Role of *Melia azedarach* leaf extract in Paracetamol Induced Hepatic damage in rats

Mohammed Fazil Ahmed*, A. Srinivasa Rao, Hameed Thayyil, Shaik Rasheed Ahemad and Mohammed Ibrahim

1Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near Ramoji Film City, Nalgonda, (AP), INDIA-508286.  
*Bhaskar Pharmacy College, Yeknapally, Moinabad(Mandal), R.R(Dist), Hyderabad-500075.

**ABSTRACT**

The hepatoprotective activity of the methanolic leaf extract of *Melia azedarach* was investigated against paracetamol induced hepatic damage in rats. Over dosing of paracetamol to rats is reported to decrease the activity of antioxidative enzymes (GPx, GST, SOD and CAT) in liver and increase the serum enzymes (SGOT, SGPT and Alkaline phosphate), bilirubin and decrease the total proteins content. *Melia azedarach* leaf extract maintained the activity of antioxidant enzymes and as well as serum enzymes to the normal level. Thus the present study ascertains that the leaf extract of *Melia azedarach* possesses significant hepatoprotective activity.

**Key words:** Hepatoprotective, Hepatic damage, Methanolic leaf extract, *Melia azedarach*, Serum enzymes and Antioxidant enzymes

**INTRODUCTION**

Liver diseases, especially viral hepatitis occur predominately in the developing world with an enormous impact on public health and economy. Plant drugs in Indian ayurvedic system and Chinese herbal medicine have long been used for liver and biliary diseases. Some plants have also been found to possess hepatoprotective activity and the underlying mechanism of action involves their anti-oxidant property. The oxidative stress is not only the causative of the liver damage but also implicated in the pathogeneses of cancer, diabetes, cardiovascular disorders as well as in the process of aging. It is established that diabetes is associated with lower level anti-oxidants and many plants show hypoglycemic activity due to their anti-oxidant potentials.

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes. The hepatoprotective activity of the leaf extract of *Alchornea cordifolia* (Schum and Thonn), a Nigerian plant on aceterminophen induced toxicity *in vivo* has been reported. The antioxidative properties revealed total phenolic content of 0.22 mg/ml and reducing power of 0.062 mg/ml as compared to vitamin E with a reducing power of 0.042 mg/ml. The authors concluded that the hepatoprotective activity of this plant on aceterminophen induced liver damage is connected to its antioxidative properties. As the plant under consideration is reported to have hypoglycemic activity it may also be useful in liver damage thus the present study was directed to investigate the hepatoprotective activity against paracetamol intoxicated rats.

Among generally used NSAIDS the frequently used is paracetamol, which is widely used analgesic antipyretic agent which is metabolized by the liver. Overdosing of paracetamol to rats is reported to decrease their sensitivity to its hepatotoxic effect, which are associated with oxidative stress. So this study on antioxidant enzymes glutathione peroxidase (GPx) glutathione-s-transferase(GST), super oxide dismutase (SOD) and catalase(CAT) have been found to be great importance in the assessment of liver damage.

*Melia azedarach* linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. *Melia azedarach* linn (synonym: *Melia dubia* Cav, Indian lilac, Persian lilac) belonging to the family *Meliaceae* is a tree found in India. It is popular as Indian lilac. Different phytochemicals present in leaf, root and stem, are meliacarpins, limonoids, sendanins, trichilins and azedarachins. The plant is traditionally used
Ahmed, et al.: Role of Melia azedarach leaf extract in Paracetamol Induced Hepatic damage in rats.

for the treatment of leprosy, inflammations, Analgesics and cardiac disorders. Its fruits extracts possess ovicidal[15] and larvicidal activity.[16] The leafs extracts also possess antiviral[17] and antifertility activity.[18] Leaf extract, paracetamol standard drug and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline. Group II received paracetamol (2 g/kg) for 7 days. A fixed dose of the leaf extract (500 mg/kg p.o) dose was given once daily to Group III animals for 7 consecutive days and paracetamol (2 g/kg, p.o) was administered on 5th day of the extract administration. Group IV & V received leaf extract (500 mg/kg, p.o) and Silymarin (25 mg/kg.p.o) for 7 days.

After 48 hours of last dose of paracetamol blood sample was collected from retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37°C and used for the assay of biochemical marker enzymes (SGOT, SGPT, and alkaline phosphatase), bilirubin and total protein. Immediately after collecting blood, the animals were sacrificed and livers dissected out for biochemical studies. SGOT, SGPT, Serum alkaline phosphatase (ALP) and bilirubin were determined by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was measured according to the method of Lowry et al, 1951.[20] Then the rats were sacrificed by cervical dislocation and liver removed immediately and transferred to ice cold container, homogenized and assayed for antioxidant enzymes such as glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase activity study using standard methods.[21-24]

Statistical Analysis
The data are represented as mean ± S.E.M. Student’s t-test is used for statistical analysis of blood serum parameters (Table 1) and one-way ANOVA followed by Tukey kramer post test was used for statistical analysis of liver enzymes (Table 2). p < 0.05 was considered significant.

RESULTS
The acute oral toxicity study of Melia azedarach showed no mortality upto 610 mg/kg. The effect of methanol extract of Melia azedarach an serum transaminases, alkaline phosphates, bilirubin and total protein level in paracetamol intoxicated rats are summarized in Table 1. There was a significant (p < 0.001) increase in serum GOT, GPT, ALP, and bilirubin levels in paracetamol-intoxicated group compared to the normal control group.
The total protein levels were significantly ($p < 0.001$) decreased to 4.98 g/dl in paracetamol-intoxicated rats from the level of 6.85 g/dl in normal group. On the other hand the group with received both leaf extract and paracetamol (Group III) and extract alone (Group IV) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 1). The leaf extract and paracetamol and extract alone treated groups also reduced the level of bilirubin to 1.45 and 1.01 mg/dl respectively from the level of 1.98 mg/dl in the untreated group.

