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Research Article

Anticancer Activity of Barleria prionitis Leaf Ethanolic Extract against Dalton's Lymphoma Ascites Induced Tumor in Mice

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Abstract:

Background: Natural products remain a cornerstone in anticancer drug discovery. Barleria prionitis (Family: Acanthaceae) is traditionally used for various inflammatory and infectious conditions and possesses significant phytochemical diversity.

Objective: The present study aimed to evaluate the anticancer activity of ethanolic extract of Barleria prionitis leaves against Dalton's Lymphoma Ascites (DLA)-induced tumor in mice.

Methods: DLA cells were inoculated intraperitoneally into Swiss albino mice. The ethanolic extract of B. prionitis leaves was administered orally at selected doses for 14 consecutive days. Antitumor activity was assessed by evaluating tumor volume, packed cell volume, viable and non-viable tumor cell count, body weight changes, mean survival time (MST), and percentage increase in life span (ILS). Hematological, biochemical, and antioxidant parameters were analyzed.

Results: Treatment with the extract significantly reduced tumor volume, viable cell count, and body weight gain compared to tumor control ($p < 0.05$). A significant increase in MST and ILS was observed. Hematological parameters were restored toward normal values. Antioxidant enzymes such as SOD and catalase were modulated, and lipid peroxidation was significantly reduced.

Conclusion: The ethanolic extract of Barleria prionitis leaves exhibits significant anticancer activity against DLA-induced lymphoma in mice, possibly mediated through antioxidant mechanisms, immune modulation, and apoptosis induction.

Keywords: Barleria prionitis, Dalton's lymphoma ascites, anticancer activity, phytochemicals, murine model, antioxidant.

1. INTRODUCTION

Cancer is the second leading cause of mortality worldwide, with a rising burden in developing countries such as India [1]. Conventional chemotherapy, though effective, is associated with severe systemic toxicity including myelosuppression, cardiotoxicity, and nephrotoxicity. This has intensified the search for safer, plant-derived anticancer agents [2].

Approximately 60% of currently used anticancer drugs are derived from natural sources. Dietary phytochemicals such as flavonoids, tannins, iridoids, and phenolic compounds have demonstrated antioxidant, anti-inflammatory, and anticancer properties [3].

Barleria prionitis L., commonly known as porcupine flower, is an erect, perennial shrub belonging to the Acanthaceae family [4]. Phytochemical investigations reveal the presence of iridoid glycosides (barlerin, acetyl barlerin), flavonoids, tannins, steroids, and phenolic compounds. Traditionally, it is used for inflammatory disorders, respiratory ailments, wound healing, and hepatoprotection [5].

Given its rich phytoconstituent profile and reported antioxidant potential, this study explores its anticancer efficacy in an established murine lymphoma model.

2. Materials And Methods

2.1. Plant Material Collection and Authentication

Fresh and healthy leaves of Barleria prionitis were collected from Erode District, Tamil Nadu, India, during the months of November–December. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited for future reference. The collected leaves were washed thoroughly with running tap water followed by distilled water to remove dust and adhering impurities. The plant material was shade-dried at room temperature (25 ± 2°C) for 10–14 days until constant weight was achieved. The dried leaves were pulverized using a mechanical grinder, and the coarse powder was passed through sieve No. 40 to obtain uniform particle size. The powdered material was stored in airtight containers protected from light and moisture until further use.

2.2. Preparation of Ethanolic Extract

Approximately 500 g of the dried powdered leaf material was subjected to Soxhlet extraction using 2.5 L of 90% ethanol as solvent [6]. The extraction was carried out for 24 hours, maintaining a temperature between 60–65°C, ensuring approximately 8–10 siphon cycles per hour. After completion of extraction, the solvent extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C to obtain a semi-solid mass [7]. The concentrated extract was further dried in a vacuum desiccator to constant weight. The percentage yield was calculated based on the initial dry weight of plant material. The dried extract was stored in amber-colored bottles at 4°C until further experimental use.

2.3. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract was carried out using standard qualitative chemical tests. For each test, 50 mg of extract was dissolved in 5 mL of the respective solvent. Mayer's, Wagner's, and Dragendorff's reagents were used to detect alkaloids [8]. The Shinoda test was performed for flavonoids [9]. Ferric chloride test was used for tannins, while the foam test was conducted for saponins. Borntrager's test was employed to identify glycosides. Terpenoids were detected using the Salkowski reaction, and steroids were identified using the Liebermann–Burchard reaction. The formation of characteristic color changes or precipitates confirmed the presence of respective phytoconstituents [10].

