Protective effect of Coccinia grandis [L] against (Diethylnitrosamine) DEN induced Heptotoxicity in Wistar Albino Rats

Chidamabaram R.1, Lakshmi Devi R.1, Senthamil Selvi R.1, Alagendran S.2 and Anusha Bhaskar1*

1Centre for Research and Development, PRIST University, Vallam, Thanjavur, Tamilnadu
2Department of Biochemistry, Constituent College Perambalur, Bharathidasan University

ABSTRACT

Diethylnitrosamine (DEN) is widely reported to induce cancer in animals and humans. The aim of the present study was to investigate the hepatoprotective and antioxidant activities of the fruit extract of Coccinia grandis Linn., against diethylnitrosamine induced liver injury in rats. An elevated level of the liver enzymes AST, ALT, ALP and ADH was observed. Liver oxidative stress was confirmed by the elevation of lipid peroxidation measured as malondialdehyde (MDA), and a decrease in enzymic and non-enzymic antioxidant activities. Oral administration of the methanolic fruit extract of Coccinia grandis for 30 days to DEN treated rats significantly improved the antioxidant levels, reduced the oxidative stress and a reversal of liver parameters. The results obtained with when treated both 100 mg and 200 mg/kg bw with C. grandis fruit extract were comparable to the standard hepatoprotective drugs silymarin.

Keywords: antioxidants; Coccinia grandis; diethylnitrosamine; hepatoprotective; lipid peroxidation; silymarin.

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body [1]. It is the seat of metabolism, secretion and storage. Its capacity to detoxify is enormous. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences [2]. A diversity of dietary [3] endogenous and environmental [4] stimuli mediate hepatocarcinogenesis.

Diethylnitrosamine (DEN) has been widely used as a precursor in initiating carcinogenesis in experimental animal models [5,6]. DEN activation takes place in the liver microsomes has been demonstrated to stimulate Kupfer cells leading to high levels of ROS which is capable of initiating peroxidative damage to the cell. This is capable of damaging liver cells and inducing hepatocarcinogenesis [7]. DEN is widely reported to be found in the environment, in tobacco smoke and is also synthesized endogenously [8,9].

Coccinia grandis (L.) J. Voigt or Ivy gourd of Cucurbitaceae, is a dioecious, perennial and herbaceous climber with glabrous stems, tuberous roots and axillary tendrils. The whole plant is traditionally used for various medicinal purposes and leaves are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation in eruptions of skin, fever, asthma and cough. Scientific investigations have shown that the crude extract possess hepatoprotective, antioxidant, anti-inflammatory, anti-nociceptive, anti-diabetic,
hypolipidemic, antibacterial and antiussive activities. In this study we report the protective role of the methanolic extract of *Coccinia grandis* (L) fruit extract against hepatotoxicity caused by DEN.

**MATERIALS AND METHODS**

**Plant material**

Fruits of the *Coccinia grandis* were procured from the local market of Ootacamund, Tamil Nadu, India and then transferred to PRIST University, Thanjavur. It was taxonomically identified and authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph College (Autonomous), Tiruchirapalli, Tamilnadu, India. The voucher specimen was deposited at the Rapinat herbarium and the voucher number is RLD 001.

The fruits were cut and shade dried and finely powdered and stored in air-tight container until further use. The powdered fruit (500g) was repeatedly extracted in a 5L round bottomed flask with 2L of methanol (60°C). The extract was concentrated by distilling off the solvent and then evaporating to dryness on a water-bath. The extracts were cooled at room temperature, and evaporated to dryness under reduced pressure in rotary evaporator [10]. The extracts were used for preliminary phytochemical and pharmacological studies.

**Animals**

Wistar albino rats (150 – 250 g body weight) were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water *ad libitum*. The work was carried out in CPCSEA approved (743/03/abc/CPCSEA dt. 3.3.03) Animal House of PRIST University, Thanjavur

**Experimental Design**

The animals were divided into 5 groups, each consisting of 6 animals.

**Group I**: the control group, serve as negative control received a control diet and a single intra peritoneal (i.p.) injection of normal saline (2.5ml/kg).

**Group II**: the DEN induced group, serve as a positive control was given a single i.p injection of DEN, 200 mg/kg of bodyweight and a control diet. Hepatocarcinogenesis was provoked by a single i.p. injection of DEN (Sigma-NO 258, USA) at a dose of 200 mg/kg body weight (mixed with olive oil). Following a two week recovery period, the promoter phenobarbital (Sigma-Aldrich, St. Louis, MO) was incorporated into the drinking water at a concentration of 0.05% for 24 successive weeks. The dose of DEN and phenobarbital and the time required to study initiation stage of hepatic carcinogenesis in rats were adopted from Bishayee et al.[11]

**Group III**: the DEN induced group, after the development of cancer, was treated with the methanolic fruit extract of *Cocinnia grandis*, 100 mg/bw.

