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Evaluation of Anti-Cancer Activity of Methanol Extract of *Nyctanthes arbor- tristis* Linn.

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ABSTRACT

Nyctanthes arbor tristis Linn Family-Oleaceae, is a well-known plant in India. The Methanol Extract (ME) of its leaves was prepared and injected into intraperitoneal route (200 and 400mg/Kg body weight) of Swiss Albino mice to observe anti-cancer activity against Ehrlich Ascites Carcinoma (EAC) cells (10^7cells/mouse). 5-Fluorouracil (5-FU) 20mg/kg body weight (b.w.) was administered into intraperitoneal (i.p.) route as standard Anti-cancer drug. After strictly maintaining the standard experimental protocol; EAC cell count (cancer cell), total tumor volume, percentage inhibition of total cell count, percentage inhibition of tumor volume, percentage inhibition of viable and non- viable cells, percentage increase in life span(%ILS) and Hematological parameter (WBC, RBC, Hb) were evaluated. These evaluated parameters were comparing with EAC control group. A significant decrease of EAC cell count, viable cell count, percentage inhibition of total cell count, percentage inhibition of tumor volume, percentage increase of life span (%ILS), percentage increase of non-viable cell count, increase of RBC count and Hemoglobin level were observed. The above evaluations of present study suggest that Methanol Extact of *Nytanthes arbor- tristis* leaves has an Anti-cancer activity against Ehrlich Ascites Carcinoma cells.

Keywords: *Nyctanthes arbor –tristis* Linn.(NA),Ehrlich Ascites Carcinoma, Methanol Extract of NA (MENA)

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INTRODUCTION

Cancer is the second leading cause of death throughout the world. According to the report of WHO, 2015 about 8.8 million deaths occur due to cancer. About 1in 6 deaths is caused by cancer according to the global scenario. Approximately 70% death occurs for the various types of cancer in Lung (69 million deaths), Liver (788000 deaths), Colorectal (774000), Breast (571000 deaths) in low and middle income group of people [1]. According to the statistical data of American Cancer Society (ACS), there will be 1,688,780 new cancer cases will be diagnosed and 600,920 cancer death may occur in the end of the year 2017[2].

Treatment of cancer is done by surgery, radiation and chemotherapy. Modern synthetic Anti-cancer drugs have maximum cost and cytotoxic effect. The people of low and middle income group of countries are maximum affected by Non-communicable diseases (NCDs) [3]. They are unable to continue the chemotherapy of cancer because of their economic problem. So we have to search phytochemical compound from natural sources having low cost and minimum cytotoxicity for the treatment of cancer of maximum affected class of people. Generally four types of phytochemical compounds are used in the United States in cancer chemotherapy. They are Catharanthus (Vinca) alkaloids (Vincristine, Vinblastin, Vinorelbin); the taxenes (docetaxel and paclitaxel), Campothecin derivatives (irinotecan and topotecan); the epipodophyllotoxine (etoposide, teniposide, etoposide phosphate) [4].

Nyctanthes arbor- tristis L. is a plant familiar as various vernacular name like Sephalika (Bengali), Parijatha (Sanskrit), Harisingar (Hindi), Night Jasmine (English), Gangasiuli (Oriya), Parijatakam (Malayalam), Parijathak (Marathi) [5]. The plant is a shrub of approximately ten meter height; bark is grey and rough; leaves are simple, rough hairy; phyllotaxy-opposite decussate; venation-reticulate unicostate; margin-entire; upper surface of the lamina is deep green and lower surface is light green [6].Fragrant flowers are white in color, five to eight lobes, corolla is orange colored centrally and corolla tube is bright orange color containing nyctanthin [7].

