Dose-response effects of *Elephantopus scaber* methanolic extract on *N*-nitrosodiethylamine-induced hepatotoxicity in rats

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**[ABSTRACT]**  **AIM:** A decoction of *Elephantopus scaber* (Asteraceae) root is used to treat liver disorders in Indian and Chinese traditional medicine. The study was designed to examine the dose response effects of *E. scaber* methanolic extract on rats exposed to *N*-nitrosodiethylamine (NDEA) induced hepatotoxicity (0.02% NDEA in water five days per week, per oral) in preventive and curative models. **METHODS:** In preventive groups, NDEA was administered for six weeks. Daily doses of *E. scaber* methanolic extract (200 and 100 mg kg⁻¹) started one week before the onset of NDEA intoxication and continued for six weeks. In curative animals, NDEA was administered for six weeks followed by treatment with the methanolic *n*-hexane extract of *E. scaber* (200 and 100 mg kg⁻¹) for ten days. **RESULTS:** *E. scaber* extract treatment significantly (*P* ≤ 0.05) reduced the levels of AST, ALT, and MDA in both experimental groups. The extract also enhanced the antioxidant enzyme and protein levels in rats intoxicated with NDEA. Treatment with the extract dose dependently protected the liver from NDEA-induced hepatotoxicity with normal hepatocytes and uniform sinusoids, but in some areas showed degenerating hepatic cells in both treatment groups. **CONCLUSION:** *E. scaber* methanolic extract dose dependently prevented and reversed the hepatotoxicity induced by NDEA in both experimental models.

**[KEY WORDS]** *Elephantopus scaber*; Asteraceae; *N*-nitrosodiethylamine; Hepatotoxicity; Root extract

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1 Introduction

*N*-Nitrosodiethylamine is a dialkyl nitrosoamine considered to be a potent hepatocarcinogen-producing hepatocellular carcinoma (HCC) after repeated administration in experimental animals [1-4]. The presence of nitroso compounds, like *N*-nitrosodiethylamine, *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine and *N*-nitrosopiperidine, has been widely reported in various foodstuffs, such as milk products, meat products, soft drinks, and alcoholic beverages [5-7]. Moreover, their reported presence in tobacco smoke accounts for one of the biggest causes for individual exposure to these nitrosamines [8]. *N*-Nitrosodiethylamine metabolism results in the formation of reactive oxygen species (ROS) leading to oxidative stress and cellular injury, which may be one of the key factors in the etiology of cancer. Development of HCC is known to be triggered by factors that lead to chronic hepatic injury and deregulation of the normal process of wound healing, which promotes the persistent stimulation of profibrotic and proangiogenic processes that lead to significant structural changes in the liver, and functional changes in hepatic physiology [9-11]. Several plant drugs have been evaluated for their potential as liver protectants against *N*-nitrosodiethylamine-induced hepatocarcinogenesis in experimental models [2-4, 12-14].

