CYTOTOXIC AND ANTITUMOR ACTIVITIES OF *PAVONIA ODORATA* AGAINST ERLICH'S ASCITES CARCINOMA CELLS BEARING MICE

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Running Title

Antitumor effect of MEPO against EAC bearing mice

Summary

Anticancer activity of the two different doses (200 and 400 mg/kg) of the methanol extract from *Pavonia odorata* (MEPO), family: Malvaceae in the mice transplanted with Erlich's ascites carcinoma (EAC) was studied. The methanol extract was further fractionated through successive solvent fractions and antitumor effect was evaluated at the doses of 140 mg/kg for chloroform fraction, 80 mg/kg for ethyl acetate fraction and 180 mg/kg for remaining hydroalcoholic fraction. 5-fluorouracil (20 mg/kg) was used as standard drug. Ethyl acetate and hydro alcoholic fractions showed *in vitro* cytotoxicity against EAC cells. Among the three fractions, hydroalcoholic and ethyl acetate fractions significantly inhibited the ascites tumor in EAC bearing mice after 9 days treatment. The average survival time (AST) and median survival time (MST) of MEPO (400 mg/kg) were 47.5 & 48.5 and for hydroalcoholic and ethyl acetate fractions were 48.3 & 49.5 and 46.2 & 47.0 respectively. Lipidperoxidation (LPx), glutathione (GSH) and activity of enzymatic antioxidant such as superoxide dismutase (SOD) and catalase (CAT) were evaluated in liver of EAC bearing mice. EAC bearing mice showed marked elevation in LPx level (541.21 ± 5.13) with decline in GSH content (23.54 ± 2.18) and enzymatic antioxidant level. Nine days treatment with MEPO and its hydroalcohlic; ethyl acetate fractions significantly inhibited LPx and restored the GSH; enzymatic antioxidant level. Blood biochemical parameters (SGOT, SGPT and ALP) and bilirubin were elevated where as total protein declined in EAC

bearing mice and it was restored to normal with the treatment of MEPO and its three fractions of *pavonia odorata*. Anemia, reduced RBCs and increased WBCs were observed in EAC bearing mice and this hematological alteration was normalized by 9 days supplementation with MEPO and its fractions. Cytotoxic effect of the MEPO and the hydroalcoholic, and ethyl acetate fractions were evaluated and the IC_{50} values were 477.71, 654.57 and 339.20 µg/ml respectively. These experimental results revealed that the MEPO and its hydroalcoholic, and ethyl acetate fractions possessed cytotoxic and anticancer activity.

Key words

Antioxidant; antitumor; cytotoxic; EAC; *pavonia odorata.*

Introduction

Cancer is considered one of the most common causes of morbidity and mortality worldwide. Most of the research carried worldwide focuses to find a way to prevent and treat the cancer. In the present day several methods exists for the treatment of cancer such as chemotherapy, radiotherapy and surgery. Among this chemotherapy is now considered as an efficient method for treatment of cancer. However, the most of the chemotherapeutic agents exhibit severe toxicity, resulting undesirable side effects [1]. Moreover many of the active molecules are highly expensive and teratogenic. Hence

there is a need to find alternative drugs which are highly effective, nontoxic. Plants are invaluable in the search of new drugs. There are tremendous historical legacies in folkore used of plant population in medicine [2]. Especially for cancer number of plants are used such as *catharanthus roseus, podophylum pellatum, P. emodi, Texus brevifolia* [3].

Pavonia odorata wild is an herb and roots have musk like aromatic odour. Roots and aerial parts are regarded in ayurveda as cooling, demulcent, carminative, diaphoretic, diuretic and fever. It is also used in inflammation, hemorrhage from internal organs. Leaves and young shoots were used as an emollient [4, 5, 6]. However, still there is no scientific report of this plant; it has been selected for its anticancer activity because of its traditional use. In the phytochemical analysis, MEPO showed the presence of terpenes and saponins more prominently and it gave positive results for flavonoids, glycosides, and carbohydrates. In this plant, the presence of sesquiterpene alcohol was already reported and called as pavonenol ($C_{15}H_{24}O$; m.p 52-55) [5]. The roots yield an essential oil that contains isovaleric acid, isovaleraldehyde, aromadendrene, pavonene, a-terpinene, azulene and pavonenol. The roots are aromatic, and possess refrigerant, antipyretic, stomachic and astringent properties. They are used in dysentery and inflammation and hemorrhage of the intestines [7].

Materials And Methods

Plant material

The whole plant of *Pavonia odorata* was collected from Kolli hills of Tamilnadu, India. The plant material was identified by the Botanical Survey of India, Kolkata, India and the voucher specimen (GMT-3) has been preserved in our research laboratory for future reference. The plant was dried under shade and powdered with a mechanical grinder. The powdered plant material was then passed through sieve # 40 and stored in an airtight container for future use.

