Hepatoprotective and Antioxidant Activities of *Amaranthus viridis* Linn

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Abstract

**Aim:** To study hepatoprotective activity of methanolic extract of whole plant of *Amaranthus viridis* Linn (MeAv) in paracetamol (PCM) induced hepatotoxicity.

**Materials and Methods:** MeAv was screened for hepatoprotective activity in PCM (3 g/kg) induced hepatotoxicity in Wistar rats for 15 days at dose of 200 and 400 mg/kg by measuring liver marker enzymes (serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase), bilirubin (total bilirubin and direct bilirubin) and albumin (ALB), total protein (TP) levels and histopathological studies. Antioxidant activities were studies by measuring malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) and total thiols (TT) in liver homogenate of treated animals.

**Results:** MeAv significantly (P<0.001) decreases the elevated liver marker enzymes (serum glutamate pyruvate transaminase and serum glutamate oxaloacetate transaminase), bilirubin (total bilirubin and direct bilirubin) and restores albumin (ALB), total protein (TP) levels. A histopathological study also showed liver protective activity of MeAv. In *in vivo* antioxidant studies, the MeAv has significantly restored the malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT) and total thiols.

**Conclusion:** In conclusion, administration of MeAv for 15 days showed liver protective activity against paracetamol induced liver damage and the potential antioxidant property of MeAv thought to be the mechanism behind its hepatoprotective activity.

Introduction

*Amaranthus viridis* Linn (Amaranthaceae), commonly called ‘Chilaka ThotaKura’ in Telugu, has been used in Indian and Nepalese traditional system to reduce labour pain and act an antipyretic [1,2]. *A. viridis* contains amino acids lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine and tryptophan [3] (Anonymous.). The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes etc [4]. Other traditional uses range from an anti-inflammatory agent of the urinary tract, venereal diseases verminfuge, diuretic, anti-rheumatic, antiulcer, analgesic, antiemetic, laxative, improvement of appetite, antileptin, treatment of respiratory and eye problems, to treatment of asthma [1, 3, 5-11].

Furthermore, the plant possesses antiproliferative and antifungal lactin properties as well as ribosome inactivating protein, beta-carotene [12-14] and antiviral activities [15]. In addition the whole plant possesses analgesic and antipyretic properties and is used for the treatment of pain and fever respectively in traditional
The main aim of the study was to investigate the liver protective activity of methanol extract of whole plant of *A. viridis* linn against paracetamol induced liver injury in rats.

### Materials and Methods

**Collection of Plant Material and Extraction**

The fresh plant of *A. viridis* was collected from Chickballapur, Karnataka, India and was authenticated by Prof. B.K.Venkatesh, Department of Botany, Government First grade College, Chickballapur, Karnataka, India. A voucher specimen (SKVCP 11) was deposited in college herbarium. The whole plant was shade dried and coarsely powdered. The coarse powder was subjected to extraction with methanol by soxhlet apparatus and extract was concentrated to dryness in vacuum (yield 34.8%).

**Preliminary Phytochemical screening**

The methanol extract of *A. viridis* was screened for the presence of various phytoconstituents like flavonoids, saponins, glycosides, terpenoids amino acids, alkaloids, carbohydrates, phenolic compounds and proteins [17].

**Animals**

Male Wistar rats weighing 150-250 g were acclimatized to the experimental room at temperature 23 ± 2 °C, controlled humidity conditions (50-55%) and 12 h light and 12 h dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V.College of Pharmacy, Chickballapur, Karnataka, according to prescribed guidelines of CPCSEA, Government of India (Reg. No. 117/2000/CPCSEA).

**Liver protective activity**

Rats were divided into five groups (n=6). Group I received a single daily dose of sodium carboxy methyl cellulose (1 ml of 1%, w/v, p.o. body weight) for 14 days. Group II animals received single dose of 3 gm/kg paracetamol (PCM) orally on 14th day, while third and fourth groups received orally 200, 400 mg/kg body weight of MeAv and fifth group received Silymarin (25 mg/kg once daily p.o) respectively for 14 days along with paracetamol single dose (3 gm/kg) as in group second. Animals were sacrificed 48 h after the paracetamol administration; blood and liver were collected [18]. The blood were collected and allowed to clot and serum was separated at 3000 rpm for 10 min to obtain serum, which was later used for the estimation of serum enzymes. Serum glutamate oxaloacetate transaminase (SGOT, U/L), serum glutamate pyruvate transaminase (SGPT U/L) [19], Albumin (ALB), total bilirubin and direct bilirubin [20] were assayed using autoanalyser (Maygun MS 500) using kits (ERBA, Transasia Biomedicals Ltd. India).