**Group IV**: the DEN induced group, after the development of cancer, was treated with the methanolic extract of *Cocinnia grandis*, 200 mg/bw.

**Group V**: the DEN induced group, after the development of cancer, was treated with the standard drug Silymarin [Sigma-SO292, USA] 100 mg/kg orally.

The duration of the treatment was 30 days. The animals were sacrificed and the abdomen was cut open to remove the liver for biochemical and histopathological studies.

**Biochemical analysis**

Aspartate transaminase, Alanine transaminase, Lactate dehydrogenase, alkaline phosphatase were estimated by using commercially available kits according to the manufacturer’s instruction.(AGGAPPE Diagnostic, Kerala and Ensure Biotech Pvt, Hyderabad, India).

**Measurement of DEN mediated oxidative stress**

The activities of enzymatic antioxidants such as SOD [12], Catalase [13] and GPx [14] were assayed in the hepatic tissue homogenate of control and experimental group of mice. Further, the levels of lipid peroxides [15] were
determined in the liver tissue homogenate of control and experimental groups of mice. The levels of non-enzymatic antioxidants such as GSH [16] and vitamin C [17] were measured in the liver tissue homogenate of control and experimental groups of mice.

Histopathological Studies
A portion of the liver was cut into two to three pieces of approximately 6mm³ size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5µm thickness of liver tissue were cut and stained with haematoxylin–eosin. The thin sections of liver were made into permanent slides and examined [18] under high resolution microscope with photographic facility and photomicrographs were taken.

Statistical Analysis
Data were evaluated with SPSS/10 software hypothesis testing methods included one way analysis of variance [ANOVA] followed by least significant difference [LSD] test P values of less than 0.05 were considered to show statistical significance. All these results were expressed as mean ± SD for six animals in each group.

RESULTS
Table 1 represents the effect of Coccinia grandis on the level of the activities of the liver enzymes viz., AST, ALT, ALP and LDH in the control and experimental groups of rats. Very high levels of these enzymes have been observed in the DEN treated groups. Oral administration of C. grandis both 100 and 200 mg/kg bw brought about a significant reduction in all the enzymes analyzed (p<0.05) comparable with that of the standard drug silymarin.

Table 2. illustrates the effect of Coccinia grandis on the level of lipid peroxide and non enzymatic antioxidant in the hepatic tissues of control and experimental groups of rats. The levels of malondialdehyde almost tripled in the DEN induced group of rats when compared with controls. When the DEN induced groups were treated with 100 and 200 mg/kg bw C. grandis fruit extract the malondialdehyde levels were reduced significantly (p<0.05). The levels of vitamin C and reduced glutathione also showed a significant decrease in levels in the DEN treated groups and on subsequent treatment with the fruit extract improved dramatically.

Table 3 represents the effect C. grandis fruit extract on the activities of enzymatic antioxidants such as SOD, catalase and GPx, in hepatic tissues of control and experimental groups of rats. The activities were significantly (p<0.05) diminished in the hepatic tissues of DEN induced group of rats. Oral treatment of Coccinta grandis similar to that of silymarin, significantly (p<0.05) attenuated the altered activities of these enzymatic antioxidants to near normalcy in hepatic tissues of DEN induced rats.
Table 3: Effect of methanolic extract of *Coccinia grandis* on enzymic antioxidant activities

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase</th>
<th>SOD</th>
<th>GPx</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>16.51 ± 0.35</td>
<td>488.04 ± 23</td>
<td>457.83 ± 5.0</td>
</tr>
<tr>
<td>DEN treated</td>
<td>12.58 ± 0.23</td>
<td>289.86 ± 26</td>
<td>277.66 ± 3.8</td>
</tr>
<tr>
<td>DEN + <em>Coccinia grandis</em> (100 mg/bw)</td>
<td>15.44 ± 0.17</td>
<td>452.33 ± 17</td>
<td>403.66 ± 5.7</td>
</tr>
<tr>
<td>DEN + <em>Coccinia grandis</em> (200 mg/bw)</td>
<td>15.85 ± 0.25</td>
<td>460.23 ± 21</td>
<td>425.25 ± 3.5</td>
</tr>
<tr>
<td>DEN + Silymarin (100mg/kg bw)</td>
<td>15.80 ± 0.30</td>
<td>458.66 ± 29</td>
<td>428.33 ± 8.2</td>
</tr>
</tbody>
</table>

Catalase (U/mg of protein), Superoxide dismutase (U/mg of protein), Glutathione peroxidase (U/mg of protein). Values of results are expressed as mean±SD for six rats.