Preparation of extract and fractions

The air-dried powdered plant material (1.5 Kg) was defatted with petroleum ether (60-80ºC) in a soxhlet extraction apparatus. The defatted plant material was extracted with methanol. The solvents were completely removed under reduced pressure to obtain a dry mass. The yields of the petroleum ether and methanol extracts were found to be 7 and 18 % w/w respectively. The concentrated methanol extract was dissolved in 1:1 ratio methanol and water. The dissolved methanol extract was successively fractionated through solvent extraction with chloroform, ethyl acetate. The remaining aqueous phase called R-H_2O Fr and the all fractions were concentrated and made it to dry mass. The percentage yield of chloroform fraction ($CHCl_3$ Fr.), ethyl acetate fraction (EtOAc Fr.) and remaining hydroalcoholic fraction (R-H_2O Fr.) of *Pavonia odorata* were 35%, 20% and 45% w/w respectively.

Phytochemical evaluation of the Extract

R-H_2O Fr and EtOAc Fr were subjected into the column and thin layer chromatographic study. R-H_2O Fr was added with excess of acetone, some brown colour mass was precipitated out. The precipitate was collected by centrifugation. Then it was purified by washing with acetone and it was found out containing mixture of saponins by TLC method using mobile phase butanol: acetic acid: water (4:1:2). The saponin mixture was subjected to column chromatographic study by using methanol and water (steep gradient elution method) as mobile phase. One single spotted brown colour amorphous powder was separated. It was identical as pavophylline with melting point (230°C), which was reported from *pavonia zeylanica* and separated by same method [8]. By other way R-H_2O Fr was carried out for column chromatographic study by using chloroform and methanol as mobile phase by steep

gradient elution method. The collected fractions were like brown oil and carried out for recoloumn chromatographic study. In the 7-15 fractions there was single spot, fine crystals and had pleasant odour. It may be a compound that has already been reported pavonenol, which have a pleasant odour [5]. EtOAc Fr showed positive for flavonoids when subjected to column chromatographic study using benzene and ethyl acetate as mobile phase. The collected fraction (10-17) gave intense yellow colour against the vapour of ammonia in TLC, which may be a flavonoid.

Preparation of test samples

The dried methanol extract (200, 400 mg /kg), fractions ($CHCl_3$ Fr. 140 mg/kg, EtOAc Fr. 80 mg/kg and R-H_2O Fr. 180 mg/kg) and reference drug 5-fluorouracil (20 mg/kg) were used in the experiment. Control group animal were administrated with normal saline.

Animals

Male Swiss albino mice weighing 20-25 g were used for the present investigation. They were housed in clean polypropylene cages and were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum.* The animals were acclimatized to laboratory condition for one week before start of experiment. All procedures described were reviewed and approved by the University animal ethical committee.

Acute Toxicity Study

Acute toxicity study was performed as per OECD-423 guidelines [9]. Swiss Albino mice (20-25 g) of either sex were used. The animals were fasted for 4 hours, but allowed

free access to tap water. The fasted mice were divided into two groups of six animals each. The group one received MEPO at the dose of 5 mg/kg.b.w of orally. The control group (group two) received a similar volume of distilled water (5 ml/kg). Mortality in each group was observed for 3 days. If mortality was not observed, the procedure was repeated for higher doses such as 50, 300 and 2000 mg/kg b.w.p.o.

Antitumor activity

Antitumor activity of the MEPO and its fractions were evaluated using ascites tumor models.

Experiment Protocol

Animals were divided into fifteen groups of six animals in each group. Groups I-VIII served for evaluating various parameters after sacrificing the animal and groups IX-XV served for determining the lifespan. All the groups were injected intraperitonially (i.p.) with $2x10^6$ viable EAC cells with ice – cold normal saline (0.9%) (aspirated from EAC bearing mice at the log phase $7–8^{th}$ day) except group I (served as normal control). After 24 hours of tumor inoculation MEPO (200, 400 mg /kg) and the fractions of MEPO ($CHCl_3$ Fr. 140 mg/kg, EtOAc Fr. 80 mg/kg and R-H_2O Fr. 180 mg/kg) were administered orally for 9 consecutive days to the group III-VII respectively. The similar treatment schedule was followed for group X-XIV. Group II and IX served as EAC control and receiving normal saline (0.9%NaCl; 10 ml/kg) and group I maintained as normal control and received normal saline (0.9%NaCl; 10 ml/kg). 5-fluorouracil (20 mg/kg body weight, i.p.) was administered to group VIII and XV and used as the standard reference drug. From the group I-VIII, blood was collected to evaluate the hematological and biochemical parameters and

in vivo antioxidant activity was determined in liver. The MST and AST were calculated as follows.

