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Anticancer Activity and *in vivo* Antioxidant Potential of *Phyllanthus niruri* (*Euphorbiaceae*) Linn. against Ehrlich Ascites Carcinoma in Swiss albino mice

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Abstract

The rising burden of cancer worldwide calls for an alternative treatment solution. Herbal medicines provide a very feasible alternative to western medicine against cancer. The present work was designed to evaluate the anticancer activity and *in vivo* antioxidant potential of the methanol extract of *Phyllanthus niruri* (MEPN) Linn. (Family: Euphorbiaceae). The effect of MEPN on cancer growth and host's survival time was studied by the parameters such as tumor volume, packed cell volume, viable and non-viable cell count and life span of the animal. MEPN was administered at a concentration of 125 and 250mg/kg b.w. once a day for 14 days, after 24 h of tumor inoculation. Decrease in tumor volume, packed cell volume, and viable cell count were observed in MEPN treated animals when compared to EAC treated animals. Administration of MEPN at a dose of 125 and 250mg/kg increased the mean survival time to 26.67 ± 3.41 and 32.15 ± 4.35 days, respectively. The plant extract also decreased the body weight of the EAC tumor bearing mice. Hematological studies reveal that the Hb content was decreased in EAC treated mouse, whereas restoration to near normal levels was observed in extract treated animals when compared to EAC treated animals. The investigation was also extended to estimate the liver biochemical parameters such as LPO, GSH, and antioxidant enzymes like SOD, CAT, etc. Treatment with MEPN decreased the levels of lipid peroxidation and increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The results obtained suggested that the methanol extract of *Phyllanthus niruri* (MEPN) exhibits significant anticancer and antioxidant activity in EAC bearing mice.

Keywords

Phyllanthus niruri, Ehrlich ascites carcinoma, Anticancer activity, *in vivo* antioxidant activity.

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INTRODUCTION

The burden of cancer rose to 18.1 million new cases and 9.6 million deaths in 2018. With 36 different types, cancer mainly affects men in the form of liver, lung, prostate, stomach and colorectal cancer and women in the form of breast, cervix, lung, thyroid, and colorectal cancer [1]. Treating cancer has become a whole new area of research. There are conventional as well as very modern techniques applied against cancers. A variety of techniques i.e., surgery, radiation therapy, and/or chemotherapy are used for treating cancer. However, all of them have some disadvantages [2]. The use of

conventional chemicals bears side effects and toxicities [3]. But, as the problem persists, new approaches are needed for the control of diseases, especially, because of the failure of conventional chemotherapeutic approaches. Therefore, there is a need for new strategies for the prevention and cure of cancer to control the death rate because of this disease.

Herbal medicine has become a very safe, non-toxic, and easily available source of cancer-treating compounds. Herbs are believed to neutralize the effects of diseases in a body because of various characteristics they possess [4]. For instance, among the many anticancer medicinal plants, *Phyllanthus niruri* (local name: Stone breaker) has been used traditionally for the anticancer properties.
Phyllanthus niruri (PN, family: Euphorbiaceae) is a small annual herb growing up to 30-40 cms in height and is indigenous to the rainforests in the Amazon and other tropical areas including the Bahamas, Southern India and China [5]. In India, it is widespread in drier tropical areas of Telangana, Andhra Pradesh, Tamil Nadu, Kerala, and Karnataka states of South India. It is named the 'stone breaker' by the indigenous people. Whole plant, fresh leaves and fruits are used to treat various ailments like dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stones, dyspepsia, anti-hepatotoxic, anti-hepatitis B, antihyperglycemic and also as antiviral and antibacterial [6]. The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres. 'Chanca Piedra' is the Spanish name for *Phyllanthus niruri* and translated, it means "Stone Breaker" or "shatter stone". It has been called Stone Breaker because it has used for generations by the indigenous peoples of the Amazon as an effective remedy to eliminate gallstones and kidney stones and for other kidney problems [7, 8 & 9]. The plant is employed for numerous other conditions including colic, diabetes, dysentery, fever, flu, tumors, jaundice, vaginitis, and dyspepsia [10]. It is also used for the treatment of hepatitis, antispasmodic, antiviral, antibacterial, diuretic, febrifugal and hypoglycemic activities [11]. It is also considered carminative, digestive, emmenagogue, laxative, stomachic, tonic and vermifuge based on its long-documented history of uses [10]. The plant contains active constituents like alkaloids (ent-norsecurinine) [11], lignans (phyllanthine and hypophyllanthine); phenols and terpenes bioflavonoids (quercetin) [12].