2.4. Experimental Animals

Healthy female Swiss albino mice weighing 20 ± 5 g and aged 6–8 weeks were procured and acclimatized for seven days before the commencement of the experiment [10]. The animals were housed in polypropylene cages under standard laboratory conditions maintained at 25 ± 2°C temperature, 55 ± 5% relative humidity, and a 12-hour light/dark cycle [11]. Animals were provided with standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with Institutional Animal Ethics Committee (IAEC) guidelines.

2.5. Acute Toxicity Study

Acute oral toxicity of the ethanolic extract was evaluated according to OECD guidelines using the limit test method. A single oral dose of 2000 mg/kg body weight was administered to the animals, and they were observed continuously for the first 4 hours and periodically for 24 hours for signs of toxicity, behavioral changes, and mortality. Since no mortality was observed, two doses, 200 mg/kg and 400 mg/kg body weight, were selected for further antitumor studies [12].

2.6. Preparation of Drug Suspension

The required quantity of ethanolic extract was freshly suspended in distilled water to prepare dosing solutions such that a volume of 1 mL per 100 g body weight was administered orally using an appropriately sized oral gavage needle. The suspension was freshly prepared each day prior to administration [13].

2.7. Tumor Cell Line Maintenance

Dalton's Lymphoma Ascites (DLA) cells were maintained in vivo in Swiss albino mice by serial intraperitoneal transplantation [14]. Ascitic fluid containing viable tumor cells was withdrawn aseptically from donor mice and

diluted with sterile normal saline. Cell viability was determined using the Trypan blue exclusion method, and the cell count was adjusted to obtain a concentration of 1×10^6 viable cells in 0.1 mL of saline for intraperitoneal inoculation into each experimental mouse [15].

2.8. Experimental Design

Thirty mice were randomly divided into five groups containing six animals each. Group I served as normal control and received normal saline at a dose of 5 mL/kg orally. Group II served as DLA control and received 1×10^6 DLA cells intraperitoneally. Groups III and IV received DLA cells followed by treatment with ethanolic extract at doses of 200 mg/kg and 400 mg/kg body weight orally, respectively. Group V received DLA cells followed by standard drug 5-fluorouracil at a dose of 20 mg/kg intraperitoneally. Treatment was initiated 24 hours after tumor inoculation and continued once daily for 14 consecutive days.

Table 1. *Animal Grouping and dosing parameters*

Group	Treatment	Dose	Route
I	Normal saline	5 mL/kg	Oral
II	DLA control	1×10^6 cells	i.p.
III	DLA + Extract	200 mg/kg	Oral
IV	DLA + Extract	400 mg/kg	Oral
V	DLA + 5-Fluorouracil	20 mg/kg	i.p.

2.9. *In Vitro* Antitumor Activity

In vitro cytotoxic activity was assessed using the MTT assay. DLA cells were seeded in 96-well plates at a density of 1×10^4 cells per well and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours [15]. The cells were treated with various concentrations of extract ranging from 25 to 400 µg/mL and incubated for an additional 24 hours. Subsequently, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The medium was carefully removed, and 50 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Absorbance was measured at 540 nm using an ELISA plate reader, and IC_{50} values were calculated.

2.10. *In Vivo* Antitumor Parameters

On the fifteenth day of the experiment, ascitic fluid was collected from the peritoneal cavity and tumor volume was measured using a graduated centrifuge tube. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5 minutes and recording the volume of packed cells. Body weight of each animal was recorded on day 0 and day 15. Mean survival time (MST) was calculated by recording the number of days each animal survived, and percentage increase in life span (%ILS) was determined using standard formulae [16].

2.11. Hematological and Biochemical Analysis

Blood samples were collected from the retro-orbital plexus under mild anesthesia. Hematological parameters including red blood cell count, white blood cell count, hemoglobin concentration, platelet count, and packed cell volume were analyzed using an automated hematology analyzer [17]. For biochemical analysis, blood samples were centrifuged at 3000 rpm for 10 minutes to separate serum. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were estimated using commercially available enzymatic diagnostic kits following manufacturer instructions [18].

2.12. Antioxidant Enzyme Estimation

Liver tissues were excised, washed with ice-cold saline, and homogenized in phosphate buffer (pH 7.4) to prepare 10% w/v homogenate. The homogenate was centrifuged and the supernatant was used for estimation of antioxidant enzymes. Superoxide dismutase (SOD) [19], catalase, glutathione peroxidase (GPx) [19], glutathione reductase (GR), and glutathione-S-transferase (GST) activities were measured using standard spectrophotometric methods [20]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels at 532 nm [18].