MST = (First death+ last death of animal)/2

AST =Sum of the animal death in different days/N

N=number of animals.

The mortality rate were noted in group IX-XV and the percent increase in life span (ILS) was calculated using the following formula

% IMLS= (1-T/C) x100 where T is mean survival time of treated group and C that of control group

% IALS= $(1-T_1/C_1)$ x100 where T_1 is average survival time of treated group and C_1 that of control group [10].

Tumor growth response

The effect of MEPO and its fractions on tumor growth were examined by studying the following parameters – tumor volume, tumor cell count and the percentage of the viable and nonviable cell count.

Hemoglobin and Biochemical estimation

Red blood cell count (RBC), haemoglobin content [11] and white blood cell count (WBC) [12] were measured from the blood obtained by intracardially. Serum glutamic oxaloacetic and glutamic pyruvic transaminase activities [13] and alkaline phosphatase [14] were determined. The total protein concentration and bilirubin were measured by the method of Lowry et al [15] and Oser [16].

In vivo antioxidant status

The antioxidant system was performed in liver and kidney. Lipid peroxidation was characterized by measuring the MDA [17] and reduced glutathione (GSH) level by Mulder et al

[18] method. Catalase activity and superoxide dismutase were assayed by the method of Luck [19] and Nishimiki et al [20] respectively.

Assay for *in vitro* cytotoxicity

The *in vitro* short term cytotoxicity of MEPO and its fractions were assayed using EAC cell lines. Briefly $1x10^6$ viable cells of cell line suspended in 0.1 ml of phosphate buffered saline (PBS) (0.2 M, pH 7.4) various concentrations of extracts (100-600 µg/ml) and phosphate buffer in a final volume of 1 ml were incubated at 37°C for 3 hours. After the incubation, the viability of the cells was determined by trypan blue exclusion method [21]. The percentage of cytotoxicity was determined by calculating IC_{50} values.

Statistical analysis

The statistical significance of differences between the groups was assessed by means of variance followed by Dunnett's tests. Values are expressed as mean ± S.E.M and p values less than 0.05 were considered as significance.

Results

Antitumor activity

The MLS and ALS were found approximately and it was 20.17 & 20.00 days respectively in the EAC control groups. After 24 hours of the EAC cells inoculation, treatment with MEPO and its different fractions significantly ($p>0.001$) increases the MST and AST. The MST of MEPO (400 mg/kg) and R-H_2O Fr were 47.50 & 48.33 respectively. The least MST was observed in the $CHCl_3$ Fr and MEPO (200

mg/kg) (27.83 & 29.67 respectively). By the treatment of MEPO (400 mg/kg) and R-H_2O Fr drastically increase the percentage of median life span (IMLS %-135.55) as well as percentage of average life span (IALS %-142.50). The 100% survival of animal were obtained from treatment R-H_2O Fr and 5-fluorouracil up to 40 days as compare to EAC control group (20 days) (Table 1).

In the EAC control groups, after the 9 days of inoculation the tumor volume increased very rapidly (4.93 ml). The proliferation of tumor cell was significantly inhibited by 9 days treatment with MEPO and its fractions. The MEPO (400 mg/kg), EtOAc Fr and R-H_2O Fr effectively prevented the development of tumor cells and the tumor volume was 1.20, 0.80 & 32.00 ml respectively. While the percentage ratio between viable and non viable cell count in the EAC control group was drastically increased and it was 75.22: 24.79% respectively. It was effectively reduced by MEPO (400 mg/kg), EtOAc Fr and the percentage ratio of viable and nonviable cells were 23.58: 76.42, 34.12: 65.88, and 32.70: 67.30% respectively (Table 2).

To study the hematological changes associated with the application of the MEPO, three parameters hemoglobin level, erythrocyte count and leukocyte count were determined sequentially in the peripheral blood of EAC bearing mice. The detailed hematological changes of normal and EAC-bearing mice expressed in Fig 1 and the hemoglobin levels in the EAC control group reduced significantly when compared to normal group. Thus, there was about 6.18 g% of hemoglobin in EAC control group and indicating that the animals were becoming anaemic ($p<0.001$). This level was restored to almost normal by the treatment with R-H_2O Fr (12.67 g %). Like the haemoglobin content, the erythrocyte count in the EAC control group ($2.7x10^6$/cu.mm) were also found to decrease gradually with the progression of tumours as expected, supporting earlier [22,23] observations. Thus, it was noted that there was an improvement in the count (4.55 & $4.53x10^6$/cu.mm) when supplemented with R-H_2O Fr and 5-fluorouracil.