This plant grows in India, Pakistan, Nepal, Bangladesh, and Thailand [8]. Leaves, flowers, seeds, stem and bark of NA contain biologically active chemical compounds like phytosterols, flavonoids, glycosides, alkaloids and saponins etc.[9, 10]which have therapeutic value. They are anti-inflammatory [11], hepatoprotective 12], antipyretic, antioxidant [13], diuretic, anti-filarial [14], anthelmintic, antibacterial [15], anti-leishmanial [16,17]. The present study is to evaluate the anti-cancer activity of the Methanol leaf extract of the plant *Nyctanthes arbor- tristis* L. (MENA).

MATERIALS AND METHODS

The twig with leaves and flowers of the plant was collected from the medicinal garden of Institute of Pharmacy, Kalyani, Nadia. A herbarium was prepared and identified as *Nyctanthes arbor- tristis* Linn, Family-Oleaceae in Central National Herbarium, Shibpur, Howrah, India (BSI, Howrah), with their certificate no. CNH/31/2013/Tech/1065. The leaves of NA plant were collected and dried under shade at room temperature. The dried leaves1.5 kg was grinded into coarse powder with the help of mechanical mixer. The powder of organized crude drug was extracted by multiple maceration using methanol as menstruum. The extract was evaporated under reduced pressure using Rotary Evaporator. Then concentrated extract was lyophilized and 80 gm solid extract was obtained. This extract was stored in an air tight container in refrigerator.

Animals

The anti-cancer in vivo study was carried out on the male Swiss Albino mice weighing 20±2gafter obtaining the permission from Animal Ethics Committee of Jadavpur University (permission letter Ref. No. AEC/PHARM/1502/12/2015).Animal experiment was carried out as per the guide line of CPCEA (Govt. of India) and UGC, India. The animals were kept under standard environmental condition, fed with standard laboratory diet and water *ad libitum.*

Acute toxicity study

The acute toxicity study of MENA in male Swiss albino mice was done as per the OECD guideline. No mortality of the mice was observed even at a dose of 2000 mg/kg and therefore considers as safe.

Study of Anti-tumor activity

The mice were divided into five groups. Each group containing 6 mice.

Group I: Normal control (Propylene glycol 5ml/kg b.w.)
Group II: EAC control (10^7cells/mouse)
Group III: Test Drug (MENA 200 mg/kg b.w.)
Group IV: Test Drug (MENA 400 mg/kg b.w.)
Group V: Standard Drug (5- Fluorouracil 20mg/kg b.w.)

EAC cells were obtained from Chittaranjan National Cancer Research Centre (CNCRI), Kolkata, India. The EAC cells were sub cultured weekly by intraperitoneal injection of 10^7 cells/mouse. On day '0', 10^7EAC cells were inoculated i.p.to all the mice of groups (II), (III), (IV) and (V). MENA was suspended with propylene glycol 80 and two doses (200mg/kg and 400mg/kg) were injected i.p. to the mice of groups (III) and (IV) just after 24 hours of inoculation of EAC cells. The standard Anti-cancer drug 5-(FU) 20mg/kg b.w. was injected i.p. to the mice of group (V). Propylene glycol 5ml/kg b.w. was injected i.p. to the mice of Group I. This treatment was continued for 9 days. The development of ascitic tumor in the mice were observed. On the tenth day, the mice were sacrificed and total number of EAC cells, tumor volume, percentage inhibition of total cell count, percentage inhibition of tumor volume, total count, viable and non-viable cell count, percentage inhibition of viable cells, increase of the life span (ILS) were evaluated [18-20].

Study of Hematological Parameters

On the eleventh day, blood was collected from the heart of the sacrificed mice from each group and WBC, RBC count and Hb level was determined. The parameters of EAC group (II) were compared to the other groups for statistical analysis [18-20].

Statistical Analysis

All data are expressed as mean ± SEM. (n = 6 mice per group). The data were analyzed by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's Multiple Comparison test.

RESULT AND DISCUSSION

The $L.D_{50}$ value of the Methanol leaf extracts of *Nyctanthes arbor- tristis* L was carried out as per the OECD guideline but no mortality of the mice was observed even at a dose of 2000 mg/kg and therefore considered as safe.