**Assessment of MDA, TP, CAT, GSH and Total thiols**

Collected Liver was homogenized in ice-cold saline-EDTA using Teflon-glass homogenizer (Remi, pvt ltd., Mumbai). The required quantity of liver homogenates was used for the estimation of lipid peroxidation [21]. The homogenate was centrifuged at 10,000 rpm for 10 min and the pellet was discarded. The supernatant was again centrifuged at 20,000 rpm for 1 hour at 4°C and the supernatant obtained was used for the estimation of total protein [22], Catalase [23], GSH and total thiols [24].

**Histopathological studies**

The livers were removed from the animals and the tissues were fixed in 10% formalin for at least 24h dehydrated with alcohol and embedded in paraffin. Thin section (5μm) were cut and stained with haematoxylin-eosin dye (H & E) for photo microscopic assessment including cell necrosis, fatty changes ballooning degeneration, focal hemorrhage and centrilobular degeneration and lymphocyte infiltration.

**Acute toxicity studies**

Methanol extract of *A. viridis* was studied for acute oral toxicity as per revised OECD (Organization for Economic Cooperation and development) guidelines No. 423. The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 200- 400 mg/kg doses of extract were used [25].

**Statistical analysis**

All the values were expressed as Mean±SEM, the results were analyzed statistically by one-way ANOVA followed by Tukey’s post-test, *P*<0.05 were considered significant.
Results and Discussion

On preliminary phytochemical analysis of methanolic extract of *A. viridis* showed the presence of flavonoids, saponins, glycosides, terpenoids aminocids, alkaloids, carbohydrates, phenolic compounds and proteins.

In present study, liver sections control groups (Group I) showed normal hepatic cell structure (Fig.1A), the hemorrhagic and necrotic findings observed in the histopathological examination of the liver of rats administered with PCM (Fig. 1 B). Whereas, MeAv 200 mg/kg treated group showed central veins and sinusoids with

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>Serum ALB</th>
<th>Serum TP</th>
<th>Bilirubin (mg/dl)</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>27.76 ± 3.50</td>
<td>61.91 ± 5.77</td>
<td>6.05 ± 0.14</td>
<td>4.28 ± 0.2</td>
<td>0.592 ± 0.05</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Paracetamol (PCM) alone (3g/kg; p.o)</td>
<td>1379.74 ± 147.45</td>
<td>1412.77 ± 109.22</td>
<td>2.99 ± 0.23</td>
<td>2.32 ± 0.17</td>
<td>2.685 ± 0.15</td>
<td>0.345 ± 0.03</td>
</tr>
<tr>
<td>PCM+ MeAv (200 mg/kg)</td>
<td>657.56 ± 100.5</td>
<td>514.7 ± 85.62</td>
<td>4.28 ± 0.14</td>
<td>2.83 ± 0.08</td>
<td>0.74 ± 0.06</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>PCM+ MeAv (400 mg/kg)</td>
<td>585.78 ± 79.82</td>
<td>345.06 ± 37.89</td>
<td>4.59 ± 0.25</td>
<td>3.02 ± 0.9</td>
<td>0.403 ± 0.03</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>PCM+ Silymarin (100 mg/kg)</td>
<td>229.23 ± 36.67</td>
<td>219.79 ± 19.31</td>
<td>5.24 ± 0.36</td>
<td>3.51 ± 0.15</td>
<td>0.442 ± 0.04</td>
<td>0.165 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±S.E.M., n=6. a *p*<0.05; b *p*<0.01; c *p*<0.001 compared with PCM group and *#* *p*<0.001 when compared to control group.