Previously, we reported the antihyperglycemic effect [13] and *in vitro* lipid peroxidation and antimicrobial activity [14] of MEPN. Since, there are no reports on anticancer effect of MEPN, in this investigation, we report the anticancer activity and *in vivo* antioxidant potential of the methanol extract of PN.

MATERIALS AND METHODS

Chemicals

Nitriblue tetrazolium (NBT), Thiobarbituric acid (TBA), Phenazonium methosulfate (PMS) and Nicotinamide adenine dinucleotide (NADH) were purchased from SISCO Research Laboratory, Mumbai, INDIA. 5,5', dithio *bis* 2-nitro benzoic acid (DTNB), reduced Glutathione and Folin-Ciocalteau phenol were purchased from Loba Chemie, Mumbai, INDIA, and bovine serum albumin from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and reagents were procured from local suppliers and were of analytical grade.

Plant material and Extract

The entire plant of *Phyllanthus niruri* was obtained from the tribal area of Karimnagar District, Telangana, India. The plant was taxonomically identified and authenticated by Dr. Alok Bhattarcharya of the Botanical Survey of India (BSI), Shibpur, Kolkata (WB), India. A voucher specimen (No. GPS-2) has been preserved in our laboratory for future reference. The entire plant was dried under shade and made to a powder using a laboratory mechanical grinder. Then, the powder was extracted initially with petroleum benzine (60-80°C) followed by methanol at 24°C for 72 hrs by hot continuous extraction method in a Soxhlet extractor [15]. The solvent was evaporated under reduced pressure at 50°C and dried in vacuum (Yield: 9.50%, methanol extract). The dried extract thus obtained was dissolved in isotonic normal saline solution and used directly for the assessment of anticancer and *in vivo* antioxidant activities.

Ethical clearance

The procedure followed in this investigation for the use of mice as an animal model for cancer was approved by the University Animal Ethical Committee, Kakatiya University, Warangal (TS), INDIA.

Previously isolated classes of compounds

The phytochemical study revealed that the MEPN contained alkaloids, flavonoids, saponins, and coumarins, polyphenols, tannins, terpenoids, lipids and lignans [16].

Acute toxicity study

The acute toxicity (LD_{50}) of the extract was calculated by the method of Litchfield and Wilcoxon [17] and UK Mazumder *et al*. (2005) [18].

Experimental animals

Male Swiss albino mice of about 8 weeks of age with an average body weight of 24±2 g was used for the experiment. The animals were bred and brought up in

our laboratory facility with 12-h cycles of light and dark at 23°C. They were fed standard laboratory diet and were given sterilized water *ad libitum*.

Tumor cells

Ehrlich ascites carcinoma (EAC) cells were obtained with a courtesy from Chittaranjan National Cancer Institute, Kolkata (WB), INDIA. The EAC cells were maintained *in vivo* in Swiss albino mice, by intraperitoneal (i.p.) transplantation of 2×10^6 cells/mouse after every 10 days. EAC cells of 9 days old were used for the screening of the MEPN.

Experimental protocol

The method followed for the determination of anticancer activity was according to the method explained in our laboratory [19].

Male Swiss Albino mice were divided into five groups of eight animals (n=8) each. The MEPN was dissolved in isotonic saine (0.9% NaCl w/v) solution and used directly in the assay. EAC cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 2×10^6 cells/ml. 0.1 ml of EAC cells per 10 g body weight of the animals was injected (intraperitoneally, i.p.) on day zero (day 0). A day of incubation was allowed for multiplication of the cells. Fourteen doses of the MEPN (125 mg and 250 mg/kg, 0.1 ml/10 g b.w.) and 5-Fluorouracil (20 mg/kg b.w.) were injected i.p. from the 1st day up to the 14th day with 24-h intervals. Control animals received only vehicle (isotonic saline solution). Food and water were withheld 18 h before sacrificing the animals. On day 15, half of the animals in each cage were sacrificed and the remaining animals were kept observing for the evaluation of life span. 5-Fluorouracil (5-FU) at a dose level of 20 mg/kg b.w. was used as standard.