*Elephantopus scaber* L. (Asteraceae) is a small herb, commonly known as elephant’s foot. In Ayurvedic medicine, a mixture of *E. scaber* with other herbs is used to treat *Vatika granthi* (minor neoplasms). Also, the leaves of *E. scaber*, *Ficus glomerata*, and *Tectona grandis* are used together with a honey-mixed fine paste of selected herbs to treat *Pittaja arbuda* (major neoplasms) [15]. It is reported that the root juice of the plant (two teaspoons three times per day) has
been consumed to overcome liver troubles\cite{16-17}. The methanolic extract from the leaves and roots is reported as an effective hypoglycemic agent against streptozotocin-induced diabetic rats \cite{18}. Furthermore, the methanolic extract exerted high inhibitory effect on the nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages \cite{19}. Teng-Khia-U (E. scaber, E. mollis, and Pseudelephantopus spicatus) is a Taiwan traditional medicine formulated for treating nephritis, edema, dampness, chest pain, fever/cough or pneumonia, and scabies/arthralgia \cite{20}. Studies have showed that Teng-Khia-U possesses anti-inflammatory and hepatoprotective activity. The aqueous extract from the whole plant at a dose of 300 mg kg\(^{-1}\) significantly inhibited the development of pad swelling in an acute experimental arthritis model, while a higher dose of the extract at 500 mg kg\(^{-1}\) was required to inhibit the development of chronic joint swelling in rats \cite{21}. The hepatoprotective effect of the E. scaber extract was studied in rats on D-galactosamine, acetaminophen, and carbon tetrachloride induced hepatic damage \cite{22-23}. Acute toxicity studies revealed the non-toxic nature of the crude extract of E. scaber \cite{18}. Further, the median lethal dose (LD\(_{50}\)) of E. scaber extract was 212.34 mg kg\(^{-1}\), with a 95% confidence limit of 1 (742.86 mg kg\(^{-1}\))–2 (503.62 mg kg\(^{-1}\)), which means that E. scaber showed relatively low acute toxicity and high safety \cite{24}. Guaianalide glucosides that include four sesquiterpene lactones are reported in E. scaber \cite{25}. Two novel germacranolide sesquiterpene lactones, namely 17, 19-dihydrodioxylexlephantopin and iso-17, 19-dihydrodioxylexlephantopin with marked anti-tumor activity have also been isolated from E. scaber \cite{20}. A published report establishes the presence of lupeol (18%), stigmasterol (8.25%) and 11, 13-dihydrodioxylexlephantopin (0.03%) in the methanolic extract of E. scaber \cite{26}. The present investigation was aimed at studying the dose response effect of E. scaber methanolic extract in preventive and curative treatments on NDEA-induced hepatotoxicity and oxidative stress in albino rats.

2 Materials and Methods
2.1 Animals
Female Wistar rats weighing between 130–160 g were used in this study. The rats had free access to food and water. Animal studies were conducted according to Institute Animal Ethics Committee (IAEC) regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. B 2442009/1) and conducted humanely. The rats received standard pellet diet and water ad libitum. The animals were maintained at a controlled temperature of 26–28 °C with a 12 h light: 12 h dark cycle.

2.2 Chemicals
N-Nitrosodiethylamine (NDEA) and silymarin were purchased from Sigma Chemical Co., St. Louis, MO., USA, Assay kits for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were purchased from Agappe, India. Hematoxylin and eosin were procured from Nice Chemicals, Kochi, India. Methanol and Tween-80 was obtained from Merck, Mumbai, India. All the other chemicals used were also of highest purity grade.

2.3 Plant extraction
Plant materials were collected from their natural habitat in Kerala, India and authenticated by the second author. A voucher specimen (SBSBRL.03) is maintained in the Biochemistry Research laboratory, School of Biosciences, M G University. Roots of the plant were separated, cleaned, chopped, shade-dried and powdered. Ten g of the dried powder was Soxhlet-extracted with methanol (400 mL). The Soxhlet extraction was continued until a drop of the solvent from the siphon tube when evaporated does not leave a residue. Then the extract was collected and the solvent evaporated under vacuum in a rotary evaporator. This step was repeated with a new set of dried powder and solvent until the required quantity was achieved. The yield of the extract was 16.6%. Extract was suspended in 5% Tween 80 to respective dosages and stored at −20 °C.

2.4 N-Nitrosodiethylamine-induced hepatotoxicity
2.4.1 Preventive effect of the extract
Thirty rats were divided into five groups, group I was normal control; group II was the NDEA control; group III was the NDEA and standard drug, silymarin (70 mg kg\(^{-1}\)) treated rats; group IV was NDEA and E. scaber methanolic extract (200 mg kg\(^{-1}\)) treated rats; and group V was the NDEA and E. scaber methanolic extract (100 mg kg\(^{-1}\)) treated rats. Hepatotoxicity induced by N-nitrosodiethylamine (NDEA) administration was studied for a period of six weeks. All of the groups, except Group I, received 0.02% NDEA in water five days per week (per oral) for six weeks \cite{27}. Oral treatments with E. scaber methanolic extract at 200 mg kg\(^{-1}\) and 100 mg kg\(^{-1}\) doses were started for group IV and V animals, respectively. Simultaneously, the standard drug silymarin (70 mg kg\(^{-1}\)) treatment was conducted in group III animals. Treatment with E. scaber extract and silymarin was started one week before the onset of NDEA administration and continued up to six weeks. The rats were sacrificed 48 h after the last dose of NDEA administration (Fig. 1a).