A marked increase in circulating leukocytes was observed to be associated with the growth of different animal tumours [24]. Similar observations and trends were noted in

Table 1. Effect of the methanol extract of *Pavonia odorata* (MEPO) on life span in Erlich's ascites carcinoma bearing mice.

Groups	Survival Time (in days)				Survival percentage (%)				
	MST	%IMST	AST	%IAST	20days	30days	40days	50days	60days
EAC control	20.17	0	20.00	0	33.33	0	0	0	0
EAC+MEPO (200mg/kg)	29.67**	47.11	29.50**	47.50	100	16.66	0	0	0
EAC+MEPO (400mg/kg)	47.50**	135.55	48.50**	142.50	100	100	83.33	33.33	0
EAC+ R-H_2O Fr (180mg/kg)	48.33**	139.68	49.50**	147.50	100	100	100	33.33	0
EAC+EtOAc Fr (80mg/kg)	46.17**	128.93	47.00**	135.00	100	100	83.33	16.66	0
EAC+ $CHCl_3$ Fr (140mg/kg)	27.83*	38.02	27.50*	37.50	100	16.66	0	0	0
EAC+ 5-Flurouracil (20 mg/kg)	52.50**	160.34	52.50**	162.50	100	100	100	50	0

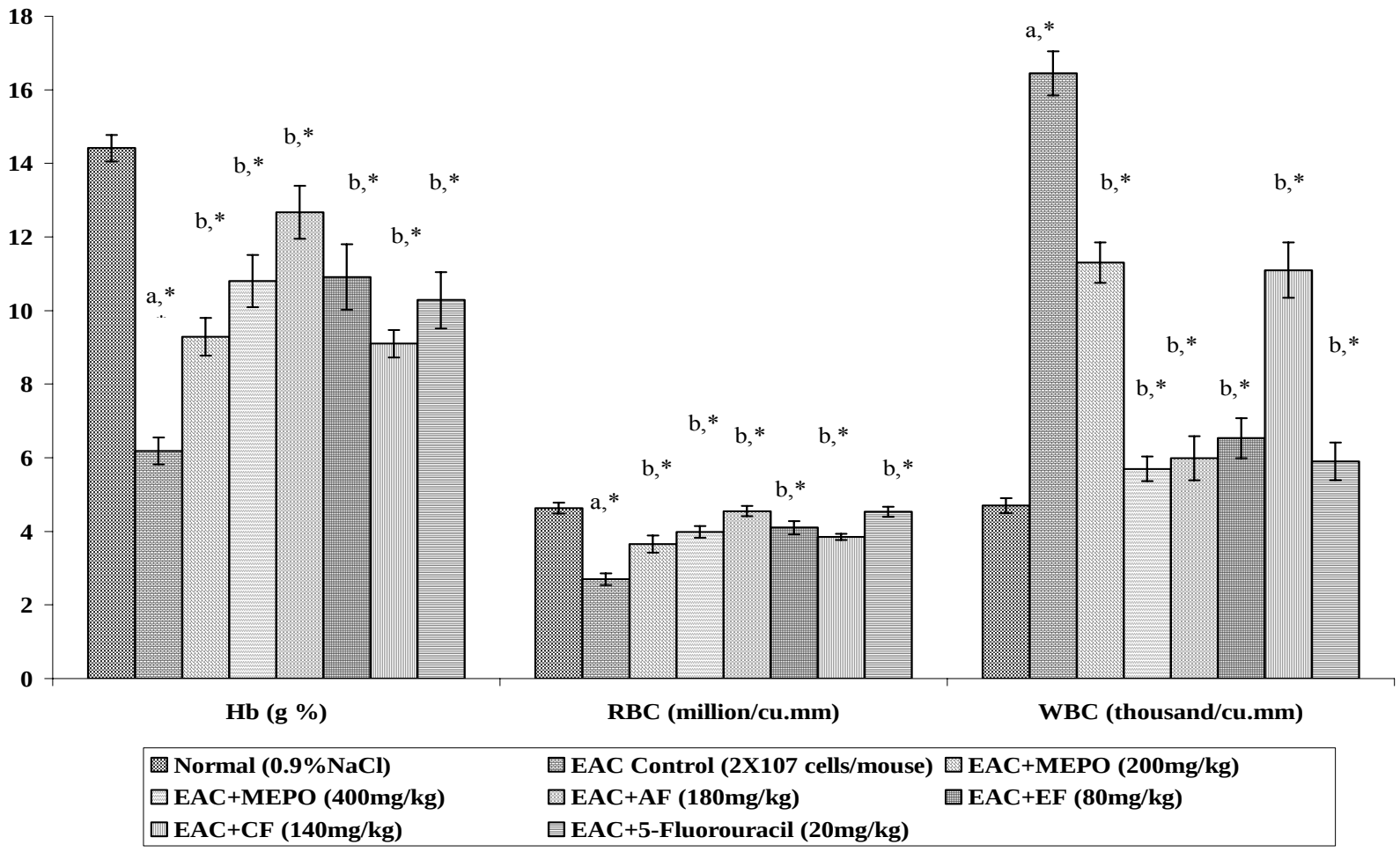
**p<0.001; *p<0.05 when compared to EAC control group where significance was performed by ANOVA followed by Dunnett's test. MST: median survival time; AST: average survival time; IMST: increase in median survival time; IAST: increase in average survival time.

