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CURATIVE EFFECT OF *ELEPHANTOPUS SCABER* LINN. ON *N'*- NITROSODIETHYLAMINE INDUCED HEPATOCELLULAR CARCINOMA IN EXPERIMENTAL RATS

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Keywords:

Elephantopus scaber, N'Nitrosodiethylamine; Hepatocellular carcinoma, Proliferating Cell Nuclear Antigen (PCNA), Cyclin D1, Vascular Endothelial Growth Factor (VEGF)

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ABSTRACT: Objective: To evaluate the curative effect of *Elephantopus scaber* Linn. on N'Nitrosodiethylamine induced hepatocellular carcinoma in experimental rats. **Methods:** Phytochemical analysis of the different solvent extracts of *E. scaber* revealed various constituents such as flavonoids, terpenoids, phenols, etc. in the methanolic extract, which was selected for further study. The curative ability of two different doses of *E. scaber* methanolic extracts (100mg/kg and 200mg/kg) was evaluated against NDEA (0.02% in water) induced liver cancer in male Wistar rats. Quantification of serum AST, ALT, ALP, and LDH was conducted to assess the extent of hepatotoxicity. Estimation of Reduced Glutathione (GSH), Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx), Catalase (CAT) and Malondialdehyde (MDA) determined the anti-oxidant activity of the plant. Histopathological examination and immunohistochemical studies for cancer markers namely Proliferating Cell Nuclear Antigen (PCNA), vascular endothelial Growth Factor (VEGF) and Cyclin D1 were conducted on liver tissues to confirm the anti-cancer properties of the plant extract. **Results:** *Elephantopus scaber* methanolic extract significantly ($p \leq 0.05$) reversed the elevation of serum AST, ALT, ALP, LDH, and tissue malondialdehyde levels. Hepatic GSH, GST, GPx, and catalase levels were remarkably increased by the treatment with the extract. Histopathological and immunohistochemical evidence also supported the dose-dependent curative effect of the plant extract upon hepatocellular carcinoma. **Conclusion:** This study demonstrated the anti-cancer and anti-oxidant activity of *Elephantopus scaber* and thus scientifically validating its use as a potential anti-cancer drug source.

INTRODUCTION: Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver and the third most deadly disease prevalent in the world [1]. Chronic infections of Hepatitis B [2] and C [3], alcoholism, toxin intake, and cirrhosis can aid in the development of hepatocellular carcinoma.

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An increased incidence has been associated with smoking and exposure to aflatoxin, a mycotoxin that contaminates peanuts and soybeans and causes mutations in the p53 tumor suppressor gene [4]. Intake of acetaminophens like drugs and certain chemicals may also lead to hepatocellular carcinoma.

A large number of molecular biological markers synthesized and secreted by HCC cells have been shown to associate with the invasiveness and recurrence of post-operational HCC and have potential prognostic significance. Various tumor-related proteins, polypeptides, and isoenzymes such

as alpha-fetoprotein (AFP), hepatoma-specific gamma-glutamyl transpeptidase (HS-GGT), *etc*., show elevated levels in the blood and are indicative of HCC invasion [5].

Other significant markers include cellular proliferation markers (PCNA, Ki-67, Mcm2, MIB1, MIA, and CSE1L/CAS protein), the p53 gene and its related molecule MDM2, other cell cycle regulators (cyclin A, cyclin D, cyclin E, cdc2, p27, p73), oncogenes and their receptors (such as ras, c-myc, c-fms, HGF, c-met, and erb-B receptor family members), apoptosis-related factors (Fas and FasL) as well as telomerase-specific markers. Enzymes such as Glutathione –S- Transferase, Glutathione Reductase, and Catalase, etc., can reduce oxidative stress in the normal tissues. Extensive liver damage causes a decrease in levels of these enzymes, suggesting high oxidative stress in the tissues.

Uses of plants and plant derivatives as an alternative medicine for many ailments are increasing on a global perspective. The hepato-protective ability of Silymarin derived from *Silybum mariannum* seeds has made it immensely useful in treating liver diseases about alcohol consumption, toxin intake, cirrhosis, *etc.* [6]

Elephantopus scaber Linn. is one among 30 species of flowering plants of the genus *Elephantopus* from the Asteraceae family whose natural habitat is subtropical or tropical moist montanes. The plant is commonly called Prickly leaved- Elephant's foot and appears to have only a bunch of leaves of 4-6 inches long and 1-2 inches wide radiating from the ground with a stalked inflorescence. The plant has been mentioned in ancient texts and has been used as a traditional medicine for many ailments in Ayurveda exploiting its analgesic, diuretic, astringent, anti-venom and antiemetic properties [7].

Furthermore, *E. scaber* extracts have been used for the treatment of rheumatism, colic pain, heart ailments, hepatitis, eczema, *etc*. and are also used in herbal formulations for treating cancer [8].

Phytochemically, the plant is rich in sesquiterpene lactones such as deoxyelephantopin, isodeoxy-elephantopin, scabertopin and sterols such as lupeol and stigmasterol that have proclaimed biological effects [9].

