Hepatoprotective Activities of *Amaranthus retroflexus* Leaves against Paracetamol Induced Hepatic Damage in Albino Rats

**Keywords:** Ethanolic extract of *Amaranthus retroflexus* leaves, serum markers

**ABSTRACT**

*Amaranthus retroflexus* is a medicinal plant. Our aim was to investigate its organ protection property. Therefore ethanolic extract of *Amaranthus retroflexus* leaves (AR) was studied and the parameters observed were SGPT, SGOT, total bilirubin, direct bilirubin, total cholesterol, HDL and ALP activities. Results of biochemical studies of blood samples of Paracetamol treated animals showed significant increase in the levels of serum markers and decrease in HDL level reflecting the liver injury caused by Paracetamol. Whereas, the animals treated with ethanolic extract of AR showed significant dose-dependent decrease in the elevated levels of serum markers and increase in HDL levels indicating the protection of hepatic cells. So these results concluded that the ethanolic extract afford significant protection against Paracetamol induced hepatocellular injury and remarkable rejuvenation of these tissues found in histopathological studies which may be attributed due to polyphenols and antioxidants present.
INTRODUCTION

Liver is an important organ in the body. It is the key organ of metabolism and excretion. During its normal physiological functioning, it metabolizes various endogenous and exogenously administered chemicals so as to terminate or inactivate these agents. Hence due to this function, it protects the whole body from the various environmental and chemical challenges. In addition to this liver has got an inbuilt mechanism to protect itself and to regenerate on several occasions, but many of these hepatotoxic challenges overpower inbuilt protective mechanism and cause hepatotoxicity resulting in the hepatic necrosis and hepatitis.

*Amaranthus retroflexus* is an edible plant which is used as vegetable which is also used by native practitioner as hepatoprotective in treating various types of jaundice. The leaves of this plant contain polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds have antioxidant property and anti-oxidants have known to possess hepatoprotective activity. Keeping the native knowledge and the above mentioned literature information¹, this plant was selected for present study to screen the leaves of this edible plant for the presence of category of phytoconstituents, antioxidant and hepatoprotective activities. This study was carried out by using 70% ethanolic extract of AR (AREE) as hepatoproectant and Paracetamol as hepatotoxicant.

MATERIALS AND METHODS

Collection and identification of plant: The plant was collected from Kusnoor village (Gulbarga district), Karnataka in the month of March and was authenticated by Dr. Srinath Rao, chairman, P.G. Department of Studies and Research in Botany, Gulbarga University, Gulbarga, Karnataka. The plant was thoroughly cleaned to remove adherent soil and other impurities, the leaves were shade dried and made into a coarse powder by rubbing in the palms.

Extraction

100 gms of shade dried leaf powder of AR was extracted in Soxhlet’s apparatus using petroleum ether for defatting and then it was extracted with 70% ethanol. The solvent was evaporated on a water bath at a low temperature (50°C) and finally, the residue was obtained.
MATERIALS

Paracetamol (Esteem Pharmaceutical Pvt. Ltd. Agra), Silymarin (SD fine chemicals, Mumbai), Ready to use diagnostic kits (Aspen Labs Pvt. Ltd., Delhi-India) 70% ethanolic extract of AR. All chemicals and reagents used were of analytical grade.

Animals used

Wistar albino rats of either sex weighing between 150-200 gms were housed in polypropylene cages and were maintained at 27° ± 2°C with 12:12 hr, light/dark cycle. They were fed with commercial diet (VRK Nutritional Laboratory, Sangli) and water at libitum during the experiment. The study was permitted by Institutional Animal Ethical Committee (Reg. No. 342).

Evaluation of AREE against Paracetamol induced hepatotoxicity:

Hepatoprotection offered by AREE, the method reported by R.R. Chattopadhyay was followed\(^2\).

In this biological screening technique, albino Wistar rats were randomly assigned into 5 groups of 6 animals each as follows:

Gp-I: Animals (-ve control) were administered 1ml/kg p.o. of saline for 7 days.

Gp-II: Animals (+ve control) were administered 1ml/kg p.o. of saline for 7 days.

Gp-III: Animals were administered with silymarin 100 mg/kg p.o. for 7 days.

Gp-IV: Animals administered with AREE 200 mg/kg p.o. for 7 days.

Gp-V: Animals administered with AREE 400 mg/kg p.o. for 7 days.

On 5\(^{th}\) day, 30 minutes after the administration of normal saline, 100 mg/kg silymarin, 200 mg/kg and 400 mg/kg of AGEE to Group- III, IV and V respectively. Paracetamol 2 gm/kg was given orally to Group- II, III, IV and V. After 48 hours of Paracetamol dosing, rats were sacrificed under mild ether anaesthesia and blood samples were withdrawn through carotid artery puncture and centrifuged immediately to get clear serum and they were subjected to various biochemical studies for evaluating the serum biochemical parameters by using Aspen diagnostic kits and liver was dissected out, the blood was blotted off, washed with saline and
stored in 10% formalin and proceeded for histopathology to evaluate the details of hepatic architecture in each group microscopically.

