EVALUATION OF CALOTROPIS GIGANTEA R. BR. FLOWER EXTRACT ON ALCOHOL INDUCED HEPATOTOXICITY

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Abstract: The protective effect of Calotropis gigantea flower extract in acute alcohol induced hepatotoxicity in rats was evaluated. A significant increase in the levels of enzyme markers of tissues damage (ALT, AST and ALP), lipid peroxidation (TBARS) and decreased serum vitamin C levels were observed in alcohol intoxicated rats as compared to control animals. Pretreatment with 250 and 500 mg/kg body weight of extract significantly decreased the levels of enzyme markers, lipid peroxidation and markedly increased serum vitamin C level in a dose-dependent manner. Post-treatment with 500mg/kg body weight of extract significantly enhanced the recovery of animals from hepatic damage compared to untreated control. Lipid peroxidation and depletion of vitamin C due to oxidative stress could be the possible mechanism of alcohol induced toxicity and the protective effect of the extract may be due to its ability to inhibit lipid peroxidation and prevent the depletion of vitamins C.

Key words: Calotropis gigantea, Hepatotoxicity, Alcohol

INTRODUCTION

Alcohol dependency is a major health and socio-economic problem throughout the world [1]. Although important progress has been made in understanding the pathogenesis of alcoholic liver disease but the current therapies are not much effective. Novel therapeutic approaches that successively correct the fundamental cellular disturbances resulting from excessive alcohol consumption are attractive [2]. Alcohol administration has been found to cause accumulation of reactive oxygen species, which in turn is the source of lipid peroxidation of cellular membranes and proteins as well as DNA oxidation resulting in hepatocyte injury [2]. Based on the hypothesis that oxidative stress occurs only when the antioxidant capacity is insufficient to cope with the generation of prooxidants, many studies have focused on the alcohol-associated changes in the liver antioxidants.

In spite of the tremendous advancement in allopathic medicine, the effective hepatoprotective medicines are scanty. Plant drugs are known to play a vital role in the management of liver diseases. At the same time, readily available plant drugs/formulations to treat severe liver disease are rare [3]. Focus on plant research has increased all over the world and there is immense potential of medicinal plants used in various traditional systems [4]. Such scientific studies have led to isolation of active principle and chemical substances with therapeutic properties. Unfortunately, a greater proportion of plants known traditionally to possess medicinal properties and used as herbal medicine have not been subjected to scientific evaluation.

Calotropis gigantea R. Br. (Asclepiadaceae) is a shrub or a small tree 8-10 feet height. It is a genus of about six species, among which C. gigantea is the species which is commonly grown in waste land throughout India [5]. The hydroalcoholic extract of flower is reported to possess hepatoprotective activity [6]. The aerial part of the C. gigantea has been
reported for its antidiarrhoeal property in castor oil induced diarrhoea [7]. Recently alcoholic extract of flower has been reported to possess analgesic property [8]. It is used as main ingredient in various hepatoprotective preparations [9]. Traditionally the C. gigantea flower are used to cure jaundice, inflammation, ulcer and asthma like diseases [10]. However, its application in hepatic protection is not known. Therefore, the present work was planned to study the protective effect of Calotropis gigantea flower extract in acute alcohol-induced hepatotoxicity in rats.

**MATERIALS AND METHODS**

**Plant material:** The flowers of Calotropis gigantea were collected from Western Ghats of Karnataka and were authenticated by Dr. Siddamallayya from Regional Research Institute (Ay.) Govt. Central Pharmacy Annex, Jayanagar, Bangalore-560011, stored in herbarium with reference no. RRI/BNG/SMP/Drug Authentication/2007-08 964.

**Preparation of extracts:** The shade dried flowers of Calotropis gigantea of about 500 g were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60-80 °C) and then extracted with 90 % ethanol using Soxhlet apparatus for about 32 h. The ethanolic extract of flowers was concentrated under vacuum to get the residues. The percentage yield of ethanolic extract was found to be 3.9% (w/w). The ethanolic extract was found to contain terpenes and flavonoids. All the test suspensions were prepared in Tween-80.