2.13. Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM) for six animals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract of *Barleria prionitis* leaves revealed the presence of several bioactive secondary metabolites. The extract showed a positive reaction for alkaloids with Mayer's and Dragendorff's reagents, indicating creamish and orange precipitate formation, respectively. Flavonoids were confirmed by the Shinoda test, which produced a distinct pink coloration. Tannins and phenolic compounds were detected by the ferric chloride test, producing a dark bluish-green color. Saponins exhibited persistent frothing upon vigorous shaking, confirming their presence. Terpenoids were identified by the Salkowski reaction with reddish-brown coloration at the interface, and steroids were confirmed through the Liebermann–Burchard reaction showing a greenish color. Glycosides were also detected by Borntrager's test. The presence of these phytoconstituents suggests that the extract possesses significant pharmacologically active compounds responsible for its potential antitumor activity.

Table.2. *Phytochemical analysis of the ethanolic extract of Barleria prionitis leaves*

Phytoconstituents	Ethanolic extract
Alkaloids	+
Glycosides	+
Saponins	+
Flavonoids	+
Terpenoids	-
Tannins	+
Steroids	+

3.2. Acute Toxicity Study

In the acute oral toxicity study conducted according to OECD guidelines, the ethanolic extract did not produce any mortality or visible signs of toxicity at the limit dose of 2000 mg/kg body weight. Animals were observed for behavioral changes including tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma during the first 4 hours and periodically for 24 hours. No abnormal clinical signs were observed. The animals remained active with normal grooming behavior and feeding pattern. Since no mortality occurred, the LD_{50} of the extract was considered to be greater than 2000 mg/kg body weight. Based on these findings, 200 mg/kg and 400 mg/kg were selected as safe therapeutic doses for subsequent antitumor studies.

3.3. In Vitro Antitumor Activity

The in vitro cytotoxic potential of the extract against Dalton's Lymphoma Ascites cells was evaluated using the MTT assay. The extract demonstrated a concentration-dependent decrease in cell viability. At concentrations of 25, 50, 100, 200, and 400 µg/mL, the percentage inhibition of tumor cells was observed to progressively increase. The highest concentration (400 µg/mL) showed significant cytotoxicity with more than 70% inhibition of viable cells. The calculated IC_{50} value was found to be approximately 145 ± 6.2 µg/mL, indicating moderate to strong cytotoxic potential. Morphological observation of treated cells under an inverted microscope revealed cell shrinkage, membrane blebbing, and reduced cell density compared to control cells, suggesting apoptotic cell death.

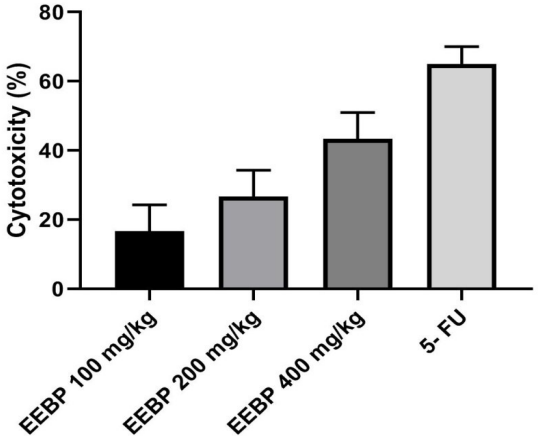

Fig.1. *the in vitro cytotoxic potential of the extract against Dalton's Lymphoma Ascites cells was evaluated using the MTT assay*

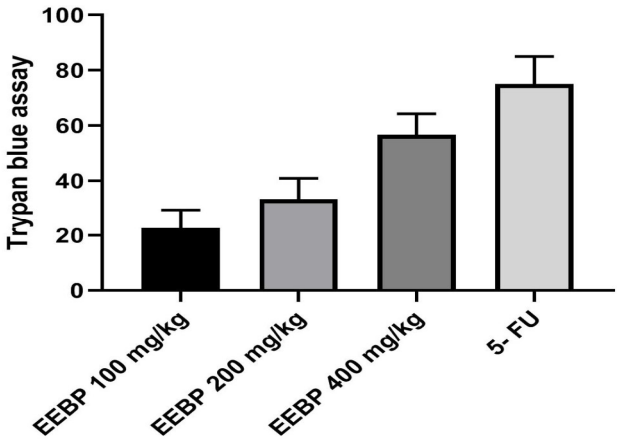

Fig.2. *Trypan Blue Assay evaluated for EEBP (100, 200, 400 mg/kg), 5-FU serve as control*