Finally, the results were compiled, tabled and graphically represented.

Table No. 1 Effect of AREE on hepatic enzymes in Paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment</th>
<th>Biochemical parameters Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGPT IU/L</td>
</tr>
<tr>
<td>1</td>
<td>Negative control (1ml/kg saline p.o.)</td>
<td>63.59 ±0.33</td>
</tr>
<tr>
<td>2</td>
<td>Paracetamol treated (positive control) (1ml/kg saline p.o + 2gms/kg p.o.)</td>
<td>206.5 ±0.57</td>
</tr>
<tr>
<td>3</td>
<td>Silymarin + Paracetamol (100mg/kg p.o. + 2gms/kg p.o.)</td>
<td>75.92 ±0.45***</td>
</tr>
<tr>
<td>4</td>
<td>AREE + Paracetamol (200 mg/kg p.o + 2gms/kg p.o.)</td>
<td>158 ±0.73***</td>
</tr>
<tr>
<td>5</td>
<td>AREE + Paracetamol (400 mg/kg p.o + 2gms/kg p.o.)</td>
<td>93.50 ±0.92***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of 6 rats/treatment.

Significance ***P< 0.001 compared to Paracetamol treatment.
Table. No. 2 Percentage recovery of biochemical parameters in Paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Groups</th>
<th>SGPT IU/L</th>
<th>SGOT IU/L</th>
<th>Total Bilirubin mg/dl</th>
<th>Direct Bilirubin mg/dl</th>
<th>Total Cholesterol mg/dl</th>
<th>HDL mg/dl</th>
<th>ALP IU/L</th>
<th>ALP IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PCM + Silymarin (100 mg/kg.p.o)</td>
<td>63.24</td>
<td>61.15</td>
<td>71.84</td>
<td>64.44</td>
<td>40.80</td>
<td>62.99</td>
<td>56.26</td>
<td>56.26</td>
</tr>
<tr>
<td>2.</td>
<td>PCM + AREE (200 mg/kg.p.o)</td>
<td>23.48</td>
<td>26.74</td>
<td>39.81</td>
<td>40</td>
<td>11.55</td>
<td>20.91</td>
<td>27.48</td>
<td>28.42</td>
</tr>
<tr>
<td>3.</td>
<td>PCM + AGREE (400 mg/kg.p.o)</td>
<td>54.72</td>
<td>57.49</td>
<td>61.05</td>
<td>58.88</td>
<td>32.62</td>
<td>59.43</td>
<td>51.40</td>
<td>52.64</td>
</tr>
</tbody>
</table>

Table No. 3 Aggregate protection in Paracetamol induced hepatotoxicity

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Paracetamol + Silymarin (100 mg/kg.p.o)</td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol + AREE (200 mg/kg.p.o)</td>
</tr>
<tr>
<td>3.</td>
<td>Paracetamol + AREE (400 mg/kg.p.o)</td>
</tr>
</tbody>
</table>

Normal Group     Paracetamol Treated     Silymarin Treated + PCM
(2gms/kg)
RESULTS

Histopathological Studies in Paracetamol induced hepatotoxicity:

- **Group A**: In the case of normal control (-ve control) hepatic globular structure, central veins, portal tract and kuffer cells look normal.

  Suggestive: **Normal liver**.

- **Group B**: In the case of paracetamol treated group (+ve control), hepatic globular architecture was normal, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Liver sinusoids were congested. Centri-lobular necrosis was observed.

  Suggestive: **Fatty liver**.

- **Group C**: In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were occasional fatty cells and few cells have shown hyaline and cytoplasm. There were occasional areas of lymphocytic infiltration and kuffer cell proliferation.

  Suggestive: **Regeneration liver**.
• **Group D:** In the case of 200 mg/kg 70% ethanolic extract of leaves of AR treated group the hepatic globular architecture was normal. A few areas show lymphocytic infiltration. Majority of hepatocytes are normal.

Suggestive: **Light regeneration of hepatocytes.**

• **Group E:** In the case of 400 mg/kg 70% ethanolic extract of leaves of AR treated group the hepatic architecture was maintained. Areas of kuffer cells proliferation and sinusoids appear to be normal.

Suggestive: **Regeneration of hepatocytes.**

Paracetamol has markedly increased in SGPT levels due to hepatocellular injury in unprotected group (206.5 IU/L). However, the SGPT levels were reversed to near normal levels (93.50 IU/L) with the treatment of 400 mg/kg of AREE. Whereas, the standard Silymarin 100 mg/kg has restored the SGPT levels significantly i.e. 75.92 IU/L.