**Animals:** Wistar albino rats of either sex, weighing 200–250 g maintained under standard husbandry conditions (temperature 23 ± 2 °C, relative humidity 55 ± 10 % and 12 h light: 12 h dark cycle) were used for all experiments. The animals were fed with a commercial diet (Amrut Feed: Mumbai) and water ad libitum. The experiments were performed after the experimental protocols approved by the Institutional animal ethics committee [11].

**Toxicity studies:** Acute toxicity study was performed for ethanolic extract according to the OECD guidelines. Female albino rats were kept fasting overnight providing only water, after which the extracts were administered orally at different doses and observed for 14 days [11].

**Chemicals:** Absolute ethanol (99.8%), was purchased from commercial source which is used as the hepatotoxin.

**Animal treatment:** Two protocols were employed to study pre-treatment and post-treatment effects of the extract together having forty animals divided into eight groups of 5 animals each.

**Protocol 1:** consisted of five groups. Group I normal control; group II extract control (extract plus isocaloric solution); group III experimental control (alcohol only); group IV and V, pretreated with flower extract 250 and 500mg/kg body weight (bw) respectively.

**Protocol 2:** consisted of 3 groups. Group I normal control; group II experimental control (alcohol only for 3 days) and group III (alcohol for 3 days followed by treatment for another 3 days). In both protocols 5g/kg bw of 25 % w/v alcohol solution, isocaloric solution and extract were administered by oral gavage.

**Collection of blood sample:** At the end of the experimental period, the animals were sacrificed after ether anesthesia and blood collected without the use of anti-coagulant for serum preparation. The blood samples were collected by direct cardiac puncture and allowed to stand for 10 min before being centrifuged at 2,000 rpm for 10 min and the serum was collected using rubber micropipette. The levels of alkaline phosphatase (ALP) was analyzed by the method of Wright et al. [12], alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analysed according to Reitman and Frankel [13]. Serum lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and serum vitamin C were determined as described by Gutterbridge [14] and Rose [15] respectively.

**Statistical analysis:** Results were presented as mean ± S.D. Students ‘t’ test was used for test of significance between two values. SPSS statistical software (Chicago, USA) was used for statistical analysis.

**RESULTS**

The levels of ALT, AST, ALP, lipid peroxidation as assayed by TBARS and ALP levels of pretreatment with or without flower extract are as
Figures 1 to 4 are histological sections of liver stained with haematoxulin and eosin. X 400

Fig. 1: Liver of rat in control group showing the normal histological structure of the hepatocytes.

Fig. 2: Liver of rat administrated ethanol only at a dose of 5g/Kg bw of 25% w/v showing degeneration of in the hepatocytes with mononuclear leucocytic inflammatory cells surrounding the central vein as well as in focal manner between the hepatocytes.

Fig. 3: Liver of rat administrated ethanol and protected by ethanol extract C. gigantea flower at a dose of 250mg/ kg bw showing moderate degeneration in the hepatocytes with mononuclear leucocytic inflammatory cells infiltration in between.

Fig. 4: Liver of rat administrated alcohol and ethanol extract Calotropis gigantea flower at a dose of 500mg/ kg bw showing few degenerative changes in the Centro lobular area of the hepatic lobules.
shown in table 1. Group III rats (treated control) developed hepatic damage compared to group I (normal control). This was evidenced by a marked elevation (p< 0.05) in the levels of hepatic enzyme marker studied. However, the levels of the enzyme markers were significantly lower (p< 0.01) in groups pretreated with flower extract 250 and 500 mg/kg bw. The levels of the enzymes (ALT, AST, and ALP) in 500mg/kg bw extract pre-treated group were significantly reduced as compared to the 250 mg/kg bw pretreated group (Table 1).