2.4.2 Curative effect of the extract
Thirty rats were divided into five groups, group I was normal control, group II was NDEA control, group III was NDEA and standard drug silymarin (70 mg kg\(^{-1}\)) treated rats, group IV was NDEA and E. scaber methanolic extract (200 mg kg\(^{-1}\)) treated rats, and group V was NDEA and E. scaber methanolic extract (100 mg kg\(^{-1}\)) treated rats. Groups II–V animals received 0.02% NDEA in water five days per week (per oral) for six weeks. After six weeks of exposure to NDEA, group IV and V rats received E. scaber methanolic extract orally (200 and 100 mg kg\(^{-1}\), respectively, suspended in 5% Tween 80) daily for 10 days. Also, group III rats were

Fig. 1 Schematic representation of experiments. a. Preventive group. Rats were administered with NDEA for six weeks and ongoing treatment with E. scaber extract at 200 and 100 mg·kg⁻¹ and silymarin. b. Curative group. Animals administered with NDEA for six weeks and then treated with E. scaber extract at 200 and 100 mg·kg⁻¹ and silymarin for 10 days. ↑ denote NDEA administration. Dotted regions denote treatment with E. scaber extracts and Silymarin.

2.5 Biochemical assays

Sera from different groups were separated by centrifugation at 2 000 r·min⁻¹ at 4 ºC for 15 min. Dissected livers were excised, and washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1 mol·L⁻¹ Tris HCl buffer (pH 7.4). The homogenate was centrifuged at 3 000 r·min⁻¹ for 20 min at 4 ºC and the supernatant was used for the estimation of reduced glutathione (GSH), glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase, lipid peroxidation (Thiobarbituric Acid Reactive Substances–TBARS), and total protein.

2.5.1 Liver function tests

AST (EC 2.6.1.1) [28] and ALT (EC 2.6.1.2) [29] levels were estimated at 340 nm by semi-auto analyzer.

2.5.2 Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was estimated by its reaction with dithio-bis-2-nitrobenzoic acid (DTNB) which gives a yellow colored complex with an absorption maximum at 412 nm [30].

2.5.3 Estimation of glutathione-S-transferase (GST)

GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB [31].

2.5.4 Estimation of glutathione peroxidase (GPx)

GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H₂O₂ and NaN₃ [32].

2.5.5 Estimation of catalase

Tissue catalase (EC 1.11.1.6) activity was determined from the rate of decomposition of H₂O₂ [33].

2.5.6 Estimation of malondialdehyde (MDA) formation

Lipid peroxidation in liver was measured by the formation of malondialdehyde (MDA) and measured by the thiobarbituric acid reactive substance (TBARS) method [34].

2.5.7 Estimation of protein

Protein content in the tissue was determined [35] using bovine serum albumin (BSA) as the standard.

2.6 Histopathological studies

Dissected livers were cut into small pieces and fixed in 10% neutral buffered formalin for histopathological analysis. The liver (5–6 mm thick pieces) fixed in buffered formalin for 12 h was processed for paraffin embedding. Five µm thick sections of the paraffin embedded liver were stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using a MotiCam 1000 camera at an original magnification of 100 ×.

2.7 Statistical analysis

Results were expressed as x ± s, and all statistical comparisons were made by means of one way ANOVA test followed by Tukey post hoc analysis. P-values less than or equal to 0.05 were considered significant.