Table 2. Effect of the methanol extract of *Pavonia odorata* (MEPO) on tumor volume and percentage ratio of viable and non viable cell in Erlich's ascites carcinoma bearing mice.
(Values are mean±SEM; six animals in each group)

Groups	Tumor volume (ml)	Viable cell count (%)	*Non viable cell count (%)*
EAC control	4.93±0.10	75.22±1.40	24.79±1.40
EAC+MEPO (200mg/kg)	3.45±0.04**	45.47±0.65**	54.54±0.65**
EAC+MEPO (400mg/kg)	1.20±0.10**	23.58±0.82**	76.42±0.82**
EAC+ R-H_2O Fr (180mg/kg)	2.00±0.09**	32.70±0.42**	67.30±0.42**
EAC+EtOAc Fr (80mg/kg)	0.83±0.15**	34.12±0.46**	65.88±0.46**
EAC+ $CHCl_3$ Fr (140mg/kg)	3.10±0.05**	42.55±0.47**	57.45±0.47**
EAC+5-Flurouracil (20 mg/kg)	0.28±0.05**	12.63±0.49**	87.37±0.49**

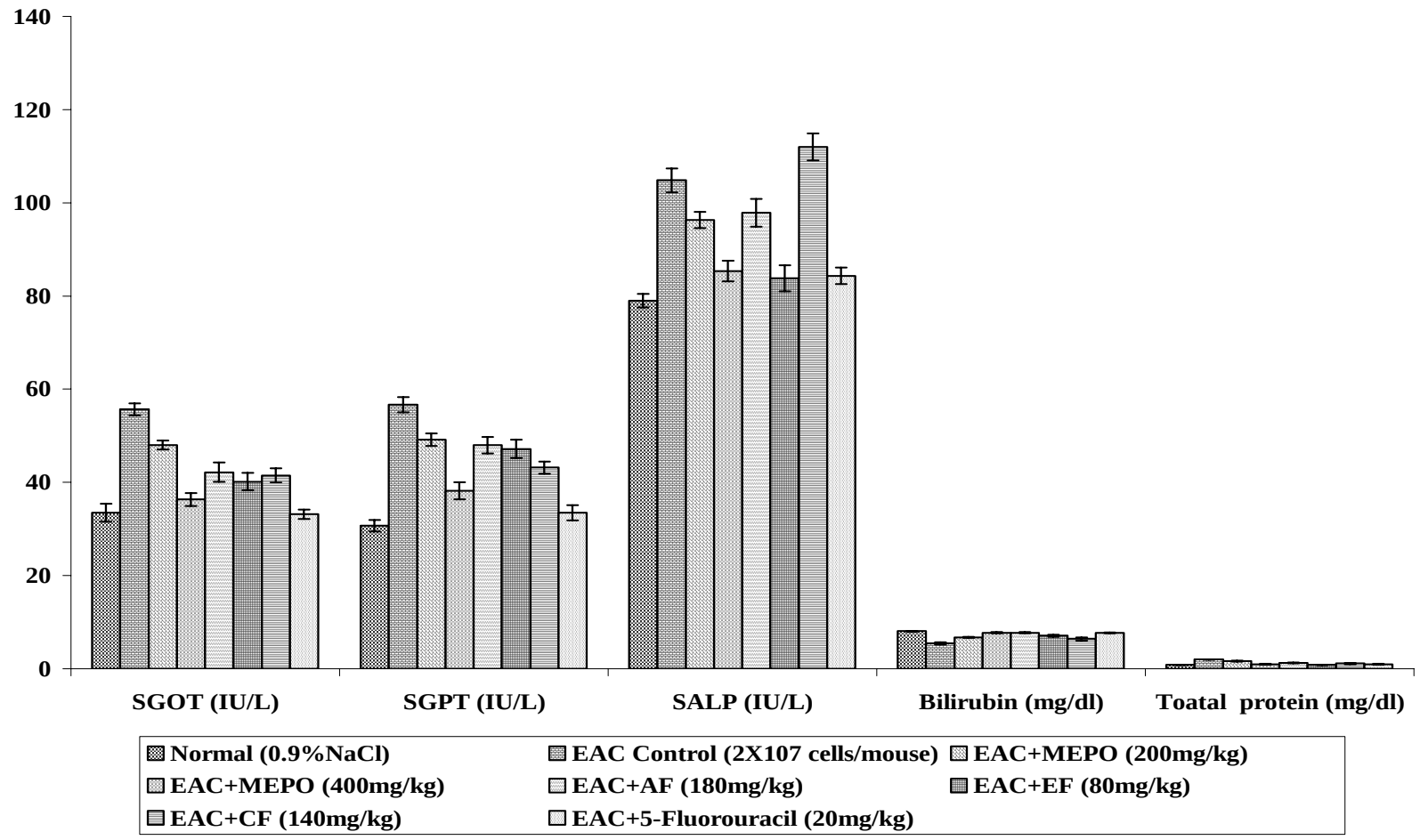
**p<0.001; *p<0.05 when compared to EAC control group where significance was performed by ANOVA followed by Dunnett's test.