The level of lipid peroxidation, as assayed by TBARS, was significantly increased in alcohol intoxicated group as compared to normal control. Lipid peroxidation was found to be significantly reduced in groups pretreated with flower extract 250 and 500 mg/kg bw. The levels of the enzymes (ALT, AST, and ALP) in 500mg/kg bw extract pre-treated group were significantly reduced as compared to the 250 mg/kg bw pretreated group (Table 1).

Administration of alcohol caused a significant decrease in the levels of serum vitamin C compared to normal control. The extract pretreated groups (250 and 500mg/kg bw) before alcohol administration showed significant (p< 0.05) increase in vitamin C levels compared to treated control group (Table 1).

Table 2 shows the effect of post treatment of flower extract on acute alcohol induced hepatotoxicity. Alcohol was observed to cause marked increase (p< 0.05) in serum enzyme markers studied in the group administered alcohol for 3 days and left for another 3 days without treatment compared to normal control. Treatment with 500 mg/kg body weight extract for 3 days after administration of alcohol was found to enhance recovery from tissue damage as seen by the significant (p< 0.01) decrease in levels of serum enzymes and also showed a significant decrease in lipid peroxidation (p< 0.05) and increased serum vitamin C concentration.

**DISCUSSION**

Alcohol, because of its addiction liabilities in acute ingestion may cause liver damage leading to other problems. Alcohol treatment of rats is known to cause the translocation of fat from the peripheral adipose tissue to liver, kidney and brain for accumulation [16]. The animals treated with alcohol (group 1) had a significant hepatic damage as indicated by the elevated levels of serum enzyme markers of tissue damage studied. The rise in the ALT level is usually accompanied by an elevation in the levels of serum enzymes and also showed a significant decrease in lipid peroxidation (p< 0.05) and increased serum vitamin C concentration.