The present work was undertaken to decipher the curative effect of *Elephantopus scaber* Linn. upon chemically induced liver cancer.

MATERIALS AND METHODS:

Chemicals: N- nitrosodiethylamine and silymarin were purchased from Sigma Chemical Co. MO, USA. 5, 5 dithiobis-{2-nitrobenzoic acid} (DTNB), bovine serum albumin, 1-chloro-2,4 dinitrobenzene (CDNB) and nitroblue tetrazolium were purchased from Sisco Research Laboratories Pvt, Ltd., Mumbai, India. Thiobarbituric acid was obtained from Central Drug House, Mumbai, India. Tween 80 was purchased from Merck, Mumbai, India. Assay kits for serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase were purchased from Agappe Diagnostic, India. All other chemicals were of analytical grade.

Collection of Plants and Preparation of Extracts: *Elephantopus scaber* plants were collected from its natural habitat in Kerala during July 2011 and authenticated. A voucher specimen (SBSBRL.03) is maintained at the institute. The plants were thoroughly washed using running tap water followed by rinsing with distilled water.

The plants were then cleaned, chopped, shade dried and powdered. A 50 g of dried powder was Soxhlet extracted with 400 mL of solvents of increasing polarity viz. petroleum ether, chloroform, methanol and ethanol for 48 h each. The extract was concentrated under reduced pressure using a rotary evaporator and was kept under refrigeration. The yield of methanolic extract was 10% (w/w).

Phytochemical Analysis: Qualitative phyto-chemical analysis was conducted on the various dried extracts of *Elephantopus scaber* to identify the various compounds in them [10, 11].

Evaluation of *in-vivo* Antioxidant Activity:

Animals and Diets: Male Wistar rats weighing 150 ± 5.5 gm (Mean ± S.D, n = 30) were used in this study. The animals were housed in polypropylene cages and given standard rat chow (Sai Feeds, Bangalore, India) and drinking water *ad libitum*. The animals were maintained at a controlled condition of a temperature of 26-28ºC with a 12 h light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics

Committee (IAEC) regulations approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. B 2442009/6) and conducted humanely.

Experimental Design and Treatment: Thirty rats were divided into five groups:

Group I: Normal control

Group II: NDEA control

Group III: Silymarin treated (100mg/kg)

Group IV: *Elephantopus scaber* methanolic extract (100mg/kg)

Group V: *Elephantopus scaber* methanolic extract (200mg/kg)

Group II-V rats received a single dose of 0.02% NDEA in water 5 days per week for 20 weeks. After treatment with NDEA, group III rats were treated with standard drug Silymarin (100mg/kg) daily for 30 days. Also, group IV – V rats received *E. scaber* methanolic extracts 100 mg/kg and 200 mg/kg, respectively for 30 days. Animals were sacrificed 48 h after the administration of the last extract.

Serum Enzyme Analysis: Hepatotoxicity was assessed by quantifying the serum levels of AST (EC 2.6.1.1), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1) and LDH (EC 1.1.1.27) by a kinetic method using the kit of Agappe Diagnostic Ltd., India. Activities of these serum enzymes were measured using semi-auto analyzer (RMS, India).

Tissue Analysis: The liver was excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1M Tris HCl buffer (pH – 7.4). The homogenate was centrifuged at 3000 rpm for 20 min at 4^{o}C, and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), reduced glutathione (GSH), lipid peroxidation (Thiobarbituric Acid Reactive Substances – TBARS) and total protein.

Tissue CAT (EC 1.11.1.6) activity was determined from the rate of decomposition of H_2O_2 [12]. GPx (EC 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 [13, 14].

GST (EC 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB [15]. Reduced GSH was determined based on the formation of a yellow colored complex with DTNB [16]. The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1'1'3'3' tetramethoxypropane as standard [17]. Protein content in the tissue was determined using bovine serum albumin (BSA) as the standard [18].

Histopathological Studies: Small pieces of liver tissues fixed in 10% buffered formalin were processed for embedding in paraffin. Sections of 5–6 μm were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The microphotographs were taken using Moticam 1000 camera at an original magnification of 100X.

Statistical Analysis: Results are expressed as mean ± S.D, and all statistical comparisons were made using one-way ANOVA test followed by Tukey's post hoc analysis and *p*-values less than or equal to 0.05 were considered significant.

Immunohistochemical Analysis: Immunohisto-chemical analysis of the three cancer markers, namely PCNA, VEGF, and Cyclin D1, were conducted upon formalin-fixed liver tissues and examined under the microscope. The microphotographs were taken using Moticam 1000 camera at an original magnification of 100X.

RESULTS:

Phytochemical Analysis: Preliminary qualitative phytochemical analysis of the various extracts of *Elephantopus scaber* Linn. showed the presence of flavonoids, phenols, carbohydrates, fixed oils, and proteins in the methanol extract **Table 1**.