The anticancer property of MENA was evaluated by their ability to inhibit cancer cell growth in ascitic fluid of Swiss albino mice. Various parameters like percentage inhibition of tumor volume (%TVI), percentage inhibition of total cell count (%TCI), percentage inhibition of viable and nonviable cell count, percentage increase in life span (%ILS) and hematological parameters were taken to be considered to establish the potency of the anticancer property of MENA.

Administrations of MENA at the doses of 200 mg /kg b.w. and 400 mg /kg b.w. significantly reduced the total cell count and tumor volume when compared to EAC control group. Percentage inhibitions of total cell count (%TCI), percentage inhibition of tumor volume (%TVI) were observed as 84.75% and 75.52% respectively at the dose of 200 mg/kg b.w. of MENA. Similarly, percentage inhibitions of total cell count (%TCI), percentage inhibition of tumor volume (%TVI) were observed as 90.36% and 96.37% respectively at the dose of 400 mg/kg b.w. of MENA. (Table 1, Figure 1 & 2). Administration of MENA at the doses of 200 mg /kg b.w. and 400 mg /kg b.w. significantly ($P < 0.01$) decreased the viable cell count (Table 2, Figure 4). Non-viable cell count was significantly ($P < 0.05$) increased in MENA treated animals when compared with EAC control animals (Table 2, Figure 3).

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Table 1: Anticancer activity of MENA against EAC bearing mice (Total EAC cells count, total tumor volume, percentage inhibition of total cell count and percentage inhibition tumor volume)

Group	Compound	Total cell count ($x10^7$)	%TCI	Tumor volume (mL)	%TVI
I	Normal	----	----	---	-----
II	EAC + Control	7.617± 0.078	0.00	6.89± 0.16	0.00
III	EAC + MENA(200mg/kg)	1.161± 0.095	84.75	1.66± 0.283	75.52
IV	EAC + MENA(400mg/kg)	0.7338 ± 0.0164	90.36	0.2495± 0.034	96.37
V	EAC + 5-FU	0.5992± 0.006	92.30	0.15±0.030	97.88

Each value represents the mean ± SEM (n = 6 mice per group). Experimental groups were compared with EAC control group ($P < 0.01$).

Table 2: Anticancer activity of MENA against EAC bearing mice (Total Count of viable, non-viable and % inhibition of viable, non-viable)

Group	Compound	Viable cell count ($x10^7$)	Non-viable cell count ($x10^7$)	% of viable cells	% of non-viable Cells
I	Normal	----	----	----	----
II	EAC + Control	6.903±0.049	0.711±0.097	90.62	9.33
III	EAC + MENA(200mg/kg)	0.794±0.064*	0.3665± 0.1345**	68.38	31.52
IV	EAC + MENA(400mg/kg)	0.3790±0.044*	0.354± 0.048**	51.64	48.24
V	EAC + 5-FU	0.1835±0.022*	0.415± 0.023***	30.62	69.28

Each value represents the mean ± SEM (n = 6 mice per group).
*Experimental groups were compared with EAC control group ($P < 0.01$).
**Experimental groups were compared with EAC control group ($P < 0.05$).
***Experimental groups were compared with EAC control group ($P > 0.05$).

Table 3: Percentage increase in life span (%ILS)

Group	Compound	MST (in days)	% ILS
I	Normal	----	----
II	EAC + Control	19.35	0.00
III	EAC + MENA(200mg/kg)	29.44	52.14
IV	EAC + MENA(400mg/kg)	35.22	82.01
V	EAC + 5-FU	43.33	123.92

Table 4: Haematological parameters- WBC, RBC and Haemoglobin content

Group	Compound	WBC count ($x10^9$/L)	RBC count (10^{12}/L)	Haemoglobin (g/dL)
I	Normal	5.415± 0.189	10.19±0.285	14.29±0.168
II	EAC + Control	17.88± 0.378	4.045± 0.222	5.117±0.2065
III	EAC + MENA(200mg/kg)	8.818± 0.4164	6.795± 0.2452	8.434±0.1687
IV	EAC + MENA(400mg/kg)	7.082 ±0.04688	8.564 ± 0.2140	12.05±0.2149
V	EAC + 5-FU	6.423±0.1601	9.020 ± 0.095	13.44±0.120

Each value represents the mean ± SEM (n = 6 mice per group).
Experimental groups were compared with EAC control group ($P < 0.01$).