Group 1: Normal control (0.9%, NaCl, w/v; 5 ml/kg b.w.); Group 2: EAC control (2×10^6 cells/mouse); Group 3: EAC (2×10^6 cells/mouse) + MEPN (125 mg/kg. b.w.); Group 4: EAC (2×10^6 cells/mouse) + MEPN (250 mg/kg. b.w.); and Group 5: EAC (2×10^6 cells/mouse) + 5-FU (20 mg/kg. b.w.).

The antitumor of the MEPN was measured in EAC animals with respect to the following parameters:

- ***Tumor volume***: The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.
- ***Tumor cell count***: The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.
- ***Viable/non-viable tumor cell count***: The cells were then stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non-viable. These viable and non-viable cells were counted.

$$\text{Cell count} = \frac{\text{No. of cells} \times \text{dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

- ***Percentage increase life span (% ILS):*** The effect of MEPN on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and % ILS was calculated.

$$\%\ \text{ILS} = \left[\frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} - 1\right] \times 100$$

$$\text{Mean survival} = \frac{\text{Day of 1st death} + \text{Day of last death}}{2}$$

- ***Body weight***: Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period in order to evaluate the relative changes.

Hematological parameters

At the end of the experimental period, all mice were sacrificed the next day after an overnight fast by decapitation. Blood was collected from freely flowing tail vein and used for the estimation of hemoglobin (Hb) content, red blood cell count (RBC) [20], and white blood cell count (WBC) [21]. WBC differential count was carried out from Leishman-stained blood smears [22].

Determination of *in vivo* antioxidant potential

After collecting the blood samples, the mice were killed by cervical dislocation. Then, the liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dried and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer and was used for the estimation of lipid peroxidation (LPO) and reduced glutathione (GSH) (after precipitating proteins with TCA). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD), catalase (CAT), and total protein.

Estimation of Lipid peroxidation (LPO) levels

The levels of Thiobarbituric acid reactive substances (TBARS) in the liver was measured by the method of Ohkhawa et al (1979) [23] as a marker for lipid peroxidation. A mixture of 0.4 ml of 10% liver homogenate, 1.5 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% aetate buffer (pH 3.5) and 1.5 ml of 0.8% TBA solution was heated at 95°C for 1 h. After cooling, 5.0 ml of *n*-butanol-pyridine (15:1) was added, and the absorbance of the *n*-butanol-pyridine layer was measured at 532 nm.

Estimation of Reduced Glutathione (GSH) levels

The tissue GSH levels was determined by the method of Beutler and Kelly [24]. Virtually all the non-protein sulfhydryl groups of tissues are in the form of reduced GSH. 0.2 ml of tissue homogenate was mixed with 1.8 ml of EDTA solution. To this, 3.0 ml precipitating reagent (1.67 g of metaphosphoric acid, 0.2 g of EDTA disodium salt, 30 g sodium chloride in 1000 ml of distilled water, after precipitating proteins with TCA was added, mixed thoroughly, and kept for 5 min before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of 0.3 M disodium hydrogen phosphate solution and 1.0 ml of DTNB reagent were added and the absorbance was read at 412 nm.

Assay of Superoxide dismutase (SOD)

The activity of SOD in tissue was assayed by the method of Kakkar [25]. The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.025 mol/L), 0.1 ml phenazine methosulfate (186 nmol/L), 0.3 ml nitro blue tetrazolium (300 mmol/L), 0.2 ml NADH (780 mmol/L) and diluted enzyme preparation and water in a total volume of 3.0 ml. After incubation at 30°C for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml *n*-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against *n*-butanol.