Serum Analysis: Administration of NDEA caused an abnormal elevation of serum enzymes AST, ALT, AFP, and LDH in the group II animals when compared to the normal control ($p \leq 0.05$). Treatment with *E. scaber* methanolic extract of 100mg/kg exerted a protection of 62.7%, 60%, 39% and 64% upon AST, ALT, ALP and LDH

levels respectively when compared to 80%, 78%, 47% and 96% protection rendered by the 200mg/kg dose of *E. scaber* methanolic extract. The standard drug Silymarin reversed the elevated AST, ALT, ALP, and LDH levels by 74%, 73%, 44%, and 90% respectively **(Fig 1: A-D)**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *E. SCABER* LINN. EXTRACTS

	Petroleum Ether	Chloroform	Ethanol	Methanol
Alkaloids	-	-	-	-
Flavanoids	-	±	±	±
Phenols	-	±	-	±
Tannins	-	±	-	±
Carbohydrates	-	±	±	±
Fixed oil	-	-	-	±
Protein	-	-	-	±
Coumarins	-	-	-	-
Terpenoids	-	-	-	±

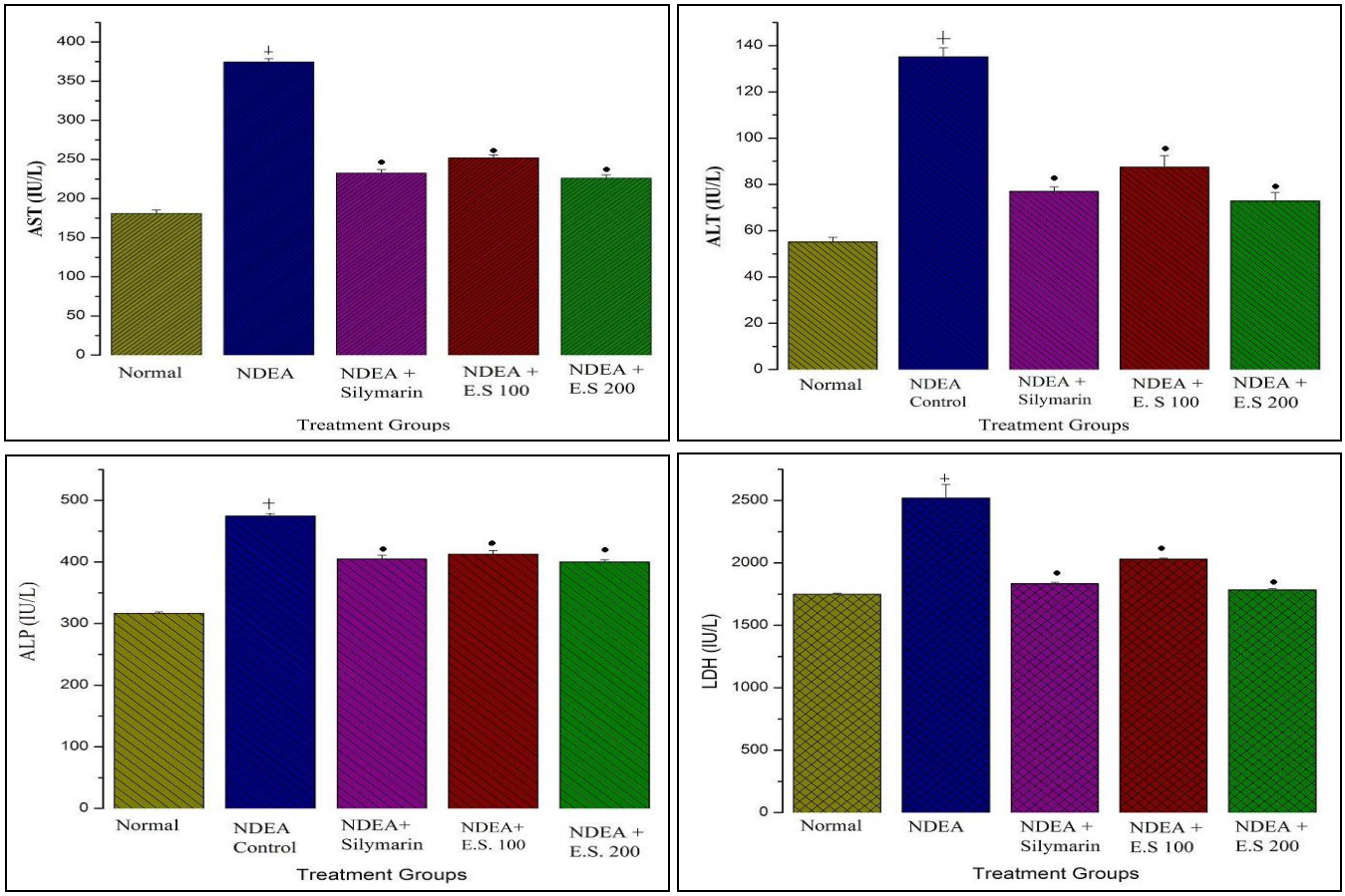

FIG. 1: EFFECTS OF E. SCABER METHANOLIC EXTRACT AND SILYMARIN POST-TREATMENT ON CHANGES IN SERUM ENZYME LEVELS OF RATS INTOXICATED WITH NDEA. A: Aspartate aminotransferase (AST); B: Alanine aminotransferase (ALT); C: Alkaline phosphatase (ALP); D: Lactate dehydrogenase (LDH)
(± - p≤0.05 vs Normal; ●- p≤0.05 vs NDEA control; values are Mean ± S.D; n=6)