<p>| Table 1: Effect of pretreatment with ethanolic extract of <em>Calotropis gigantea</em> flowers on alcohol induced hepatotoxicity. Values are Mean ± SD, n = 5, * (p&lt;0.05), ** (p&lt;0.001). AST, ALT, ALP and TBARS (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and thiobarbituric acid reactive substances). |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (u/l)</th>
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<th>TBARS (nmol/h)</th>
<th>Vitamin C mg/100ml</th>
</tr>
</thead>
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<tr>
<td>Normal control + Isocaloric solution</td>
<td>28.0 ± 6.20</td>
<td>16.80 ± 2.77</td>
<td>94.20 ± 7.07</td>
<td>28.56 ± 1.37</td>
<td>0.356 ± 0.001</td>
</tr>
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<td>200mg extract + Isocaloric solution</td>
<td>24.60 ± 4.56</td>
<td>14.80 ± 1.64</td>
<td>101.80 ± 4.55</td>
<td>28.52 ± 1.30</td>
<td>0.372 ± 0.007</td>
</tr>
<tr>
<td>Alcohol (5g/kg body weight of 25% w/v Alcohol)</td>
<td>50.60 ± 9.06*</td>
<td>22.00 ± 3.00*</td>
<td>110.00 ± 6.81*</td>
<td>35.65 ± 0.054*</td>
<td>0.322 ± 0.003*</td>
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<td>250mg extract + Alcohol 30 min later</td>
<td>31.4 ± 4.50**</td>
<td>17.20 ± 5.02**</td>
<td>98.20 ± 7.50**</td>
<td>30.46 ± 1.53**</td>
<td>0.338 ± 0.005**</td>
</tr>
<tr>
<td>500mg extract + Alcohol 30 min. later</td>
<td>23.0 ± 4.00**</td>
<td>15.20 ± 2.49**</td>
<td>91.40 ± 6.91**</td>
<td>23.40 ± 0.61**</td>
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<p>| Table 2: Effect of post treatment with ethanolic extract of <em>Calotropis gigantea</em> flower on alcohol induced hepatotoxicity. Values are Mean ± SD, n = 5, * (p&lt;0.05), ** (p&lt;0.05) |</p>
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<td>27.46 ± 1.8 7*</td>
<td>0.362 ± 0.002*</td>
</tr>
<tr>
<td>Alcohol (5g/kg body weight of 25% w/v Alcohol) for 72h and left for another 72h</td>
<td>51.00 ± 9.17**</td>
<td>20.00 ± 4.58**</td>
<td>106.60 ± 8.89**</td>
<td>24.46 ± 0.18**</td>
<td>0.338 ± 0.008**</td>
</tr>
<tr>
<td>Alcohol (5g/kg body weight of 25% w/v Alcohol) for 72h + 500mg extract for another 72h</td>
<td>32.20 ± 5.20**</td>
<td>16.60 ± 3.91**</td>
<td>90.60 ± 11.30**</td>
<td>21.63 ± 0.38</td>
<td>0.358 ± 0.002**</td>
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<p>| Table 2 | Effect of post treatment with ethanolic extract of <em>Calotropis gigantea</em> flower on alcohol induced hepatotoxicity. Values are Mean ± SD, n = 5, * (p&lt;0.05), ** (p&lt;0.05) |</p>
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Acute alcohol ingestion is known to promote oxidative stress in animals and humans [18]. In the present study, significant increased levels of lipid peroxidation in blood serum of rats treated with alcohol were observed, indicating the activation of the lipid peroxidation system. The latter is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane lipids. The high vulnerability of neutral tissues to oxidative damage is partly due to its high lipid content [19]. Pre-treatment with extract before alcohol administration significantly decreased the levels of lipid peroxidation in the blood. The antiperoxidative effect may be due to the presence of flavonoids, triterpenoids, and volatile long chain fatty acids like bioactive constituents, which have been isolated from flowers as earlier reported [20-22]. The latex of Calotropis gigantea was found to contain cysteine proteases designated as FI, FII [23] and calotropins DI and DII [24]. The protease family has drawn special attention for drug target for cure of several diseases such as jaundice, osteoporosis, arthritis and cancer [25]. The flowers of Calotropis gigantea are rich in latex.

Post treatment with the extract also resulted in marked decrease in the level of lipid peroxidation compared to the group left without post treatment. Thus, the extract also has the ability not only to protect the animals from the effect of lipid peroxidation induced by alcohol but also to scavenge the already produced radicals and reverse the effect of the observed lipid peroxidation. Increased lipid peroxidation under ethanol treated conditions of rats left without pre- or post-treatment was due to increased oxidative stress as a result of depletion of the antioxidant scavenger system which might have resulted in changes in the cellular metabolism of the liver [18].

The observed decrease in the level of serum vitamin C (an antioxidant) in alcohol treated group could be as a result of increased utilization of this antioxidant in scavenging the free radicals generated or produced during acute alcohol induction [26]. Pre-treatment of rats with the extract at different doses administered resulted in significant increase in the levels of vitamin C. This indicates that the extract was able to raise the vitamin C levels of the pre-treated animals, and increased the ability to combat toxins/hazards produced by free radicals. Raised level of serum vitamin C after post-treatment of extract enhanced the recovery of the animals from alcohol-induced damage compared to untreated group.

In conclusion, the study has further strengthens the fact that acute alcohol intake induces hepatotoxicity. The probable mechanisms of alcohol induced tissue damage were found to be due to increased lipid peroxidation and depletion of antioxidant reserves as assayed by serum vitamin C level. Pretreatment with the extract was also found to protect the liver from acute alcohol induced damage, while post-treatment with the flowers extract of Calotropis gigantea exhibited a therapeutic effect. Both protective and curative effects of the extract have been attributed to the rich antioxidant nature of the flower extract, which exhibited its effects in a dose dependent manner.

REFERENCES