Serum SGOT levels were also elevated in the Paracetamol treated group (270.55 IU/L). Treatment with standard Silymarin 100 mg/kg has brought back the SGOT levels to the near normal levels i.e. 105.1 IU/L. Meanwhile, treatment with the AREE restored the SGOT levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 198.2 and 115 IU/L respectively, which are statistically significant.

On treatment with Paracetamol, there was an increase in the total and direct bilirubin (4.12 and 0.90 mg/dl respectively). Treatment with 400 mg/kg of AREE could reverse the total and direct bilirubin serum levels to 1.64 mg/dl and 0.37 mg/dl respectively, which are statistically significant, when compared with Paracetamol treated group. The reversal by standard Silymarin 100 mg/kg was obviously significant i.e. 1.16 mg/dl (total bilirubin) and 0.32 mg/dl (direct bilirubin).

Serum total cholesterol levels were also elevated in the Paracetamol treated group (195.6 mg/dl). Treatment with standard Silymarin 100 mg/kg has brought back the total cholesterol levels to the near normal levels i.e.115.8 mg/dl. But, treatment with the AREE restored the levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 173 and 131.8 mg/dl respectively, which are statistically significant.
Serum HDL levels were reduced in the Paracetamol treated group (4.35 mg/dl). Treatment with standard Silymarin 100 mg/kg has brought back the HDL levels to the near normal levels i.e. 7.09 mg/dl. However, treatment with the AREE restored the levels in a dose dependent manner at both the doses (200 mg/kg and 400 mg/kg) to 5.26 and 6.74 mg/dl respectively, which are statistically significant.

Similarly, rise in ALP serum levels due to Paracetamol challenge was remarkable (339.5 IU/L) and the same was brought back significantly by both doses of AREE to near normal level i.e. 246.2 IU/L and 165 IU/L respectively, which are statistically significant. As expected standard Silymarin 100 mg/kg responded well and restored the ALP levels to 148.5 IU/L. Various biochemical markers were significantly lowered by both extracts & Silymarin treatment. These results are summarized in Table no. 1 and shown in Fig. No. 1.

On the basis of the results presented in Table no. 1, an attempt was made to translate results into percentage protection (Table no. 2 & 3) in comparison with positive control. Later, these parameters of test extracts & standard were averaged to estimate aggregate protection offered by them. Wherein, Silymarin has shown 60.10% protection, followed by 53.66 % & 27.14% protection exhibited by 400mg/kg & 200mg/kg of AREE respectively.

DISCUSSION

The model selected to assess organ protection for ethanol extract of AR was tested against Paracetamol induced hepatotoxicity in rats. The PCM treated group exhibited extensive fatty changes, congestion of sinusoids, necrosis etc. upon histopathological observations. Treatment with PCM showed serum levels of biochemical markers of hepatocellular damage like SGPT, SGOT, bilirubin (total and direct), ALP, cholesterol were increased as a mark of fatty change, congestion, inflammation etc.

The animals treated with AREE for 7 days showed remarkable rejuvenation of hepatocellular architecture, the AREE reversed the elevated serum markers of liver damage proportionate to the doses employed. Protection was offered by silymarin, (100 mg/kg), 200 mg/kg and 400 mg/kg of AREE respectively (compared to positive control w.r.t. biochemical parameters).

Paracetamol is usually safe at therapeutic doses but, produces centrilobular or massive hepatic necrosis along with damage to subcellular organelle like mitochondria followed by congestion and failure at the dose of 2gm/kg (p.o). This happens due to exhaustion and non-
availability of safer ways of eliminating Paracetamol such as sulphation and glucuronidation. As a result, a number of isoenzymes of CYP-450 namely CYP 2E1, CYP 1A2, CYP 2A6, CYP 3A4, CYP 2D6 are released and produce N-acetyl p-benzoquinine amine (NAPQI). This toxic electrophile donates an electron to surroundings, generating hydroxyl radical and ROS, meanwhile it gets itself converted to highly reactive semiquinone radical\textsuperscript{3-5}.

Paracetamol treatment (2gm/kg) causes centrilobular or massive hepatic necrosis along with damage to subcellular organelle resulting in the leakage of biochemical markers into the bloodstream thereby the levels of biochemical markers in the serum was raised in the present study.\textsuperscript{6}

The animals treated with AREE dose showed praise-worthy reversal of all the elevated parameters indicating the prevention of liver damage. The animals pretreated with Silymarin and AREE showed regeneration of hepatocytes. It is clear that the free radicals mediated assault made by NAPQI forms the central dogma of Paracetamol induced pathogenesis.\textsuperscript{7,8}

**CONCLUSION**

AREE has a powerful organ protection property and significantly it has good in-vitro antioxidant properties which are attributed due to presence of antioxidant phyto-constituents like flavonoids, phytosterols and other polyphenolic constituents. Therefore the above findings reveals that the use of *Amaranthus retroflexus* leaves in our food protects our liver.

These findings adds strength to our claim.

**ACKNOWLEDGEMENTS**

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