Tissue Analysis:

Estimation of Reduced Glutathione: Reduced glutathione (GSH) levels were lowered significantly ($p \leq 0.05$) in rats exposed to NDEA. Treatment with *E. scaber* extract (200mg/kg) caused an increase in the levels of GSH by a significant ($p \leq 0.05$) 81% while the 100mg/kg treatment caused a 50% increase in GSH levels as compared to the NDEA control group **Table 2**.

Estimation of Glutathione-S-Transferase: When compared to NDEA treated animals, post-treatment with *E. scaber* methanolic extract (200mg/kg) caused an increase in the levels of GST by 81% while the 100mg/kg extract exerted 59% protection significantly ($p \leq 0.05$). Silymarin treatment caused an increase of 74% in GST levels towards normal when compared to the NDEA control **Table 2**.

Estimation of Glutathione Peroxidase: In NDEA exposed rats, significant ($p \leq 0.05$) lowering of GPx levels were observed. *E. scaber* methanolic extract (200mg/kg) treatment exerted its protection by 94%, and 100mg/kg treatment showed 51% protection when compared to NDEA control. Silymarin exerted 82% protection on GPx levels when compared to NDEA control **Table 2**.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *E. SCABER* LINN. ON TISSUE PARAMETERS

GROUPS	GSH (nmol / mg protein)	GST (nmol CDNB-GSH conjugate /min/mg)	GPx (nmol of GSH oxidized/min/mg protein)	CAT (U/mg protein)	MDA (mg/100gm tissue)	PROTEIN (mg/100gm tissue)
Group I (Normal)	24.5 ± 0.5	74.6 ± 1.2	291.3 ± 8.7	51.8 ± 1.5	45.1 ± 1.3	43.3 ± 2.5
Group II (NDEA toxic)	14.2 ± 0.4 *	35.23 ± 0.9*	174.2 ± 0.5*	33.6 ± 1.3*	74.9 ± 3.1*	20.5 ± 0.9*
Group III (NDEA ± SIL)	21.1 ± 0.3 Φ	64.31 ± 0.9Φ	271 ± 3.8 Φ	46.0 ± 0.8 Φ	58.3 ± 0.6 Φ	38.4 ± 1.3Φ
Group IV (NDEA ± *E.scaber* M100)	18.5 ± 0.3 Φ	58.45 ± 0.6 Φ	211.6 ± 1.0 Φ	41.27 ± 1.3 Φ	62.5 ± 1.0 Φ	33.8 ± 2.3Φ
Group V (NDEA ± *E.scaber* M200)	22.3 ± 0.2 Φ	67.11 ± 0.7 Φ	284.5 ± 4.2 Φ	49.7 ± 0.7 Φ	51.5 ± 1.7 Φ	39.7 ± 1.4 Φ

* $p \leq 0.05$ versus Normal Control; Φ $p \leq 0.05$ versus NDEA Control (Values are Mean ± S.D, n=6).

Estimation of Catalase: Catalase levels were lowered significantly ($p \leq 0.05$) in rats exposed to NDEA. *E. scaber* methanolic extract (200mg/kg) treatment exerted its protection by 88% while the treatment with 100mg/kg showed a 43% protection when compared to the NDEA Control. Silymarin protected the animals by about 68% when compared to NDEA control **Table 2**.

Estimation of Tissue Malondialdehyde: In NDEA administered animals, significant ($p \leq 0.05$) elevation of malondialdehyde (MDA) levels was observed. *E. scaber* methanolic extract (200mg/kg) treatment exerted its protection by 79% while the 100mg/kg extract treatment provided 42% protection. The standard drug Silymarin showed 58% protection against NDEA control **Table 2**.

Estimation of Total Protein: Protein levels were lowered significantly ($p \leq 0.05$) in rats exposed to NDEA. *E. scaber* methanolic extract (200mg/kg) brought about an 81% increase in protein levels whereas 100mg/kg brought about an increase of 63% when compared to NDEA control. Silymarin administered at 100mg/kg showed an increase in protein levels by 71% when compared to NDEA control **Table 2**.