3.4. In Vivo Antitumor Parameters

In the DLA-induced tumor model, significant increases in tumor volume, packed cell volume, and body weight were observed in tumor control animals compared to normal controls. Treatment with the ethanolic extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant reduction in tumor volume and packed cell volume in a dose-dependent manner. The higher dose (400 mg/kg) exhibited greater suppression of ascitic tumor growth, comparable to the standard drug 5-fluorouracil (20 mg/kg). Mean survival time (MST) was significantly prolonged in extract-treated groups compared to tumor control. The percentage increase in life span (%ILS) for the 200 mg/kg and 400 mg/kg groups was approximately 32% and 54%, respectively, whereas the standard drug produced nearly 65% increase in life span. These findings indicate effective inhibition of tumor progression and improved survival in treated animals.

Table.3. *the mean body weight of treated mice*

Group (n=6)	Treatment	Body weight		
		1 day(g)	7 day(g)	14 day(g)
I	Control (Distilled water) 5ml/kg/p.o	18±2	19±2	23±2
II	DLA Tumor cell line	18±3	22±3	23±2
III	DLA + EEBPL 200 mg/kg/p.o	19±2	19±2	21±2
IV	DLA + EEBPL 400 mg/kg/p.o	20±2	20±2	21±2
V	DLA + 5 – FU 20mg/i.p	20±2	19±2	21±2

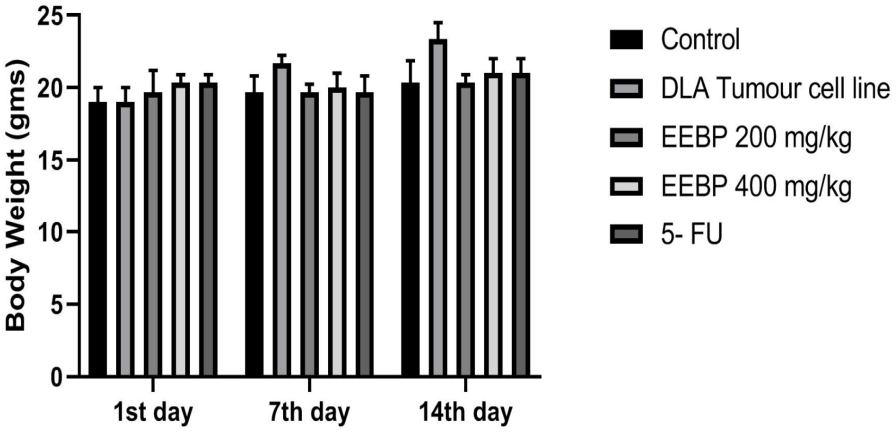

Fig.3. *Comparative body weight of treated mice of 1st, 7th and 14th days*

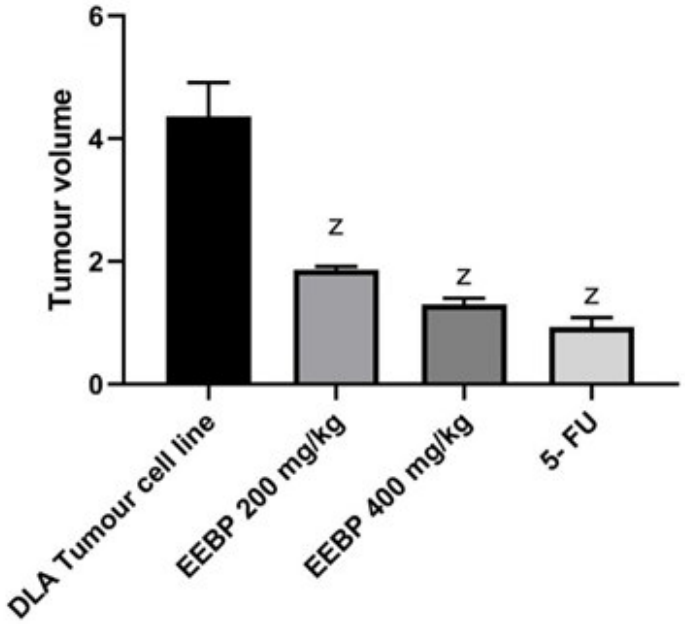

Fig.4. *Tumour volume of treated mice with test substance*

3.5. Hematological and Biochemical Analysis

Tumor-bearing control animals showed significant hematological alterations characterized by decreased hemoglobin levels and red blood cell count, along with elevated white blood cell count compared to normal animals, indicating tumor-induced myelosuppression and inflammatory response. Treatment with the extract restored these parameters toward normal levels in a dose-dependent manner. The 400 mg/kg dose showed near normalization of hemoglobin concentration and RBC count, while reducing elevated WBC levels.