The effect of *Melia azedarach* on GPx, GST, SOD and catalase activity is shown in Table 2. It showed that GPx, GST, SOD and catalase activity were significantly decreased in the paracetamol treated group compared to normal control. The leaf extract and extract alone treated groups showed an increased activity of these enzymes.

### Table 1: Effect of *Melia azedarach* on some serum chemical parameters of paracetamol intoxicated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Paracetamol</th>
<th>Extract + paracetamol</th>
<th>Extract</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.82 ± 0.21</td>
<td>1.98 ± 0.37**</td>
<td>1.45 ± 0.79**</td>
<td>1.01 ± 2.4**</td>
<td>1.02 ± 0.30**</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>59.12 ± 10.28</td>
<td>186.33 ± 16.77**</td>
<td>150.00 ± 2.66*</td>
<td>102 ± 3.20***</td>
<td>85.27 ± 10.53***</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>46.59 ± 6.71</td>
<td>99.13 ± 10.51*</td>
<td>79.04 ± 5.3*</td>
<td>63.69 ± 2.80***</td>
<td>51.08 ± 5.31***</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>133.60 ± 1.50</td>
<td>373.94 ± 7.62*</td>
<td>225.00 ± 1.21**</td>
<td>181.00 ± 3.40***</td>
<td>153.52 ± 9.26***</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.85 ± 0.13</td>
<td>4.98 ± 0.72*</td>
<td>6.69 ± 2.50*</td>
<td>6.75 ± 0.80*</td>
<td>6.72 ± 0.06**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. number of rats = 6. Paracetamol control group compared with normal, control group °$p < 0.001$. Experimental groups compared with Paracetamol control group *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$.

### Table 2: Effect of *Melia azedarach* extract on antioxidant enzymes in liver of rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>Paracetamol</th>
<th>Extract + paracetamol</th>
<th>Extract</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (µU/gp)</td>
<td>19.60 ± 1.20</td>
<td>11.00 ± 0.80*</td>
<td>18.2 ± 1.90</td>
<td>18.90 ± 0.70</td>
<td>20.20 ± 1.40</td>
</tr>
<tr>
<td>GST (µm/gpp)</td>
<td>1290.00 ± 52.00</td>
<td>903.00 ± 52*</td>
<td>1187.00 ± 59.00</td>
<td>1342.00 ± 67.00</td>
<td>1302.00 ± 43.00</td>
</tr>
<tr>
<td>SOD (µU/gp)</td>
<td>56.50 ± 2.30</td>
<td>39.60 ± 2.70*</td>
<td>52.20 ± 3.10</td>
<td>65.90 ± 1.80</td>
<td>62.70 ± 1.50</td>
</tr>
<tr>
<td>CAT (µU/gp)</td>
<td>68.40 ± 3.90</td>
<td>52.10 ± 3.10*</td>
<td>66.70 ± 1.90</td>
<td>74.10 ± 2.30</td>
<td>67.20 ± 2.60</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; $n = 6$ in each group. *Significantly different with compared to saline and other treatment groups. GPx = Glutathione peroxidase (µU = µmoles GSH utilized/min). GST = Glutathione-S-transferase (µU = nmole CDNB-GSH conjugate/min). SOD = superoxide dismutase (µU = ukat) CAT = Catalase (µU = nmole of H$_2$O$_2$ decomposed/sec).
GST, SOD and catalase activity were significantly ($p < 0.001$) decrease in paracetamol-intoxicated rats when compared with those animals in normal control group. On the other hand, the group which received leaf extract and paracetamol (Group III), the values of above enzymatic parameters were near normal compared to Group I animals and were significantly different from their paracetamol treated control group (Group III Vs Group II). But there was no significant difference between Group I and Group IV animals. The results are well compared with Silymarin standard drug treated group (Group V).

**DISCUSSION**

Paracetamol is one of the most commonly used hepatotoxin. The covalent binding of N-acetyl-p-benzoquinoneimine (oxidation product of paracetamol) to sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity induced by paracetamol have been reported earlier.\(^{[25,26]}\)

Although serum enzyme levels are not a direct measure of hepatic injury they show the status of liver. The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.\(^{[27]}\) Thus lowering of enzyme content in serum is a definite indication of hepatoprotective action of a drug. High level of SGOT indicates liver damage such as due to viral hepatitis. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage.\(^{[28]}\) Serum ALP and bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis in presence of increasing biliary pressure.\(^{[29]}\)

In the present study, *Melia azedarach* has been found to reduce SGOT, SGPT, ALP and bilirubin in the treated groups compared with the untreated one (Table 1). It is well documented that the hepatocellular enzymes (GPx, GST, SOD and CAT) serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity. Administration of *Melia azedarach* leaf extract significantly enhanced the hepatic level of glutathione dependent enzymes and superoxide dismutase and catalase activity (Table 2).

**CONCLUSION**

In conclusion, the results of present study demonstrate that *Melia azedarach* leaf extract has potent hepatoprotective activity against Paracetamol induced liver damage in rats. The results also imply that the hepatoprotective effects of *Melia azedarach* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituent(s) responsible for hepataprotective effect.

**ACKNOWLEDGMENTS**

My sincere thanks to Dr. A Srinavasa Rao, Principal, Bhaskar Pharmacy College and Dr. Mohammed Ibrahim, Principal, Nizam Institute of Pharmacy, for rendering their suggestions and helping me in each and every step of completing this research work successfully.

**REFERENCES**