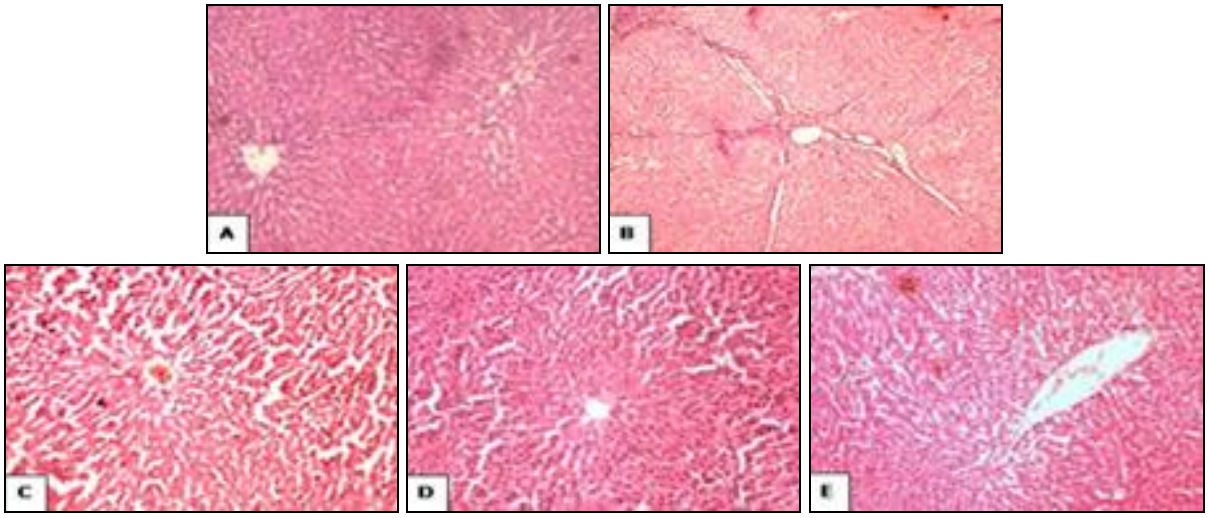

FIG. 2: HISTOPATHOLOGICAL CHANGES OCCURRED IN RAT LIVER AFTER NDEA INTOXICATION AND RECOVERY BY THE TREATMENT WITH *E. SCABER* METHANOLIC EXTRACT AND SILYMARIN (HEMATOXYLIN AND EOSIN, 100X). (A) Normal control; (B) NDEA control, (100 mg/kg s.c.); (C) Silymarin (100 mg/kg) + NDEA; (D) *E. scaber* (100 mg/kg) + NDEA; (E) *E. scaber* (200 mg/kg) + NDEA.

Histopathological Study: Histopathological analysis of normal rat liver showed uniformly arranged liver plates with oval hepatocytes of uniform size. Administration of NDEA brought about distortion in the normal cellular physiology forming irregular cell plates with more damages to the central vein regions. Enlarged nuclei are a prominent feature of the toxic group tissues. *E. scaber* extracts given at a dose of 200 mg/kg showed normal hepatocytes with uniform sinusoids. However, in some areas degenerating hepatic cells were detected in rats treated at a dose of 100 mg/kg. This was comparable with that of the standard hepatoprotective drug, silymarin at a dose of 100 mg/kg.

Immunohistochemical Analysis: Immuno-histochemical analysis of the normal rat tissue showed regularly stained nucleus indicating a normal expression level of Proliferating Cell Nuclear Antigen (PCNA), Cyclin D1, and Vascular Endothelial Growth Factor (VEGF).

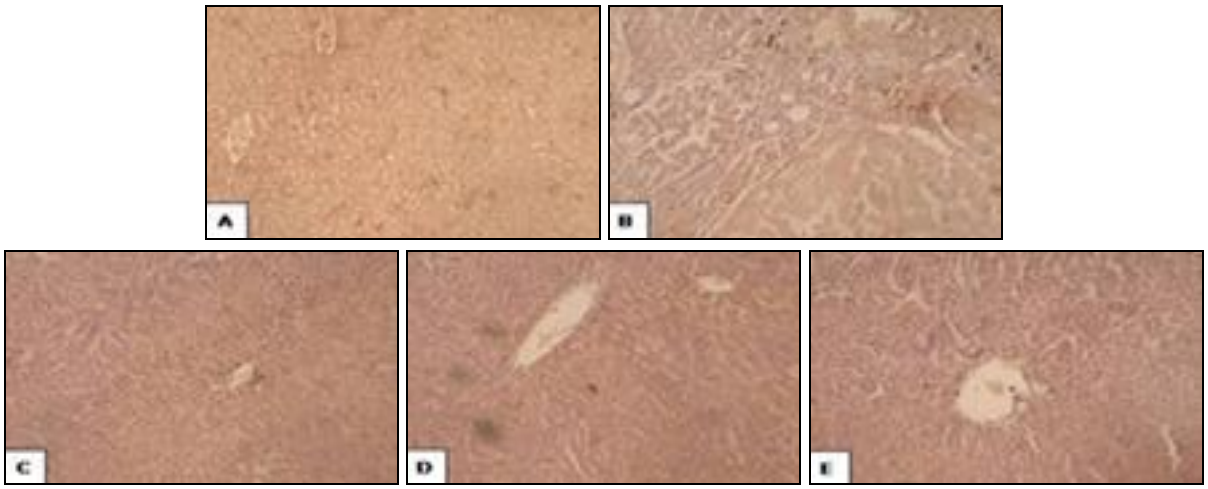

FIG. 3: IMMUNOHISTOCHEMICAL ANALYSIS OF PCNA IN RAT LIVER AFTER NDEA INTOXICATION AND RECOVERY BY THE TREATMENT WITH *E. SCABER* METHANOLIC EXTRACT AND SILYMARIN (HEMATOXYLIN AND EOSIN, 100×). (A) Normal control; (B) NDEA control, (100 mg/kg s.c.); (C) Silymarin (100 mg/kg) + NDEA; (D) *E. scaber* (100 mg/kg) + NDEA; (E) *E. scaber* (200 mg/kg) + NDEA