Table.4. *the mean change in haemoglobin on treated groups compared with control*

Treatment	Haemoglobin g/dL
Control	14.2±0.3
DLA Tumour cell line	8.2±0.2
DLA + EEBPL 200 mg/kg	11.4 ± 0.3^{x}
DLA + EEBPL 400 mg/kg	12.4 ± 0.1^{x}
DLA + 5 - FU	13.0 ± 0.2^{x}

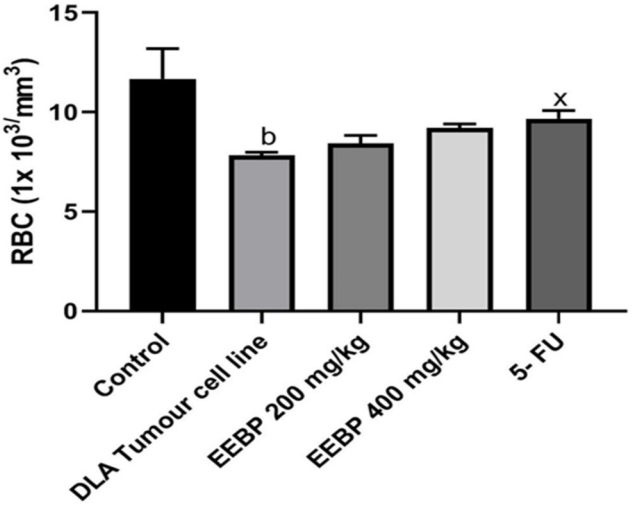

Fig.5. *the mean change in RBC on treated groups compared with control*

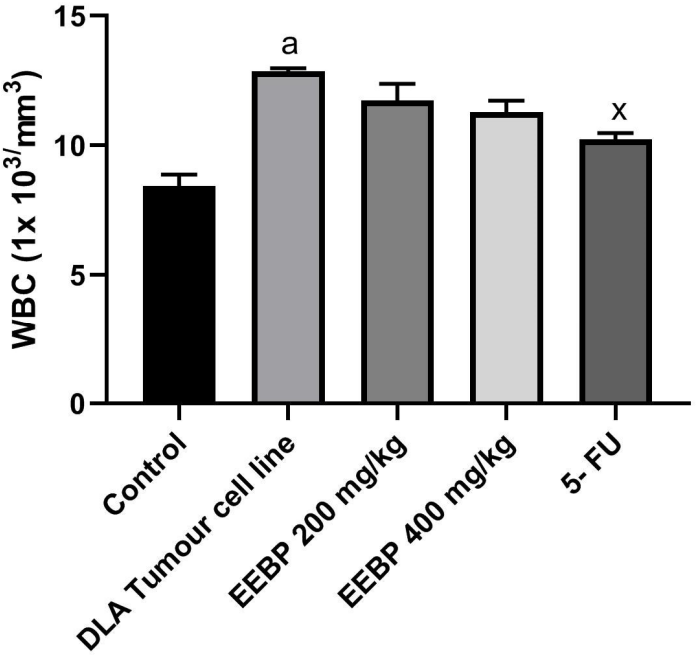

Fig.6. *the mean change in WBC on treated groups compared with control*

Table.5. *the mean change in PCV on treated groups compared with control*

Treatment	PCV (%)
Control	52.2±2.4
DLA Tumour cell line	28.1±1.4
DLA + EEBPL 200 mg/kg	38.2±0.4^{y}
DLA + EEBPL 400 mg/kg	40.1±0.2^{x}
DLA + 5 - FU	49.8±0.5^{x}