Assay of Catalase (CAT)

Catalase was assayed according to the method of Maehly and Chance [26]. The estimation was done spectrophotometrically following the decrease in absorbance at 230 nm. The tissue was homogenized in M/150 phosphate buffer (pH 7.0) at 1-4°C and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer (pH 7.0), 2 mM H_2O_2 and the enzyme extract. The specific activity of catalase is expressed in terms of units/mg protein. A unit is defined as the velocity constant per second.

Estimation of Total proteins

The protein content of tissue homogenates was measured by the method of Lowry [27]. 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10% TCA and centrifuged for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOH. From this an aliquot was taken for protein estimation. 0.1 ml of aliquot was mixed with 5.0 ml of alkaline copper reagent and allowed to stand at room temperature for 10 min. 0.5 mL of Folin's phenol reagent was added and the blue color developed was read after 20 min at 640 nm.

Statistical analysis

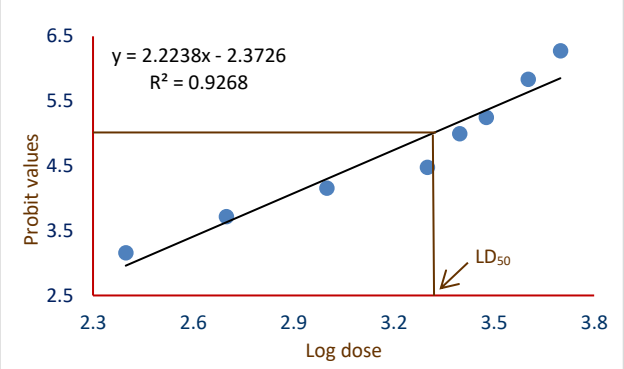
The experimental results were expressed as mean ± SEM. Data was assessed by ANOVA followed by the student's *t*-test, *p*-value < 0.05 was considered as statistically significant.

RESULTS

Acute toxicity

LD_{50} value of the MEPN was evaluated in Swiss albino mice. The drug was administered intraperitoneally. As shown in Fig. 1, the LD_{50} value of the MEPN was found to be 1400.27 mg/kg b.w.

Fig. 1: LD_{50} determination of methanol extract of *Phyllanthus niruri* (MEPN) in mice



Anticancer activity

Tumor growth response of MEPN on tumor and packed cell volume, viable and non-viable cell count and % increase in life span.

Antitumor activity of MEPN against EAC tumor bearing mice was assessed by the parameters such as tumor volume, packed cell volume, cell count (viable and non-viable), mean survival time, and % increase life span. The results are illustrated in Table 1. The tumor volume, packed cell volume and viable cell count were found to be significant ($p < 0.01$) increased and non-viable cell count was significantly ($p < 0.01$) low in EAC control animals when compared with normal control animals. Administration of MEPN at the dose of 125 and 250 mg/kg significantly ($p < 0.05$) decreased the tumour volume, packed cell volume and viable cell count. Non-viable cell count was significantly ($p < 0.05$) higher in MEPN treated animals when compared with EAC control

animals. Furthermore, the mean survival time was increased to 26.67 ± 1.41 (% ILS = 20.36) and 32.15 ± 0.35 (% ILS = 45.24) on administration of MEPN at 125 and 250 mg/kg, respectively. Finally, the change in body weights (Table 2) of the animals suggests the tumor growth inhibiting property of MEPN. All these results clearly indicate that the MEPN has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line in a dose-dependent manner in experimental animals.

Hematological parameters

Hematological parameters (Table 3) of tumor bearing mice on day 14 were found to be significantly altered compared to the normal group. The total WBC count was found to be increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. In differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval on MEMP 250mg/kg treatment restored in all the altered hematological parameters to almost near normal. MEPN 125 mg/kg treatment also recovered these altered depleted parameters towards normal though MEPN 250mg treatment was found to be more effective.

Table 1: Effect of methanol extract of *Phyllanthus niruri* (MEPN) on tumor volume, packed cell volume, tumor cell count, mean survival time and % increase in life span in EAC tumor bearing mice.