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One of the most reliable criteria for judging the efficiency of any anticancer drug is the prolongation of the life span of animals. Life span was also increased in MENA treated animals (group II and group III) when compared with EAC control animals (Group II) (Table 3, Figure 5).

Treatment with MENA at the doses of 200 mg/kg b.w. and 400mg/kg b.w.in EAC bearing mice significantly ($P < 0.01$) increased both the level of RBC and hemoglobin (Hb) while significantly ($P < 0.01$) reduced level of WBC when compared with EAC control group (Table 4, Figure 6, 7, 8).

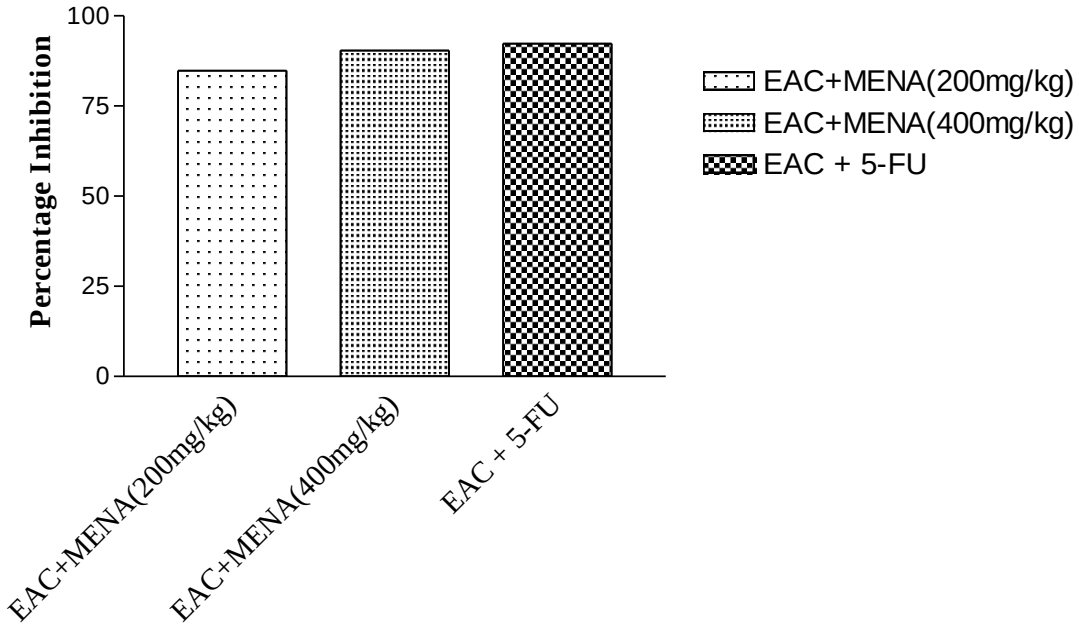

Figure 1: Percentage Inhibition of Total Cell Count

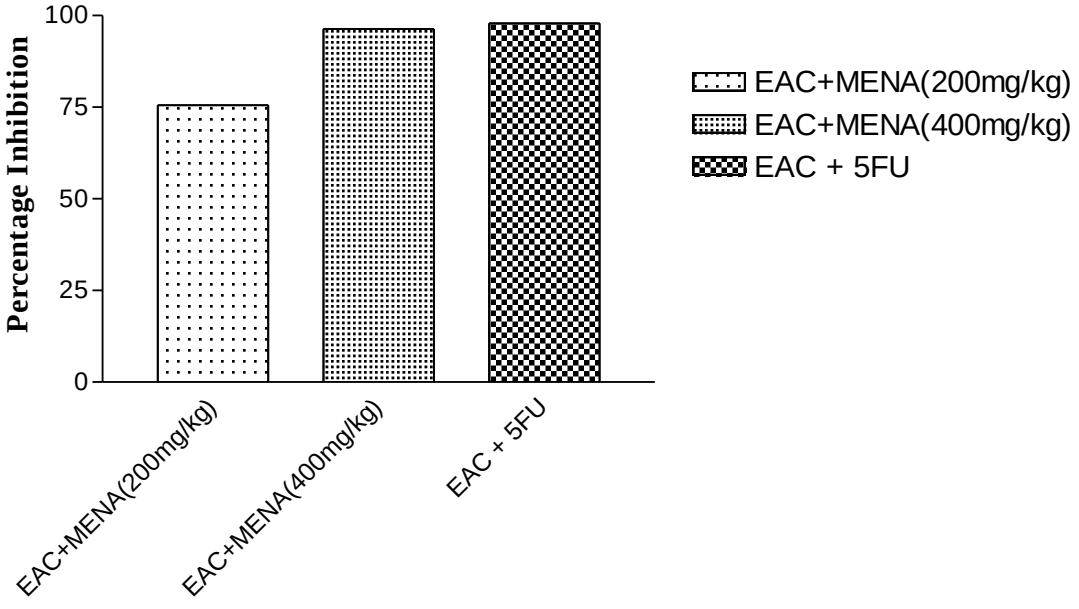

Figure 2: Percentage Inhibition of Tumor Volume

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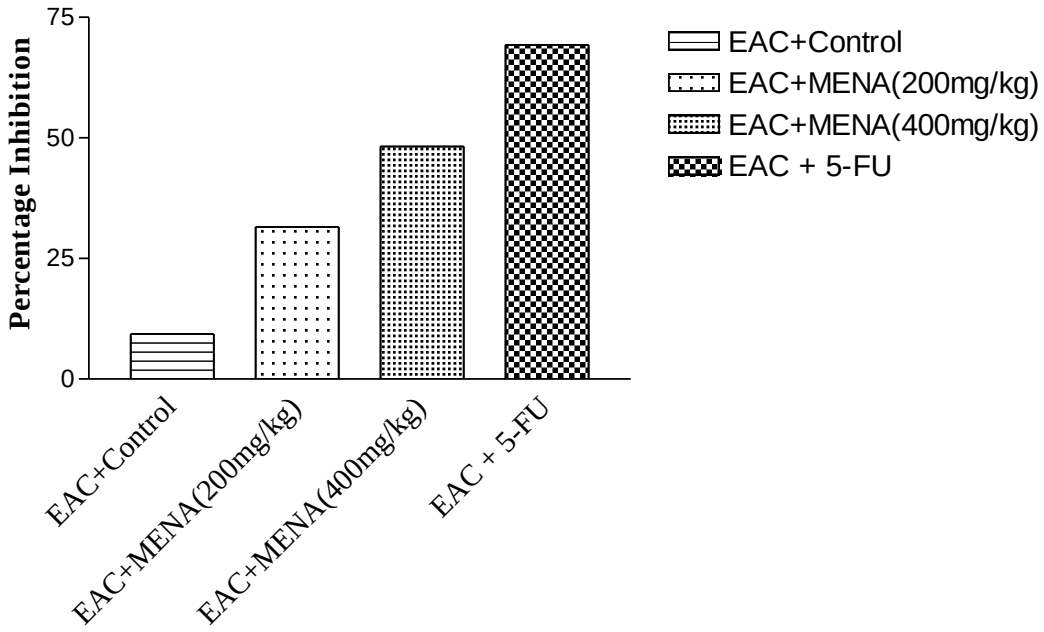