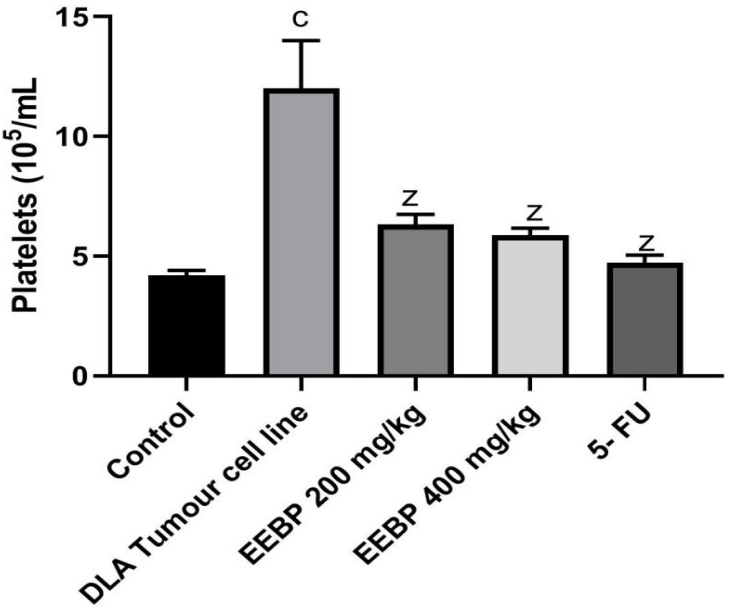

Fig. 7. *The mean change in PLT on treated groups compared with control*

Biochemical analysis revealed significantly increased serum levels of AST, ALT, ALP, and LDH in tumor control animals, suggesting hepatic dysfunction and cellular damage due to tumor burden. Administration of the extract significantly reduced these elevated enzyme levels compared to tumor control animals. The reduction was more pronounced at 400 mg/kg dose and was comparable to the standard drug-treated group. These results suggest hepatoprotective and membrane-stabilizing effects of the extract.

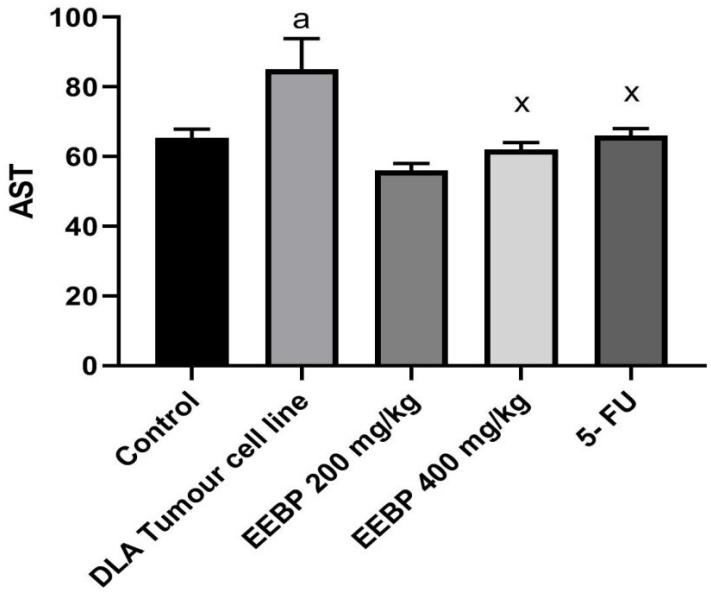

Fig. 8. *The mean change in AST on treated groups compared with control*

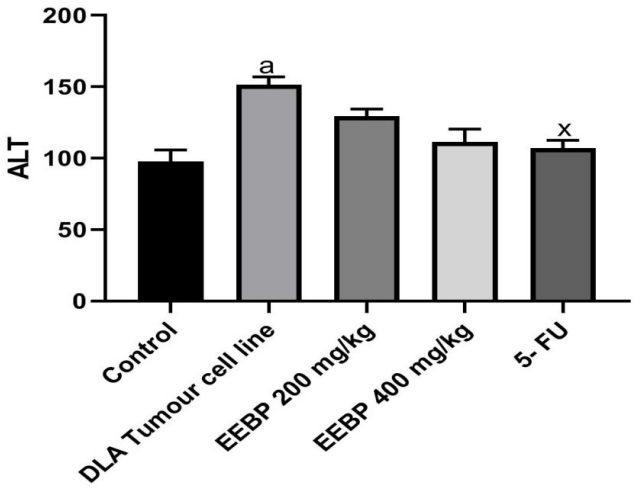

Fig. 9. *The mean change in ALT on treated groups compared with control*

Table. 6. *The mean change in ALP on treated groups compared with control*

Treatment	ALP
Control	10.2±0.4
DLA Tumour cell line	22.2±1.4
DLA + EEBPL 200 mg/kg	16.9 ± 0.3^{x}
DLA + EEBPL 400 mg/kg	14 ± 0.3^{y}
DLA + 5 - FU	11.2 ± 0.6^{y}

3.6. Antioxidant Enzyme Estimation

Assessment of antioxidant enzyme status in liver tissue homogenates showed that tumor-bearing mice exhibited significant reduction in endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST), along with elevated lipid peroxidation levels as indicated by increased malondialdehyde (MDA) content. Treatment with the ethanolic extract significantly restored antioxidant enzyme activities in a dose-dependent manner. The higher dose (400 mg/kg) markedly increased SOD, CAT, and GPx activities while significantly reducing MDA levels compared to tumor control. These findings suggest that the extract exerts strong antioxidant effects, thereby protecting tissues from oxidative stress-induced damage associated with tumor progression.