Treatment	Dose	Tumor Volume (In ml.)	Packed cell volume (in ml.)	Tumor cell count (2×10^6 cells)		Mean survival time (in days)	% increase in life span (in days)
				Viable	Nonviable		
Normal	0.9%NaCl w/v, 5ml/kg	-	-	-	-	-	-
EAC	2×10^6 cells/mouse	3.12±0.20	1.99±0.12	8.91±0.45	0.33±0.31	22.11±2.05	-
EAC + MEPN	125mg/kg	2.41±0.27*	1.38±0.18*	4.86±0.36*	0.59±0.33*	26.67±3.41*	20.36#
EAC + MEPN	250mg/kg	1.90±0.32#	0.67±0.21#	2.75±0.19#	0.47±0.77#	32.15±4.35#	45.24#
EAC + 5-FU	20mg/kg	0.8 5±0.11#	0.30±0.14#	0.95±0.43#	0.13±0.28#	37.79±5.10#	70.58*

(Values are mean ± SEM, n = 8); #$P < 0.01$ Statistically highly significant when compared with EAC control group.
*$P < 0.05$ Statistically significant when compared with EAC control group.

Table 2: Effect of methanol extract of *Phyllanthus niruri* (MEPN) on body weight of EAC tumor bearing mice.

Treatment	Body weight (in g)						
	Initial (day 0)	Day 5	Day 10	Day 15	Day 25	Day 35	Day 45
Normal (0.9%NaCl w/v.)	18.53±2.75	18.75±1.50	18.75±2.00	19.00±3.00	19.50±3.00	21.25±3.00	22.75±2.50
EAC (2 x 10^6 cells)	19.00±1.50	19.75±1.50	21.25±1.50	23.75±2.00	-	-	-
EAC + MEPN (125mg/kg)	19.50±1.75[a]	21.00±1.50[a]	22.25±1.50[a]	23.00±1.75[a]	22.25±1.75[a]	-	-
EAC + MEPN (250mg/kg)	19.50±2.50[a]	21.25±2.50[a]	21.00±1.50[a]	20.75±2.00[a]	20.75±2.00[a]	-	-
EAC + 5-FU (20mg/kg)	19.00±2.25[a]	19.25±1.75[a]	20.50±2.00[a]	21.50±2.50[a]	21.00±3.00[a]	20.50±2.50[a]	-

(Values are mean ± SEM, n = 8); [a]$P < 0.05$ Statistically significant when compared with EAC control.

Table 3: Effect of methanol extract of *Phyllanthus niruri* (MEPN) on hematological profile of EAC tumor bearing mice.

Treatment	Hb Content (In g)	RBC (Cells × 10^6)	WBC (Cells × 10^3)	Differential count		
				Neutrophils (%)	Monocytes (%)	Lymphocytes (%)
Normal (0.9% NaCl w/v.)	13.58±0.53	5.33±0.72	7.88±0.81	68.00±4.33	2.20±0.35	27.22±4.11
EAC (2×10^6 cells/mouse)	10.00±0.72	3.95±0.80	15.49±0.69	35.00±3.52	1.62±0.25	65.25±4.47
EAC + *MEMP* (125mg/kg)	11.13±2.41[a]	3.90±0.67[a]	13.62±2.29[a]	48.00±3.79[a]	1.53±0.37[a]	54.55±5.34[a]
EAC + *MEMP* (250mg/kg)	12.21±2.83[a]	4.32±1.62[a]	11.90±2.25[a]	61.00±4.22[a]	1.97±0.48[a]	37.11±3.53[a]
5-Fluorouracil (20 mg/kg)	13.10±2.73[a]	4.43±0.72[a]	8.51±3.04[a]	64.00±3.29[a]	1.70±0.8[a]	30.23±4.17[a]

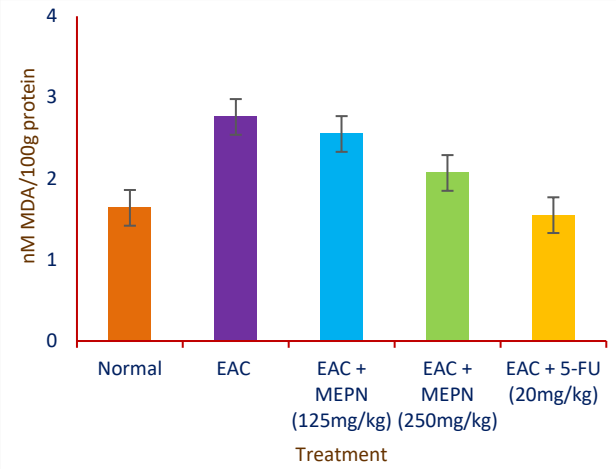
(Values are mean ± SEM, n = 8); [a]$P < 0.05$ Statistically significant when compared with EAC control group.