Figure 3:Percentage Inhibition of Nonviable Cell Count

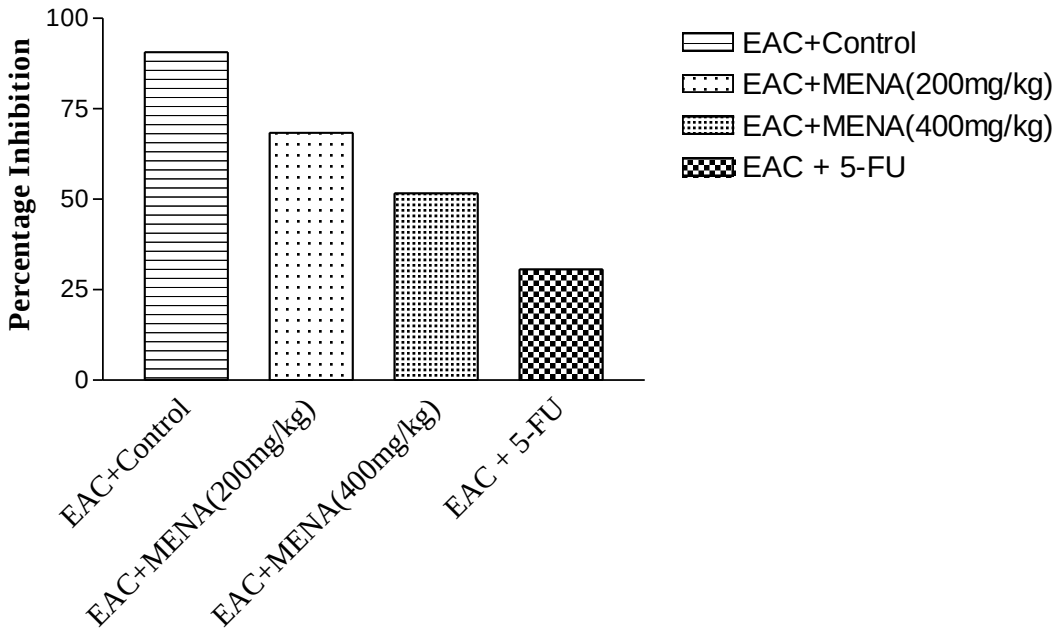

Figure 4: Percentage Inhibition of Viable Cell Count

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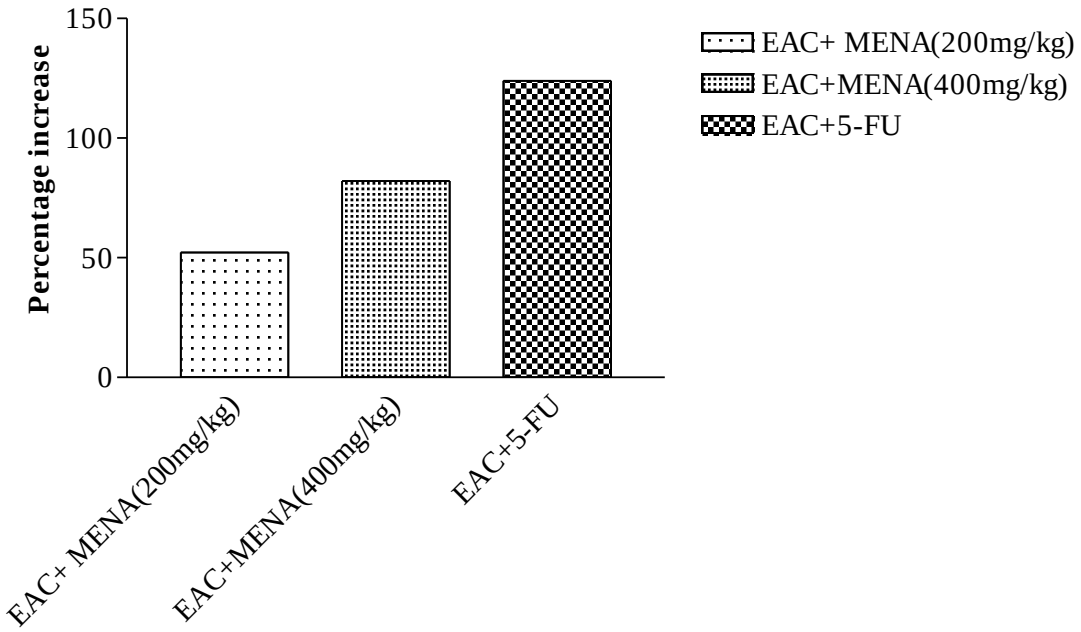