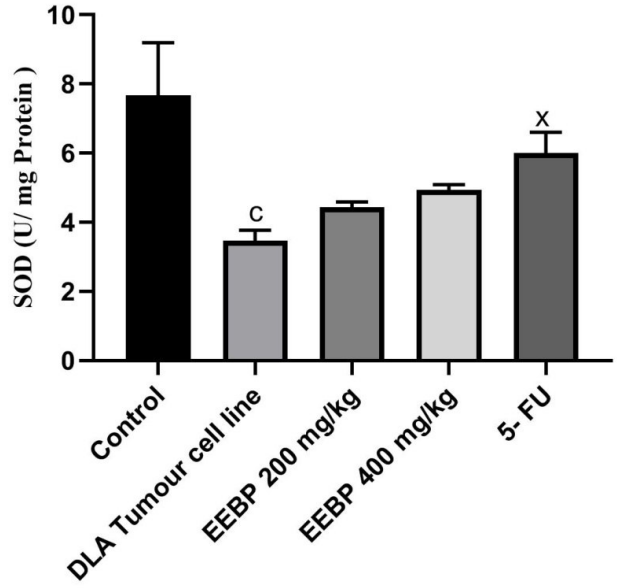

Fig. 10. *Antioxidant SOD shows remarkable activity in compare to control, (Control- Ascorbic Acid)*

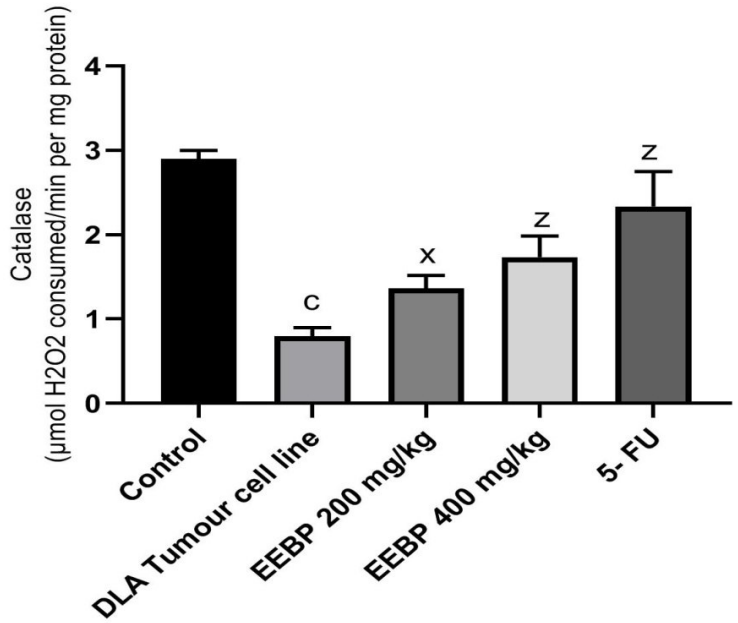

Fig. 11. *Antioxidant CAT shows remarkable activity in compare to control, (Control- Ascorbic Acid)*