Tissues of NDEA administered toxic control showed irregular staining pattern indicating the over-expression of antigens. Administration of *E. scaber* extract of 100mg/kg showed a reduction in the irregular staining pattern as depicted in control. The plant extract given at a concentration of 200 mg/kg showed a remarkable lowering of irregular nuclear staining and was comparable to the effect rendered by silymarin at a concentration of 100 mg/kg **Fig. 3, 4, 5**.

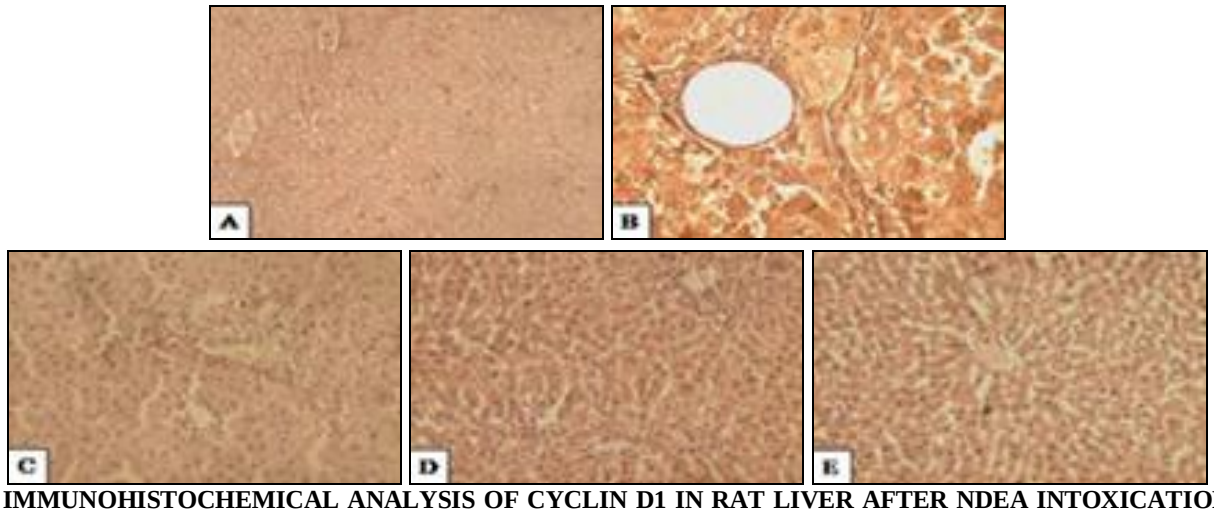

FIG 4: IMMUNOHISTOCHEMICAL ANALYSIS OF CYCLIN D1 IN RAT LIVER AFTER NDEA INTOXICATION AND RECOVERY BY THE TREATMENT WITH *E. SCABER* METHANOLIC EXTRACT AND SILYMARIN (HEMATOXYLIN AND EOSIN, 100×). (A) Normal control; (B) NDEA control, (100 mg/kg s.c.); (C) Silymarin (100 mg/kg) + NDEA; (D) *E. scaber* (100 mg/kg) + NDEA; (E) *E.scaber* (200 mg/kg) + NDEA