Values are expressed as mean ± S.D (n=6).

* a P<0.05 DEN treated with *C. grandis* (100 mg/bw) compared with DEN induced

* b P<0.05 DEN treated with *C. grandis* (200 mg/bw) compared with DEN induced

Fig. 1 A–D represents the photomicrographs of hematoxylin–eosin staining of hepatic tissues of control and experimental groups of rats. A. shows the section of hepatic tissue of control rat. B. Hepatic tissues of DEN induced group. C. Hepatic tissues of DEN induced group of rats treated with *C. grandis*. D. Hepatic tissues of DEN induced group of rats treated with silymarin.

Fig. 1 A–D represents the photomicrographs of hematoxylin–eosin staining of hepatic tissues of control and experimental groups of rats. Fig. 1 A shows the section of hepatic tissue of control rat exhibiting a concentric arrangement of the hepatocytes with sinusoidal cards around the central vein and portal tracts. The portal tracts show portal triad with portal vein, hepatic artery and bile duct. Fig. 1 B portrays the section of hepatic tissues of DEN induced group of mice exhibiting distortion in the arrangement of hepatocytes around the central vein, periportal fatty infiltration with focal necrosis of hepatocytes, congestion of sinusoids around central vein regions, granular degeneration, microvesicular vacuolization, focal necrosis, hyperemia in the sinusoids and portal tract inflammation. Fig. 1C demonstrates the section of hepatic tissues of DEN induced group of rats treated with *C. grandis* (200 mg/kg bw) presenting the normal hepatocytes arrangement around the central vein with abridged necrosis, declined fat accumulation and mild sinusoidal dilatation. Similarly, the hepatic tissues of DEN induced group of rats treated with silymarin shows similar pattern of hepatocytes arrangement (Fig. 1D) and are comparable with control group of rats.
DISCUSSION

The administration of DEN has shown to increase the levels of liver tissue LPO during hepato carcinogenesis and this vigorous action may be lead by an uncompromised production of free radicals [19].

The increase in the levels of the enzymes ALT, AST, ALP and LDH are indicative of hepatic injury [20]. DEN hepatic injury is related to the disturbance in hepatocytes membrane instability and metabolism resulting in alterations of the serum levels of these enzymes. The increase of ALT and AST serum levels are specific to hepatocellular disturbance[21]. An increase in the ALP denotes disturbances in biliary flow. Treatment with C. grandis to the DEN induced group brings about a significant decrease in these enzyme levels.

It is well documented that DEN induces hepatic damage through the generation of reactive oxygen species (ROS) and a concomitant decrease in the enzymic and non-enzymic antioxidants. In the present study a 3-fold increase in the level of LPO and highly pronounced decreased levels of SOD, CAT and GPx were observed during DEN administration. Further, the observed decrease in the levels of non-enzymatic antioxidant (vitamin C and GSH) indicates the complete disruption of the antioxidant defense mechanism of the liver. The administration of the fruit extract of C. grandis at 200 mg/ kg body weight showed improved levels of antioxidants in DEN induced hepatotoxicity.

The biochemical findings are supported by histopathological observations of the liver. The histopathological patterns of liver injury observed in rats treated with DEN was found to be more pronounced. Centrilobular necrosis, ballooning of hepatocytes, infiltration of lymphocytes and steatosis of liver cells were characteristic alterations occurred due to DEN Moreover treatment with C. grandis decreased the hepatocytes degeneration and reduced the lymphocytic infiltration in liver and showed regenerative effects. This could be used as functional development of hepatocytes, which may possibly due to stimulate the regeneration of parenchymal cells or less damage in the presence of C. grandis.

Therefore, the present investigation throws light on the hepatocytes protective nature of C. grandis methanolic fruit extract in DEN induced oxidative stress. The oral administration of C. grandis to DEN induced rats exhibited significant ameliorative potential probably by attenuating the DEN mediated oxidative stress and preserving the structural and functional integrity of hepatocytes. Further detailed investigations are in progress to elucidate the exact mechanism by which C. grandis methanolic extract elicit its various effects.

REFERENCES


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