Paracetamol is one of the commonest drugs used for the treatment of minor to moderate pain in humans. However, the intake of a single dose of 10 g or higher causes centrilobular liver necrosis [26]. PCM is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [27]. However, these metabolites in the liver are converted into a reactive metabolite known to be toxic for the liver, namely N-acetyl-p-benzoquinoneimine, by the liver cytochrome P-450 enzyme system. Paracetamol administration to rats produced hepatotoxicity shown by significant increase in the levels of SGOT, SGPT, ALB and decreases TP and Bilirubin in comparison to control group. The methanol extract of *A. viridis* showed significant decrease in the SGOT, SGPT, Bilirubin (*p*<0.001) and also significantly improved the levels of ALB and TP when compared to paracetamol group (Table 1).

In vivo antioxidant studies are showed in Table 2. Paracetamol showed significant (*p*<0.001) increase in lipid peroxidation (MDA) and decrease in GSH, CAT and Total thiols levels in group II compared to control group. Pretreatment with MeAv has significantly (*p*<0.001) restored these changes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (n moles/g of tissue)</th>
<th>GSH (n moles/mg of protein)</th>
<th>Catalase (U/mg of protein)</th>
<th>Total thiols (µ moles/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>9.8 ± 0.43</td>
<td>69.44 ± 4.35</td>
<td>76.49 ± 3.68</td>
<td>0.75 ± 0.021</td>
</tr>
<tr>
<td>Paracetamol (PCM) alone (3g/kg; p.o)</td>
<td>59.60 ± 2.1</td>
<td>10.47 ± 1.13</td>
<td>20.48 ± 2.56</td>
<td>0.08 ± 0.018</td>
</tr>
<tr>
<td>PCM+MeAv (200 mg/kg once daily; p.o.)</td>
<td>34.19 ± 2.23</td>
<td>30.48 ± 1.76</td>
<td>30.98 ± 2.38</td>
<td>0.24 ± 0.009</td>
</tr>
<tr>
<td>PCM+MeAv (400 mg/kg once daily; p.o.)</td>
<td>20.15 ± 2.14</td>
<td>45.39 ± 2.18</td>
<td>43.75 ± 2.43</td>
<td>0.40 ± 0.012</td>
</tr>
<tr>
<td>PCM+Silymarin (100 mg/kg once daily; p.o.)</td>
<td>16.29 ± 1.6</td>
<td>54.18 ± 1.95</td>
<td>51.27 ± 2.67</td>
<td>0.58 ± 0.017</td>
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</table>

Each value represents Mean ± S.E.M., n=6. "p"<0.001 compared with PCM alone group. "*p"<0.001 compared with control group.
Figure 1: Histopathology of liver tissues (A) liver section of normal rat. (B) PCM treated group showing; section shows patches of liver cell necrosis with inflammatory collections. (C) PCM+ MeAv (200 mg/kg) treated group which showed central veins and sinusoids show reasonable congestion and focal haemorrhage or extensive centrilobular degeneration and diffuse inflammatory process. (D) & (E) showed central veins and sinusoids show less congestion and focal haemorrhage or small centrilobular degeneration with slight degree of inflammatory process; less portal triaditis and spotty necrosis in PCM+ MeAv (400 mg/kg) and PCM+ Silymarin (100 mg/kg) groups.

reasonable congestion and focal hemorrhage or extensive centrilobular degeneration and diffuse inflammatory process (Fig. 1 C). MeAv (400 mg/kg) and silymarin (100 mg/kg) treated groups showed central veins and sinusoids show less congestion and focal hemorrhage or small centrilobular degeneration with slight degree of inflammatory process; less portal triaditis and spotty necrosis, also treated group showed absence of cell necrosis (Fig. 1D & E).
The present study shows the liver protective activity of MeAv in PCM induced hepatotoxicity, may be due to the presence of amino acids [28-30], flavonoids [31], terpenoids [32], saponins [33], glycosides [34] and phenolic compounds [35]. In the acute toxicity study, MeAv did not show any mortality up to a dose of 2000 mg/kg body weight.

Conclusion
Our investigation suggests that methanolic extract of whole plant of *Amaranthus viridis* Linn, possess liver protective activity against paracetamol induced hepatotoxicity in rats. Therefore, further work could be done on isolation of active constituents and study of its liver protective activity.

Acknowledgment
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