3 Results

3.1 Body weight

The body weight of the NDEA-treated animals declined significantly (P ≤ 0.05) by the end of the 6th week of exposure when compared with the normal rats. Ongoing treatment with E. scaber extract prevented the decline of animal body weight remarkably in rats exposed to NDEA for six weeks (Fig. 2a). In post-treatment animals, E. scaber extract improved the body weight during a period of ten days after intoxication with NDEA for six weeks (Fig. 2b). The standard drug silymarin (70 mg·kg⁻¹) also showed a remarkable gain in body weight from NDEA-induced liver damage in both treatments.

3.2 Liver function

AST and ALT activities in the serum of NDEA administered rats were elevates in contrast to the normal rats. In the preventive treatment, E. scaber remarkably prevented the rise of AST and ALT levels, and in the curative treatment animals,
Fig. 2 Graph showing the body weight pattern of rats administered with NDEA and E. scaber extract in preventive (a) and curative (b) treatments. The mean of each of the groups is represented, with error bar indicating the standard deviation. Group I- Normal control, Group II- NDEA control, Group III- Silymarin and NDEA treated, and Group IV- E. scaber 100 mg·kg⁻¹ and NDEA treated animals. Significant difference (0.05) observed between normal control and NDEA control and between NDEA control and treated groups during final body weight of animals.

it reversed the toxic effect of NDEA as evidenced by the normal levels of AST and ALT displayed. The results were comparable to standard drug silymarin.

3.2.1 Estimation of aspartate aminotransferase (AST)

Administration of E. scaber methanolic extract (200 mg·kg⁻¹) exerted its protection by 81% and 94% in the preventive and curative groups, respectively. E. scaber methanolic extract (100 mg·kg⁻¹) treatment exerted its protection by 61% and 86% in preventive (Fig. 3a) and curative (Fig. 4a) groups, respectively. In the preventive and curative treatments, silymarin (70 mg·kg⁻¹) exerted its protection by 62% and 70%, respectively when compared to the normal control.

3.2.2 Estimation of alanine aminotransferase (ALT)

E. scaber methanolic extract (200 mg·kg⁻¹) treatment exerted its protection by 85% and 90% in preventive and curative groups, respectively compared to the normal control. Administration of E. scaber methanolic extract (100 mg·kg⁻¹) exerted its protection by 62% and 78% in the preventive (Fig. 3b) and curative (Fig. 4b) groups, respectively. The standard drug silymarin (70 mg·kg⁻¹) exerted its protection by 65% and 82% in the preventive and curative groups, respectively when compared to the normal control.

3.3 Estimation of reduced glutathione

Reduced glutathione (GSH) levels were lowered significantly (P ≤ 0.05) in rats exposed to NDEA compared to normal control. Percent protection induced by E. scaber methanolic extract at a dose of 200, 100 mg·kg⁻¹ and silymarin (70 mg·kg⁻¹) treatment caused an increase in the levels of GSH by 58, 36, and 50%, respectively in the preventive groups (Table 1). In the curative groups, the percent protection yielded was 71, 51, and 57%, respectively for doses of 200, 100 mg·kg⁻¹ and silymarin (70 mg·kg⁻¹) when compared to the normal control (Table 2).

3.4 Estimation of GST

Rats administered NDEA alone were found to have significantly (P ≤ 0.05) lowered levels of GST. In the preventive groups, treatment with 200 and 100 mg·kg⁻¹ methanolic extract exhibited a significant increase i.e., 54% and 39%, respectively in the GST levels compared to untreated rats (Table 1). Silymarin treatment also prevented the lowering of GST level by 46%. In the curative groups, treatment with 200 and 100 mg·kg⁻¹ exerted a significant increase of 82 and 63%, respectively in GST levels (Table 2). Silymarin exhibited a 70% increase in GST levels when compared to normal control.