Fig 1. Effect of the methanol extract of *Pavonia odorata* (MEPO) on hematological parameters in Erlich's ascites carcinoma bearing mice.



[a]Comparison of EAC control group *vs* normal group *p<0.05; [b]comparison of EAC control group *vs* all treated groups normal group *p<0.05: where significance was performed by ANOVA followed by Dunnett's test.

Fig 2. Effect of the methanol extract of *Pavonia odorata* (MEPO) on biochemical parameters in Erlich's ascites carcinoma bearing mice.



$p>0.05$ when compare the EAC control group *vs* normal group and compare the EAC control group *vs* all treated groups normal group, where significance was performed by ANOVA followed by Dunnett's test.

EAC control group from total WBC counts. In the EAC control group, WBC count ($16.45x10^3$/cu.mm) was significantly increased which were decreased to normal by 9 days treatment with MEPO (400 mg/kg), R-H_2O Fr and 5-fluorouracil (5.70, 5.98 & $5.90x10^3$/cu.mm) (Fig 1).

In order to evaluate the drug induced hepatotoxicity and EAC cell impact on the liver, the levels of SGPT, SGOT, SALP, total bilirubin and total protein were biochemically measured in normal mice, EAC bearing mice and treated mice. The data are showed in Fig 2 and stated that SGPT, SGOT, SALP and Total bilirubin level of the EAC control group(55.67, 56.67, 104.83, & 5.45) increased when compare to normal group (33.50, 30.67, 79.00 & 8.07) respectively. These levels were reduced by the 9 days treatment with the MEPO and its fractions. Mainly the MEPO (400 mg/kg) and standard were reduced these levels more effectively when compare to other treatment.

Level of LPx, GSH and activity of SOD &CAT in liver

Effects of MEPO treatment on the level of LPx (measured as MDA in liver of nonimplanted animals or tumor bearing mice) are shown in Table 3. There was significant change in liver MDA levels between the non implanted animals (95.26 ± 1.97) and the implanted group (541.21 ± 5.13). EAC control animals had a marked elevation in MDA level in liver which was more pronounced on day 9 as compared with the control values of the corresponding period. Similar patterns of MDA levels were observed from treated groups especially MEPO (400 mg/kg) had MDA levels (133.77 ± 9.49) close to control values. However, the moderate reduction of MDA level observed in the 5-fluorouracil treated animals (264.42 ± 6.70). Among the fractions, R-H_2O Fr and $CHCl_3$ Fr were significantly reduced the elevated MDA levels 135.33 ± 9.63 and 113.42 ± 5.53 respectively.

GSH level in liver was reduced drastically in the EAC bearing mice on the 9^{th} day when compare to the normal mice. Treatment with MEPO revealed significant changes in

Table 3. Effect of the methanol extract of *Pavonia odorata* on liver lipid peroxidation, glutathione and antioxidant enzymes in EAC treated mice.

(Values are mean±SEM; six animals in each group)