Figure 5: Percentage increase in life span (%ILS)

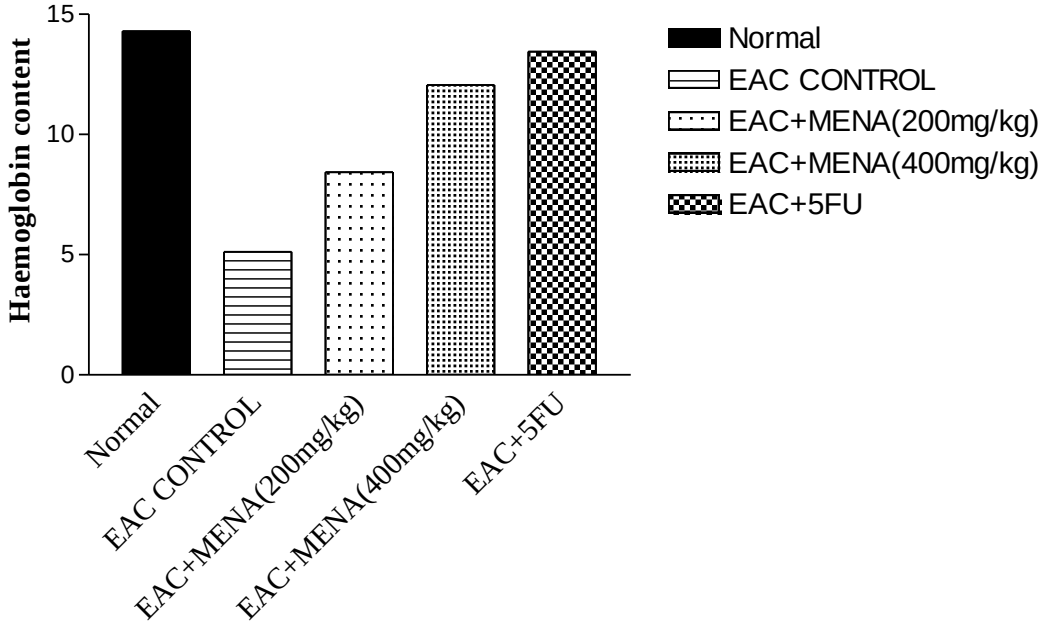

Figure 6: Haemoglobin

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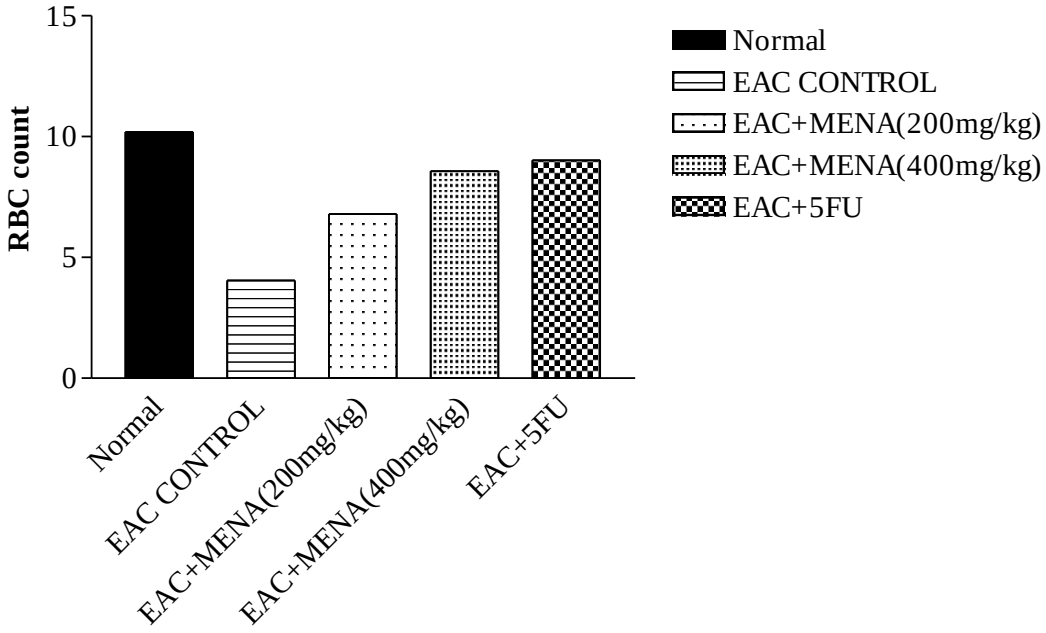

Figure 7: Red Blood Cell

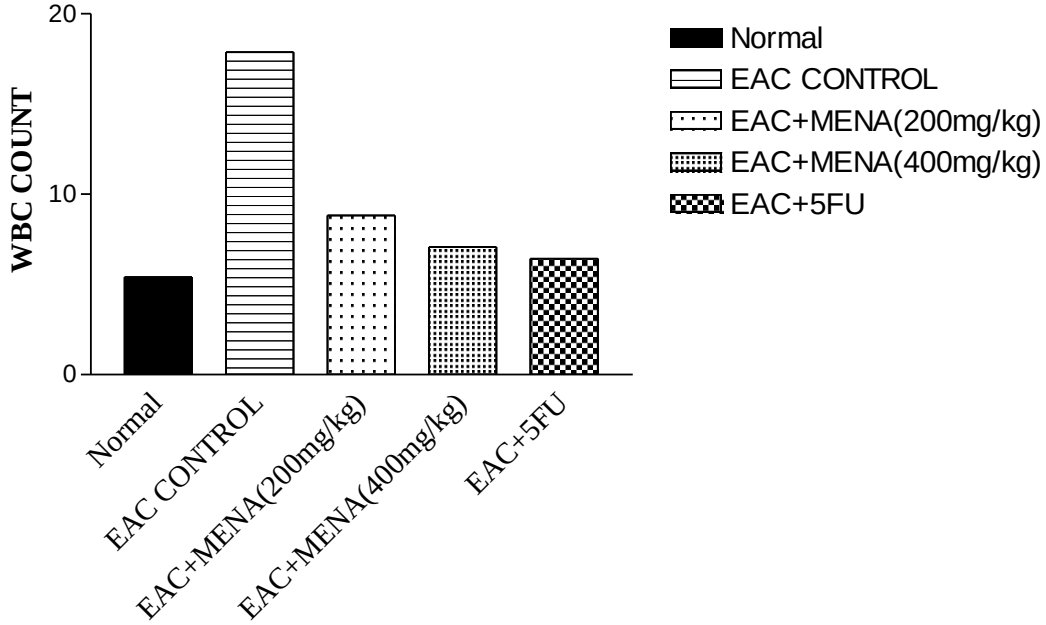

Figure 8: White Blood Cell

CONCLUSION

In the present investigation it was noted that MENA significantly reduced tumor volume, viable cells and normalized the hematological profiles, increasing life span as compared with EAC control group. These findings indicate that methanol extract of *Nyctanthes arbor- tristis* Linn leaves have anti-cancer activity.

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