Fig. 3  Effect of E. scaber methanolic extract on (a) aspartate aminotransferase (AST) levels and (b) alanine aminotransferase (ALT) in the serum of preventive treatment groups. *P ≤ 0.05 vs normal control, † P ≤ 0.05 vs NDEA control.
3.5 Estimation of GPx

In NDEA-exposed animals, a significant \( P \leq 0.05 \) lowering of GPx level compared to normal control was observed. *E. scaber* treatment at 200 mg \( \cdot \) kg\(^{-1} \) increased the GPx level by 77% and 84% in the preventive and curative groups, respectively. Administration of *E. scaber* methanolic extract (100 mg \( \cdot \) kg\(^{-1} \)) yielded its protection level by 67% and 77% in the preventive and curative groups, respectively when compared to the normal control (Tables 1 & 2).

3.6 Estimation of catalase

Catalase levels were lowered significantly \( P \leq 0.05 \) in rats exposed to NDEA compared to the normal control. In the pretreatment groups, *E. scaber* methanolic extract at 200 and 100 mg \( \cdot \) kg\(^{-1} \) exhibited significant \( P \leq 0.05 \) protection by 69% and 39%, respectively in catalase levels (Table 1). Silymarin-treated rats also prevented the lowering of catalase by 72%. In post-treatment groups, *E. scaber* methanolic extract at 200 and 100 mg \( \cdot \) kg\(^{-1} \) exhibited significant \( P \leq 0.05 \) reversal by 79 and 55%, respectively in catalase levels (Table 2). Silymarin treated rats also reversed the decline of catalase by 85% (Table 2).

### Table 1  Effect of *E. scaber* methanolic extracts and Silymarin on different parameters in NDEA exposed rats (preventive treatment groups) \( (\bar{x} \pm s, n = 3) \)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>GSH (mg/100 mg tissue)</th>
<th>GPx (min/mg protein)</th>
<th>GST (enzyme unit/mL)</th>
<th>Catalase (millimoles/min/mg protein)</th>
<th>TBARS (mmol/100 mg tissue)</th>
<th>Protein (mg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.26 ± 0.07</td>
<td>2.53 ± 0.69</td>
<td>0.73 ± 0.11</td>
<td>1.03 ± 0.19</td>
<td>1.33 ± 0.24</td>
<td>46.54 ± 9.18</td>
</tr>
<tr>
<td>NDEA control</td>
<td>0.59 ± 0.08*</td>
<td>1.25 ± 0.39</td>
<td>0.35 ± 0.05*</td>
<td>0.45 ± 0.14*</td>
<td>4.26 ± 0.95*</td>
<td>26.83 ± 6.38*</td>
</tr>
<tr>
<td>Silymarin (70 mg kg(^{-1} )) + NDEA</td>
<td>0.87 ± 0.05*</td>
<td>2.11 ± 0.70*</td>
<td>0.52 ± 0.06*</td>
<td>0.74 ± 0.10*</td>
<td>2.19 ± 0.09*</td>
<td>41.03 ± 8.06*</td>
</tr>
<tr>
<td><em>E. scaber</em> (200 mg kg(^{-1} )) + NDEA</td>
<td>0.97 ± 0.10†</td>
<td>2.23 ± 0.58†</td>
<td>0.56 ± 0.06†</td>
<td>0.85 ± 0.10†</td>
<td>2.03 ± 0.10†</td>
<td>42.23 ± 9.35†</td>
</tr>
<tr>
<td><em>E. scaber</em> (100 mg kg(^{-1} )) + NDEA</td>
<td>0.83 ± 0.09†</td>
<td>2.01 ± 0.63†</td>
<td>0.50 ± 0.07†</td>
<td>0.68 ± 0.08</td>
<td>2.25 ± 0.13†</td>
<td>40.03 ± 9.11†</td>
</tr>
</tbody>
</table>

\* \( P \leq 0.05 \) vs normal control, \( \dagger P \leq 0.05 \) vs NDEA control