Groups	Lipid peroxidation (nM of MDA/mg wet liver tissue)	Glutathione (nM/ mg wet liver tissue)	Superoxide dismutase (IU/ mg wet liver tissue)	Catalase (nM of H_2O_2 decomposed/ min/ mg wet liver tissue)
Normal (0.9% NaCl)	95.26±1.97	49.76±096	9.46±0.25	52.66±0.75
EAC control	541.21±5.13[a,**]	23.54±2.18[a,**]	3.94±0.12[a,**]	22.53±0.79[a,**]
EAC+MEPO (200mg/kg)	326.40±10.74[b,**]	33.22±1.62[b,*]	5.66±0.08[b,**]	39.96±0.41[b,**]
EAC+MEPO (400mg/kg)	133.77±9.49[b,**]	83.88±3.25[b,**]	9.55±0.19[b,**]	51.94±0.59[b,**]
EAC+ R-H_2O Fr (180mg/kg)	135.33±9.63[b,**]	69.37±3.61[b,**]	10.09±0.35[b,**]	48.42±0.50[b,**]
EAC+EtOAc Fr (80mg/kg)	293.28±2.44[b,**]	52.94±1.08[b,**]	8.68±0.19[b,**]	48.11±0.64[b,**]
EAC+ $CHCl_3$ Fr (140mg/kg)	113.42±5.53[b,**]	43.98±0.69[b,**]	7.17±0.27[b,**]	39.93±0.39[b,**]
EAC+5-Flurouracil (20 mg/kg.b.w)	264.42±6.70[b,**]	41.88±1.83[b,**]	11.46±0.17[b,**]	53.85±0.59[b,**]

[a]EAC control group *vs* normal group, [**]$p<0.001$, [*]$p<0.05$; [b] treated group *vs* EAC control group, [**]$p<0.001$, [*]$p<0.05$, where significance was performed by ANOVA followed by Dunnett's test.

liver GSH content. A marked depletion in GSH level was improved to more than the GSH level of normal mice by the supplementation with MEPO (400 mg/kg) and R-H_2O Fr (Table 3).

As shown in Table 3, The SOD and CAT activity in the EAC control group had a marked decrease in liver on day 9. MEPO treatment markedly elevated SOD and CAT activity in liver to normal control levels at the corresponding time intervals. Treatment with 5-fluorouracil more effectively elevated SOD and CAT activity in liver. Among the fractions R-H_2O Fr was improved the antioxidant enzymes activity. The high dose of the MEPO (400 mg/kg) also significantly increase the SOD and CAT levels in liver when compare to EAC control group.

Assay for *in vitro* cytotoxicity

The EtOAc Fr and MEPO (400 mg/kg) showed marked cytotoxicity effect. The IC_{50} value of EtOAc Fr and MEPO (400 mg/kg) were 339.20 & 477.71 µg/ml. However R-H_2O Fr had moderate effect and the IC_{50} value is 654.57 µg/ml. The $CHCl_3$ Fr had not prominent cytotoxicity effect up to 1mg ml as compare to other fractions.

Discussion

Human have always relied on the nature to survive, which has been the main source of food, clothing and remedies. Natural products have been regarded as important source that could produce potential chemotherapeutic agents [25]. In particular anti cancer therapy have special place and recently some newer chemotherapeutic agents available for uses in a clinical setting include paclitaxel, vincristine, podophyllotoxin and camptothecin. Obviously natural products are extremely important as a source of medicinal agents. The acute toxicity study, MEPO has been concluded that it is non-toxic and safe up to 2000 mg/kg. By this basis

the present study deals with cytotoxic and anti tumor activity of the different fractions of *Pavonia odorata* by *in vivo* and *in vitro* method in EAC bearing mice. The lifespan is the important parameter to assess the antitumor activity. The zero percentage survival time was observed in EAC control group after 30days, where in MEPO (400 mg/kg), R-H_2O Fr and 5-fluorouracil were observed after 60 days. MEPO (200 mg/kg) and $CHCl_3$ Fr failed to produce effective survival time and zero percentage survival time were observed after 40 days. The ratio between the viable and non viable cell count was effectively reduced by standard and MEPO. The administration of various fractions and crude extract caused retardation in the tumor development, as it is evident by the increase of MST and AST and reduced tumor volume and viable count. This depicts the effectiveness of MEPO in restricting tumor cell multiplication and growth. Cytotoxicity is one of the main targets by chemicals to produce antitumor activity [26]. Number of anticancer drugs possesses significant cytotoxicity activity. In *in vitro* cytotoxicity study, EtOAc Fr was effectively produced the cytotoxicity compare to other fractions.

Preliminary phytochemical examination of EtOAc Fr shows detectable amount of flavanoids. Most number of flavonoids possesses remarkable antitumor activity [27]. Although the clear mechanism of flavonoids is not well known, they are reported to decrease the high glycolytic activity of EAC cells by inhibition of Na^+ and K^+ ATPase in the plasma membrane [28]. This may be one of the reasons for the inhibition of tumor growth. The R-H_2O Fr shows the presence of the saponins and it is well known that saponin has the hemolytic and cytotoxicity property. This may also contributes the antitumor activity of R-H_2O Fr [29].