Antitumor effect of MEPN on thiobarbituric acid (TBARS) levels

Reactive oxygen species (ROS) in cancer tissues results in lipid peroxidation and subsequently to increase in malondialdehyde (MDA) level. Fig. 2 depicts the levels of TBARS in liver tissue of experimental animals. In the present study, the levels of MDA were significantly ($p < 0.01$) increased in EAC control animals when compared with normal control animals. After treatment with MEPN at 125 mg and 250 mg/kg and 5-FU at 20 mg/kg significantly ($p < 0.05$) reduced the MDA levels when

compared with EAC control animals. Results were expressed as nmoles MDA/mg proteins/ml.

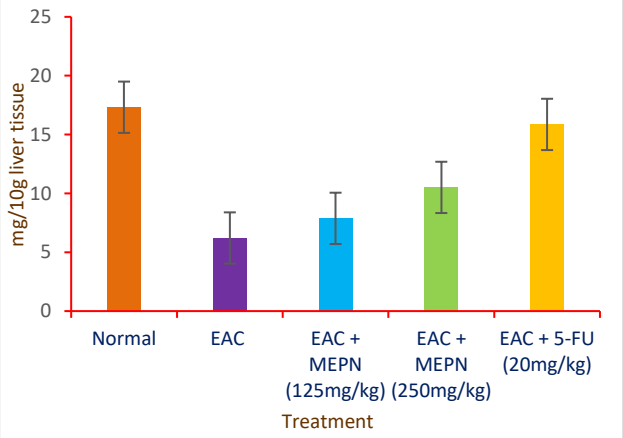
Fig. 2: Effect of MEPN on Lipid peroxidation



Antitumor effect of MEPN on Reduced glutathione levels

Fig. 3 depicts the levels of reduced GSH in experimental groups. The levels of reduced GSH were significantly ($p < 0.01$) decreased in EAC control group when compared with normal control group. The levels of reduced GSH were found to be increased on administration of MEPN at 125 mg and 250 mg/kg and 5-FU at 20 mg/kg when compared with EAC control group.

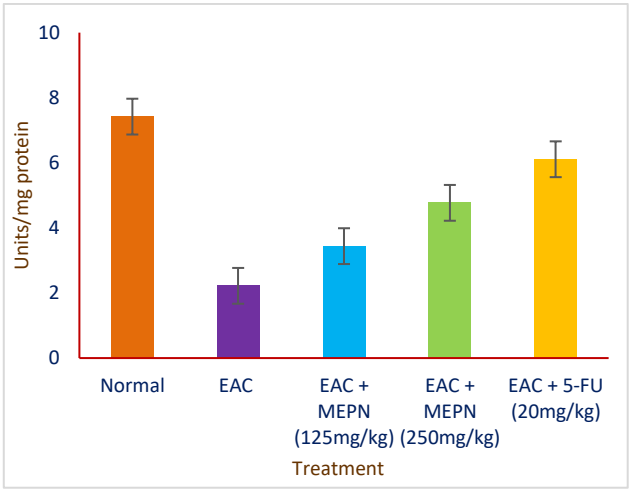
Fig. 3: Effect of MEPN on GSH levels



Antitumor effect of MEPN on superoxide dismutase (SOD) levels

Fig. 4 demonstrates the activity of SOD in liver tissue of experimental groups. There was a significant ($p < 0.01$) reduction in the levels of liver SOD in EAC control animals. Administration of MEPN at 125 mg and 250 mg/kg and 5-FU 20 mg/kg increased the levels significantly ($p < 0.05$) as compared with EAC control animals.