### Table 2  Effect of *E. scaber* methanolic extracts and Silymarin on different parameters in NDEA exposed rats (curative treatment groups) \( (\bar{x} \pm s, n = 3) \)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>GSH (mg/100 mg tissue)</th>
<th>GPx (min/mg protein)</th>
<th>GST (enzyme unit/mL)</th>
<th>Catalase (millimoles/min/mg protein)</th>
<th>TBARS (mmol/100 mg tissue)(mg/mg tissue)</th>
<th>Protein (mg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.27 ± 0.15</td>
<td>1.68 ± 0.41</td>
<td>1.07 ± 0.22</td>
<td>1.05 ± 0.21</td>
<td>1.20 ± 0.26</td>
<td>56.63 ± 4.99</td>
</tr>
<tr>
<td>NDEA control</td>
<td>0.71 ± 0.14*</td>
<td>0.94 ± 0.17*</td>
<td>0.67 ± 0.06*</td>
<td>0.62 ± 0.08*</td>
<td>3.15 ± 0.74*</td>
<td>41.5 ± 1.86*</td>
</tr>
<tr>
<td>Silymarin (70 mg kg(^{-1} )) + NDEA</td>
<td>1.03 ± 0.05*</td>
<td>1.51 ± 0.42*</td>
<td>0.95 ± 0.14*</td>
<td>0.88 ± 0.12*</td>
<td>1.56 ± 0.27*</td>
<td>54.43 ± 4.99*</td>
</tr>
<tr>
<td><em>E. scaber</em> (200 mg kg(^{-1} )) + NDEA</td>
<td>1.11 ± 0.12†</td>
<td>1.57 ± 0.37†</td>
<td>1.00 ± 0.18†</td>
<td>0.96 ± 0.18†</td>
<td>1.42 ± 0.22†</td>
<td>55.50 ± 5.13†</td>
</tr>
<tr>
<td><em>E. scaber</em> (100 mg kg(^{-1} )) + NDEA</td>
<td>1.00 ± 0.02†</td>
<td>1.51 ± 0.38†</td>
<td>0.93 ± 0.13†</td>
<td>0.86 ± 0.13†</td>
<td>1.58 ± 0.26†</td>
<td>53.16 ± 4.47†</td>
</tr>
</tbody>
</table>

\* \( P \leq 0.05 \) vs normal control, \( \dagger P \leq 0.05 \) vs NDEA control
3.7 Estimation of malondialdehyde

A significant \((P \leq 0.05)\) increase in tissue MDA level was observed in the NDEA-treated rats. In the preventive treatment, NDEA-induced elevation of tissue MDA concentrations, was lowered by 76% and 69% in rats treated with \(E. scaber\) extract at a dose of 200 and 100 mg·kg\(^{-1}\), respectively (Table 1). This effect was comparable to that of silymarin (71%). In the curative treatment, \(E. scaber\) methanolic extract at 200 and 100 mg·kg\(^{-1}\) exerted its protection by 88% and 80%, respectively (Table 2). Silymarin (70 mg·kg\(^{-1}\)) treated rats exerted its protection by 81%.

3.8 Estimation of protein

Protein levels were lowered significantly \((P \leq 0.05)\) in rats exposed to NDEA. \(E. scaber\) methanolic extract (200 mg·kg\(^{-1}\)) treatment caused an increase in the levels of protein by 78% and 93% in the preventive and curative groups. \(E. scaber\) methanolic extract (100 mg·kg\(^{-1}\) treatment caused an increase in the level of protein by 67% and 77% in the preventive and curative groups, respectively. Silymarin (70 mg·kg\(^{-1}\)) treated rats caused an increase in the level of protein by 72% and 85% in the preventive and curative groups, respectively compared to the normal control (Tables 1 & 2).

3.9 Histopathological studies

Histopathological analysis of normal rat liver showed uniformly arranged cell plates with oval hepatocytes of the same size. In both experimental groups, NDEA administered rat liver showed irregularly formed cell plates with more damage towards the central vein region. Further, scattered masses of necrotic tissues were detected in most of the areas. Enlarged nuclei were also spotted in NDEA treated rats. Rats in preventive groups administered with \(E. scaber\) methanolic extract at a dose of 200 and 100 mg·kg\(^{-1}\) showed normal hepatocytes with uniform sinusoids, but in some areas degenerating hepatic cells were detected (Fig. 5). In the curative groups, \(E. scaber\) extract treatment completely protected the liver from NDEA-induced hepatotoxicity as evidenced by normal hepatocytes with uniform cell plates (Fig. 6).