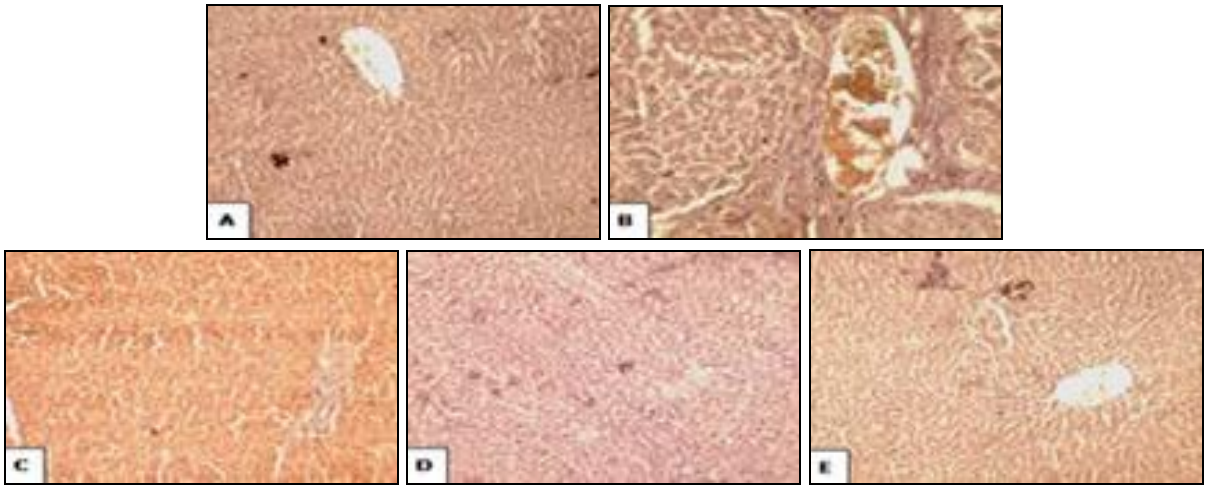

FIG. 5: IMMUNOHISTOCHEMICAL ANALYSIS OF VEGF IN RAT LIVER AFTER NDEA INTOXICATION AND RECOVERY BY THE TREATMENT WITH *E. SCABER* METHANOLIC EXTRACT AND SILYMARIN (HEMATOXYLIN AND EOSIN, 100×). (A) Normal control; (B) NDEA control, (100 mg/kg s.c.); (C) Silymarin (100 mg/kg) + NDEA; (D) *E. scaber* (100 mg/kg) + NDEA; (E) *E.scaber* (200 mg/kg) + NDEA

DISCUSSION: N-nitroso diethylamine (NDEA) is a potent carcinogen that is widely used in experiments to induce cancer in experimental animals. NDEA is known to disrupt cellular machinery, causing oxidative stress and injury due to the enhanced formation of free radicals. It is also associated with the increase in biochemical parameters such as serum glutamyl pyruvate transaminase, alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase, *etc*., and also decreases the levels of total proteins and uric acid. NDEA is also bioactivated by cytochrome P450, following which it forms DNA adducts rapidly, bringing about mutation and fragmentation that may lead to the formation of micronuclei [19].

Tissue damage caused by several factors, including cancer can cause the cells to break apart or increase its permeability, thereby releasing enzymes into the bloodstream. Elevated levels of liver function enzymes such as AST, ALT, LDH, and ALP are indicative of liver damage.

ALT produced mainly in the liver catalyzes the transfer of amino groups between L-alanine and glutamate for physiological purposes while AST catalyzes the transfer of amino and keto groups between α- amino acids and keto amino acids. ALT is specific for hepatic damage. AST levels may increase due to a variety of reasons owing to the presence of the enzyme in many tissues. Alkaline Phosphatases are a family of zinc metalloenzymes with serine at the active center and found histochemically in many tissues including microvilli of bile canaliculi and the sinusoidal surface of hepatocytes. Upon liver damage, the enzymes may leak into the blood or due to the rupture of normal metabolism; they may not be eliminated from the blood leading to an increase in the levels of these enzymes in blood [20]. The levels of these serum enzymes showed a drastic increase upon treatment with NDEA. The administration of *E. scaber* methanolic extract was shown to decrease the increased serum enzyme levels to near normal values indicating the ability of the plant extract to curb the adverse effects of an already established liver cancer.

The oxidative stress in livers of HCC-induced rats may be attributed to the fact that NDEA is mainly metabolized in the liver by the action of cytochrome p450 enzymes and the reactive metabolites are primarily responsible for its hepatotoxic effects. NDEA is bioactivated to ethyldiazonium ion which alkylates DNA bases to form promutagenic adducts such as O^6-ethyldeoxyguanosine and O^4 and O^6-ethyldeoxythymidine and these ROS induce oxidative stress and cytotoxicity by damaging biomolecules such as DNA, lipids, and proteins [21].

The data obtained in the present study indicate that administration of NDEA augments the oxidative stress and impairs the antioxidant defense mechanism as implied by the derangement of the oxidative stress marker (MDA) and free radical scavenging enzymes such as GR, GPx, GST, GSH, and CAT from their normal values.

The present study showed that levels of MDA in rat liver tissue were found to increase owing to an electrophilic counterattack provoked by NDEA treatment when compared to the normal rat liver tissue MDA levels. The administration of *E. scaber* methanolic extract brought about the lowering of the MDA levels suggesting that the plant extract possesses the capability to lower the ROS levels elevated by the toxin.

Glutathione (γ – glutamylcysteinyl glycine) is a tripeptide found in all living organisms and its reduced state, serves several vital functions including GST mediated detoxification of activated xenobiotic metabolites and scavenging of free radicals. Reduced glutathione (GSH) readily interacts with free radicals by donating a hydrogen ion. Such reactions protect by neutralizing reactive hydroxyl ions, which are considered to be a major source of free radical damage.

The major anti-oxidant mechanism of the cell comprises of GPx, GR, GST, and catalase. Glutathione S Transferases catalyze the conjugation of reduced glutathione via a sulphydryl group to electrophilic centers on a wide variety of substrates. This activity detoxifies endogenous compounds such as peroxidized lipids as well as xenobiotics. GST's may bind to toxins and facilitate their transport as inactivated compounds [22]. Glutathione Reductase (GR) is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. The activity of GR is used as an indicator for oxidative stress and is monitored by NADPH consumption levels which rise due to cellular damage [23].

