</tr>
<tr>
<td>10.</td>
<td>11.07</td>
<td>5-Isopropyl-6-methyl-hepta-3-dien-2-ol</td>
<td>C11H20O2</td>
<td>168</td>
<td>0.25</td>
<td>Alcoholic compound</td>
</tr>
<tr>
<td>11.</td>
<td>12.47</td>
<td>Dibutyl phthalate</td>
<td>C22H44O4</td>
<td>370</td>
<td>0.40</td>
<td>Plasticizer compound</td>
</tr>
<tr>
<td>12.</td>
<td>15.45</td>
<td>Phytol</td>
<td>C20H40O</td>
<td>296</td>
<td>0.28</td>
<td>Diterpene</td>
</tr>
<tr>
<td>13.</td>
<td>19.41</td>
<td>Hexaneedioic acid, bis(2-ethylhexyl) ester</td>
<td>C22H42O4</td>
<td>370</td>
<td>0.40</td>
<td>Ester compound</td>
</tr>
<tr>
<td>14.</td>
<td>21.48</td>
<td>1,2-Benzenedicarboxylic acid, diisocetyl ester</td>
<td>C24H38O4</td>
<td>390</td>
<td>0.67</td>
<td>Plasticizer compound</td>
</tr>
<tr>
<td>15.</td>
<td>26.79</td>
<td>Tetrazol-5-amine, N-{(3,4-dimethoxybenzyl)-</td>
<td>C10H13N5O2</td>
<td>235</td>
<td>5.14</td>
<td>Amino compound</td>
</tr>
<tr>
<td>17.</td>
<td>27.80</td>
<td>Bufa-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-, (3,4,5,15,16)- [Synonyms: Cinobufagin, deacetyl-]</td>
<td>C24H32O5</td>
<td>400</td>
<td>17.67</td>
<td>Hydroxy compound</td>
</tr>
<tr>
<td>18.</td>
<td>29.13</td>
<td>2-Allyl-3,6-dimethoxybenzyl alcohol</td>
<td>C12H16O3</td>
<td>208</td>
<td>1.71</td>
<td>Alcoholic compound</td>
</tr>
<tr>
<td>19.</td>
<td>29.37</td>
<td>1,2-Dimethoxy-4-n-propylbenzene</td>
<td>C11H16O2</td>
<td>180</td>
<td>2.57</td>
<td>Aromatic compound</td>
</tr>
</tbody>
</table>

Fig. 2: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of SGOT (IU/L), SGPT (IU/L) and MDA (nM/mg of protein) [Lipid peroxidation (LPO)] level of hepatic tissue during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

Fig. 3: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of alkaline phosphotase (ALP) (IU/L) & total protein and hepatic levels of CAT (U/mg protein), GSH (U/mg protein) and GPX (mg of glutathione utilized/min/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively
Fig. 4: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg) on hepatic levels of SOD (units of activity/mg protein) & GST (Units/mg protein) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

Fig. 5: Effect of ethanolic extract of *Atropa acuminata* and silymarin (standard drug, (25 mg/kg)) on serum levels of total bilirubin (mg/dl) during acetaminophen treated hepatotoxicity and oxidative stress in rats. Values are mean ± S.D. (n = 6). **p < 0.01, *p < 0.05, respectively

6 (a) Normal photomicrograph of liver tissue of control rat showing normal hepatic cells with central vein and sinusoidal dilation. (H and E 100X)

6(b) Liver section of rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation. (H and E 100X)
DISCUSSION

Acetaminophen (N-acetyl-p-aminophenol, Paracetamol), a widely used analgesic, antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses. The laboratory features of hepatotoxicity induced by APAP resemble other kinds of acute inflammatory liver disease with prominent increase of GOT, GPT, and ALP levels.

In the present study, the serum level of hepatic enzymes GOT, GPT, ALP and total bilirubin levels were increased and reflected the hepatocellular damage in the APAP-induced hepatotoxicity animal model. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver.

However, the total protein level was decreased. There was a significant (P<0.01) restoration of these enzyme levels on administration of the ethanol extract of Atropa acuminata 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 ethanol extract of Atropa acuminata may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.

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

This indicates the anti-lipid per oxidation and/or adaptive nature of the systems as brought about by ethanol extract of Atropa acuminata against the damaging effects of free radical produced by APAP.

Previous studies have demonstrated that oxidative stress is a major mechanism in the development of APAP-induced hepatotoxicity. In APAP overdose, the compound is converted to toxic form, NAPQI (N-acetyl-p-benzoquinoneimine) which rapidly reacts with glutathione (GSH) and leads to 90% total hepatic GSH depletion.
Both reductions of GST & GSH activity APAP-treated rats as observed in this study indicate the damage to the hepatic cells.

Administration of Atropa acuminata extract promoted the reactivation of hepatic glutathione reductase enzyme in APAP-treated rats. The restoration of GSH level to such APAP treated rats indicates the protective effect after the administration of ethanol extract of Atropa acuminata.

Furthermore, the level of MDA was increased in the group receiving APAP administration, but treatment with the Atropa acuminata reduced the amount of MDA. This result indicated that decreasing the formation of lipid peroxidation is also one of the events in preventing the oxidative toxicity by APAP.

In conclusion, ethanol extract of Atropa acuminata significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and ALP. The reduced levels of parameters of SOD, CAT, GSH, GPX and GST in acetaminophen-treated rats were significantly increased by treatment with Atropa acuminata. It may be due to synergistic effect of the chemical compounds present in them, making them good source for hepatoprotective and antioxidant activities against APAP induced hepatotoxicity and has the potential for use as a hepatoprotective agent. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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REFERENCES


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