Fig. 5: Histopathological features of liver in the preventive treatment group (A) Normal rat liver (B) NDEA control (C) NDEA + Silymarin (70 mg·kg\(^{-1}\)) treated rats (D) NDEA + \(E. scaber\) methanolic extract (200 mg·kg\(^{-1}\)) (E) NDEA + \(E. scaber\) methanolic extract (100 mg·kg\(^{-1}\))
4 Discussion and Conclusion

Weight loss is one of the major symptoms of hepatotoxicity [36]. Published reports indicate that repeated administration with NDEA reduces the body weight of animals, but stimulates the body weight by the treatment with plant extracts [4, 37]. In the present investigation, a severe weight loss was observed in rats exposed to NDEA in both experimental groups. It is important to note that the treatment with E. scaber methanolic extract enhanced the body weight in both experimental groups, indicating its ability to protect the animals from NDEA-induced toxicity.

Glutathione (GSH) is a key player in reduction processes in the cell. It also plays a role in the reduction of NTPs to dNTPs, and in the detoxification of endogenous and exogenous compounds, it serves as a cofactor for various enzymes, stores and transports cysteine, and may be involved in cell cycle regulation and thermotolerance [38-39]. Lipid peroxidation, initiated in the presence of NDEA, and resulting in the production of malondialdehyde (MDA), directly produces oxidative stress [40]. In a previous study, the E. scaber extract showed medial antioxidant capacity with moderate electron donating activity and reducing capacity [19]. In the present study, the levels of GSH, GPx, GST, catalase, MDA, and protein were improved to normal levels in rats treated with E. scaber extract in both the preventive and curative groups which reveals the antioxidant potential of the extract.

Amino transferases (AST and ALT) are the important cytoplasmic enzymes present in the liver. During chemical intoxication, the membrane integrity is lost due to necrosis, and these enzymes move into the circulatory system followed by elevated levels of aminotransferases in the serum [41]. Increased levels of serum AST and ALT was detected in NDEA-treated rats, and was effectively down-regulated by both treatments with the E. scaber methanolic extract indicating the protective role of E. scaber in treating...
NDEA-exposed hepatotoxicity. These biochemical restorations may be due to the inhibitory effects of *E. scaber* extract on the process of cellular damage in the liver. The phytochemicals identified are more potent in inhibiting free radicals, and this could be protective against the progression of NDEA-induced hepatotoxicity. Thus protection yielded by this extract may be due to the combined effects of these compounds or fractions rather than any single component.

Histopathological observation also supported the protective role played by *E. scaber* in NDEA-treated rats, as was evidenced by the presence of normal hepatocytes and small emboli of degenerating hepatic cells in both experimental groups. Thus the results presented in this study indicate that the hepatotoxicity induced by N-nitrosodiethyamine was effectively inhibited by the treatment with *E. scaber* extract in a concentration dependent manner in both the preventive and curative models. When compared to the pre-treatment group, the post-treatment group showed better efficacy against NDEA-induced liver injury. This might be due to the self-recovery of the animals during ten days after the toxin NDEA was administered.

The present study has unveiled the pharmacological actions of *E. scaber* against NDEA-induced hepatotoxicity for the first time. This study has successfully drawn several conclusions that determine the hepatoprotective effect of *E. scaber*. The present study reveals the root extract to have hepatoprotective properties against toxic chemicals that cause chronic liver injury, which seems to validate the plant use in traditional medicine. The result of the experiment indicates that *E. scaber* methanolic extract can normalize the situation by bringing the AST and ALT levels, as well as the antioxidant status and curative models. Therefore, the results presented in this study indicate that the protective effect of *E. scaber* against NDEA-induced liver injury. This might be due to the self-recovery of the animals during ten days after the toxin NDEA was administered.

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