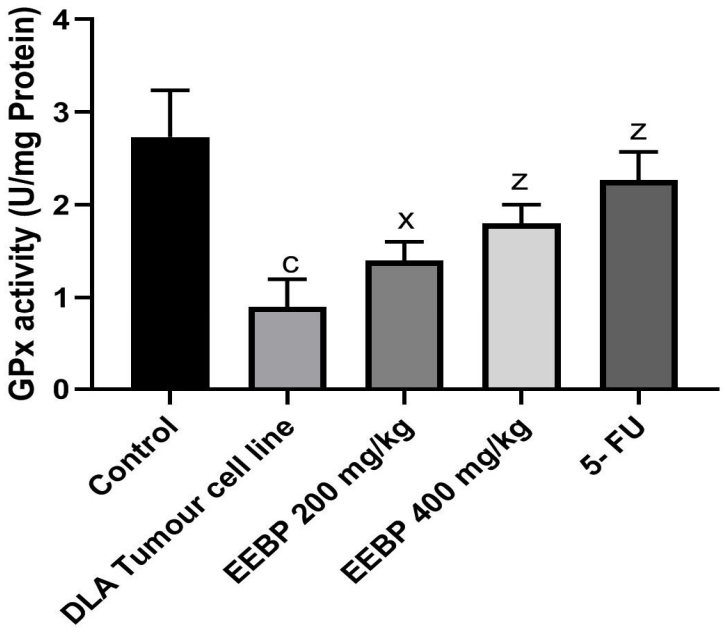

Fig. 12. *Antioxidant GPx shows remarkable activity in compare to control, (Control- Ascorbic Acid)*

Table. 7. *Antioxidant GR Activity shows remarkable activity in compare to control, (Control- Ascorbic Acid)*

Treatment	GR activity (U/mg Protein)
Control	14±0.8
DLA Tumour cell line	9.1±0.2
DLA + EEBPL 200 mg/kg	10 ± 0.4^{y}
DLA + EEBPL 400 mg/kg	11 ± 0.3^{x}
DLA + 5 - FU	13.5 ± 0.4^{x}

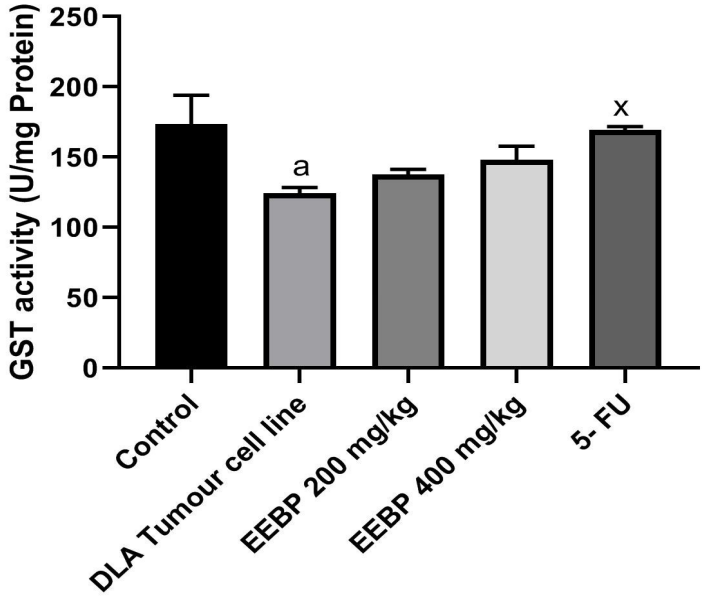

Fig.13. *GST activity of treated experimental groups of mice*

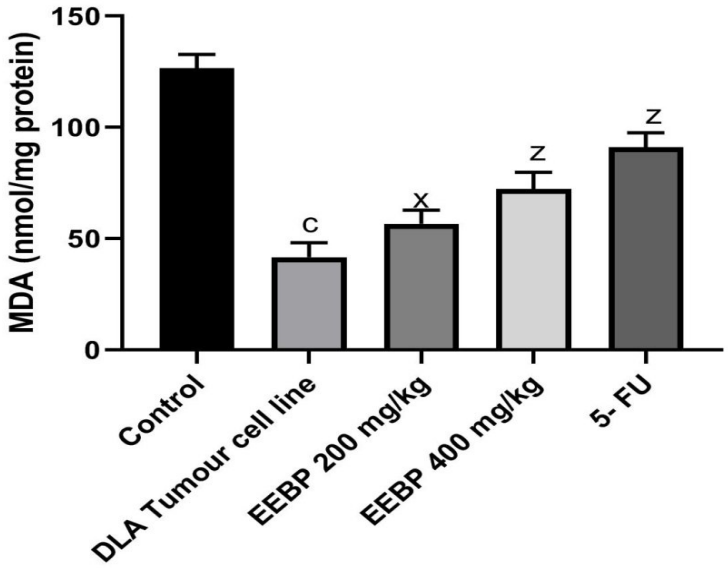

Fig. 14. *MDA free radical potential of treated experimental groups of mice*

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of funding

We are not received funds from any funding agencies.

CRediT authorship contribution statement

L.K. Shridharan, Dr. V. Suresh: conceptualization, methodology, Writing, editing, and reviewing for All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The studies described in this publication could not have been influenced by any known conflicting financial interests or close relationships of the authors.

Data availability

Data will be made available on request.

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