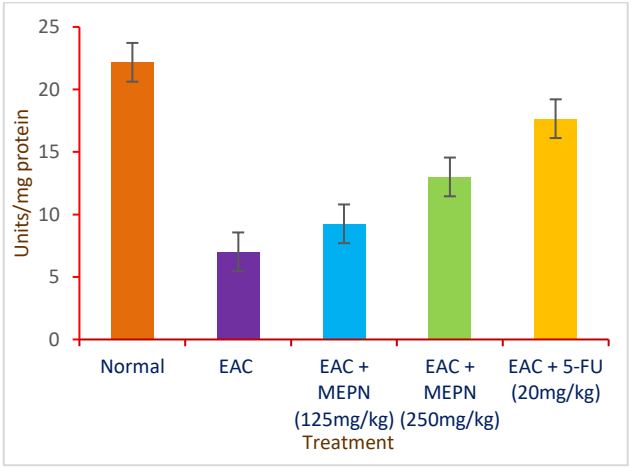
Fig. 4: Effect of MEPN on SOD levels



Antitumor effect of MEPN on catalase (CAT) levels

Fig. 5 shows the activity of catalase (CAT) in liver tissue of experimental mice. A significant ($p < 0.01$) reduction was observed in the activity of catalase in EAC control groups. Feeding with MEPN at 125 mg and 250 mg/kg and 5-FU 20 mg/kg increased the levels significantly ($p < 0.05$) as compared with EAC control mice.

Fig. 5: Effect of MEPN on CAT levels



DISCUSSION

The present study was carried out to evaluate the antitumor activity and antioxidant status of methanol extract of *Phyllanthus niruri* (MEPN) in EAC tumor bearing mice. The MEPN treated animals at the doses of 125 and 250 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor (viable) cell count, and brought back the hematological parameters to more or less normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor-bearing mice to near normal levels.

In EAC tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [28]. Treatment with MEPN inhibited the tumor volume, viable tumor cell count, and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [29]. It may be concluded that MEPN by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC-bearing mice. Thus, MEPN has antitumor activity against EAC bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [30]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [31]. Treatment with MEPN brought back the hemoglobin (Hb) content, RBC, and WBC count more or less to normal levels. This clearly indicates that MEPN possess protective action on the hemopoietic system.

Malonaldehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals [32], which is accepted as an indicator of lipid peroxidation [33]. MDA, the end product of lipid peroxidation, was reported to be higher in cancer tissues than in non-diseased organ [34]. Our findings indicate that TBARS levels in the tested cancerous tissues are higher than those in normal tissues. These results are in agreement with the published data [35 & 36]. This emphasizes the reduction in free radical yield and the subsequent decrease in harm and damage to the cell membrane and decrease in MDA production.

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in particularly high concentration in the liver and is known to have a key function in the protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage to macromolecules, for example, lipid peroxidation *in vivo* [37]. It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially the liver, even when the site of the tumor does not interfere directly with organ function [38]. In our study, GSH levels in experimental mice were found to be significantly lower than that in the EAC control mice.

SOD, CAT, ang Glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H_2O_2). SOD catalyses the diminution of superoxide into H2O2, which has to be eliminated by glutathione peroxidase and/or catalase [39]. Further, it has been reported that a decrease in SOD activity in EAC bearing mice may be due to loss of Mn^{2+} containing Sod activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver [40]. A small amount of catalase (CAT) in tumor cells was reported [40]. The inhibition of SOD and CAT activities as a result of tumor growth were also reported [41]. Similar findings were observed in our present study in EAC bearing mice. The administration of MEPN at two different doses significantly increased the SOD and CAT levels in a dose dependent manner. It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells [42] and antitumor activity in experimental animals [43]. Antitumor activity of these antioxidants is either through induction of apoptosis [44] or by inhibition of neovascularization [45]. The implication of free radicals in tumors is well documented [46 & 47]. The free radical hypothesis supported the fact that the antioxidants effectively inhibit the tumor, and the observed properties may be attributed to the antioxidant and antitumor principles present in the plant extract.

In conclusion, the present investigation demonstrates that the methanol extract of *Phyllanthus niruri* (MEPN) increased the life span of EAC tumor bearing mice and decreased lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. All these parameters suggest that the methanol extract of *Phyllanthus niruri* seeds exhibits potential antitumor and antioxidant activities.

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