NDEA administration causes depletion in GSH level and GSH dependent enzymes, GPx and GR, which may be attributed to the reduction in their biosynthesis owing to either hepatocellular damage or their excessive utilization in scavenging the free radicals formed during the metabolism of NDEA. Alternatively, the decreased levels of cellular GSH might have caused a reduction in the activities of GSH dependent enzymes, GPx and GR, as GSH is a vital co-factor for these enzymes [24].

The administration of Silymarin and the methanolic extract of *E. scaber* significantly ($p \leq 0.05$) increased the GSH values. This increase in GSH levels may be responsible for reducing the DNA-carcinogen interaction by providing a large nucleophilic pool for the electrophilic carcinogen (NDEA). GSH neutralizes the required electrophilic site by providing sulphydryl (SH) groups and renders the metabolite more water soluble. It may also increase the bioactivities of other anti-oxidant enzymes such as GPx, GR, *etc*; thus rendering a higher anti-oxidant status to the tissue [25].

Elevation of glucose uptake by the cells, which serves as fuel for both pentose phosphate pathway and oxidative phosphorylation brings up the cellular levels of NADPH/NADP+. Increasing the levels of NADPH may tend to increase GR activity, thereby raising the levels of the GSH, the substrate for GPx which ultimately leads to the jump start of an efficient anti-oxidant mechanism as brought about by Silymarin [26].

The present study showed that treatment with *E. scaber* extract alleviated the levels of GPx and GST when compared to NDEA treated animals significantly ($p \leq 0.05$) stressing the anti-oxidant capability of the plant.

Hydrogen peroxide (H_2O_2) is an important ROS formed during the reaction catalyzed by superoxide dismutase. Catalase is the enzyme responsible for the breakdown of H_20_2. NDEA treatment reduces the activity of catalase, thereby increasing the levels of H_2O_2. *E. scaber* supplementation in this study significantly ($p<0.05$) elevated the catalase levels when compared to the NDEA control indicating the efficacy of the antioxidant activity of the plant.

Histopathologic analysis of NDEA administered rat tissues exhibited cytomegaly, vacuolated hepatocytes, irregular blebbing of nuclei towards the central vein region. These symptoms were reduced in the Silymarin and *E. scaber* extract treated tissues. The results signify the anti-cancer potential of the herb, *Elephantopus scaber*.

The proliferating cell nuclear antigen (PCNA) is a 36 kDa protein with a ring-shaped homotrimeric structure that acts as a DNA sliding clamp. Both by and p53-independent and -independent regulations, PCNA interact with multiple proteins that play a key role in DNA-synthesis and repair, cell cycle

regulation, chromatin remodeling, and apoptosis [27]. Functionally, PCNA may be presenting itself in nucleoplasmic-free/soluble/DNA-unbound and in detergent-resistant/insoluble/DNA-bound fractions, the latter tightly associated with chromatin and actively involved in DNA-synthesis and repair [28].

PCNA modifications such as phosphorylation, acetylation, methyl esterification, mono, and poly-ubiquitylation, *etc*. are often found in response to DNA damage. Due to its multiple partner binding capabilities, analysis of PCNA and its abnormalities are relevant in cancer studies. PCNA upregulation is observed in cancerous tissues such as hepatocellular carcinoma [29].

PCNA immunostaining conducted in the present study depicted an upregulation of the protein which appeared as brown stained nuclei in the sections of NDEA treated rat livers while significant downregulation was observed when treated with *E. scaber* (200 mg/kg) depicting the anti-cancer effect of the plant.

The cyclin families of proteins are highly conserved nuclear cell cycle regulatory proteins of which the cyclin D family (Cyclin D1, D2, and D3) play pivotal roles in progression through the G1 phase of the cell cycle. Overexpression of Cyclin D1 has been associated with a variety of cancers, including HCC [30]. In the present study, overexpression of cyclin D1 was significantly reduced by the administration of *E. scaber* methanolic extract, which further elucidates the anti-tumor capability of the herb.

VEGF is an endothelial cell mitogen that induces and promotes angiogenesis and endothelial cell proliferation, which plays an important role in regulating angiogenesis [31], and which was initially identified as a vascular permeability factor [32]. VEGF expression also plays an important role in the development of hepatocellular carcinoma [33]. In the present study, treatment with *E. scaber* extracts showed significant down-regulation of VEGF in the cancerous tissues examined immuno-histochemically suggesting the potential of the plant as an effective anti-cancer remedy.

CONCLUSION: The present study aimed at the evaluation of the efficiency of *Elephantopus scaber* Linn., a traditional medicinal plant in curing established liver cancer. The methanolic extract of *E. scaber* proved its efficiency in normalizing the harmful effects of NDEA induced hepatocellular carcinoma. The extract brought about tremendous changes in the serum enzyme, antioxidant status, and cancer marker levels of NDEA challenged experimental animals to near normal levels in a dose-dependent response when compared to the standard drug Silymarin. The study has unveiled the herb as a potentially effective source of remedy for cancer.

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CONFLICT OF INTEREST: Nil

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