A literature survey reveals [30] that tumorigenesis and its progression has been accompanied by the following changes compared with normal cytogenesis (1) gradual decrease in haemoglobin content, erythrocyte count and bone marrow cellularity, (2) gradual increase in leukocytes, thrombocytes and splenic cellularity, and (3) reversal of the lymphoid-myeloid ratio in the differential WBC count. As noted in Fig. 1, it was observed that there was significant decrease in the haemoglobin level and erythrocyte count in

EAC control group. This reduction was effectively improved by the MEPO and its fractions except $CHCl_3$ Fr. By agree with Roy et al statement the leucocytes was drastically increased in the tumor bearing mice, and it significantly reduces towards normal level with treatment of MEPO. Thus, the results indicate that MEPO and its fractions do not adversely affect haematopoiesis at its optimum dose while it does inhibit tumor growth markedly.

It is well known [31] that there are significant elevations in the levels of SGPT, SGPT, SALP and bilirubin in liver diseases and disorders and in hepatocellular damages caused by a number of agents. An increase in these enzyme levels is observed in patients with cardiac damage due to myocardial infarction and with liver disorders [31]. Biochemical measurements of these parameters showed that some extent of hepatotoxicity was associated after 9 days inoculation with EAC. Treatment with the MEPO and its fractions depicts that values remain near the normal range in the treated groups (Fig. 2). From this it states that tumor cell was inducing the hepatotoxicity to some extent and the damage protected by extract supplementation

Oxidative stress as result of overproduction of reactive oxygen species (ROS) has been implicated in the pathogenesis of a number of human diseases including cancer [32]. Number of studies has been indicated that tumor growth can cause antioxidant disturbance in certain tissues of the tumor host [33, 34, 35, 36]. So for the present study deal with antioxidant status of the EAC control group and treated groups. Moreover antioxidant status was measured by oxidative stress markers, which are lipid peroxidation and total antioxidant status.

Lipidperoxidation (LPx) is one of the important markers to determine the antioxidant status and measured by thiobarbituric acid reacting substances (TBARS). Abu zeid et al reported that in EAC bearing mice the lipid peroxidation in liver was elevated after 9 days of tumor inoculation [37]. The present data also document marked increase of MDA level in EAC control group. After 9 days treatment with MEPO and its fractions significantly reduce the elevated MDA level.

The present data reveals marked depletion of GSH level in liver of EAC control mice. There is a correlation

between depletion of GSH and the increase of LPx [38]. In addition relation between GSH and GSSG was reported that during cancer growth, glutathione redox (GSH/GSSG) decrease in the blood and liver of EAC bearing mice. It is mainly due to an increase of GSSG in blood result of oxidative stress [35]. In this study, the reduction of GSH and increase of GSSG may be due to oxidation of GSH and leads to increase of GSSG release from liver tissue. The oxidation of GSH is mainly due to peroxide production by tumor cells [35]. Further the reduced liver GSH level in tumor bearing mice may be by decrease of glutathione reductase activity. Glutathione reductase is responsible for increase GSH/GSSG ratio. On the other contrary, Wong HK et al [39] observed a reduced SOD activity and glutathione redox in tumor bearing mice. The supplementation by MEPO and its fractions restored the GSH content in liver. From this view the MEPO and its fractions may increase the GSH production by increasing glutathione reductase or inhibit the peroxide production in tumor by either prevent the formation or scavenge it. By any of the above mentioned way MEPO and its fractions may reduce oxidation of GSH and restore it in liver.

The present work also demonstrated the enzymatic antioxidant such as SODs and CAT activity. Both SOD and CAT provide a defense against the potentially damaging reactive oxygen species (ROS) such as $O_2^{\cdot-}$ and H_2O_2 in cells. However the extreme oxidative stress as a result of tumor growth leads to degrade ROS scavenging enzymes such as SODs and CAT [40]. SODs are the first and most important line of antioxidant enzyme defense system against ROS and particularly superoxide anion radical. At present three distinct isoforms of SOD have been identified. Two isoforms of SOD have Cu and Zn in their catalytic center and localized in intracellular and extracellular elements called SOD_1 and SOD_3 respectively. The third isoform of SODs has manganese (Mn) as a cofactor and has been localized in mitochondria of aerobic cells called SOD_2 [41]. SOD_2 plays an important role in promoting cellular differentiation and tumorgenesis [42] and protecting hyperoxia induced pulmonary toxicity [43]. The decreased level of SODs in the EAC control animals were significantly increased by

supplementation with MEPO and its fractions. The reduction of SODs in the EAC control group may be due to mitochondrial damage in liver and leads to degrade the SODs and the MEPO and its fractions may protect the mitochondria damage induced by tumor and inhibit the degrade of the SODs in liver.

CAT is a haem containing enzymes and plays pivotal role in the oxidative stress. In EAC control group the level of CAT was significantly reduced when compared to normal group and this study is agreement with previous studies of sun et al [44] and markuland et al [45] who was found the reduced CAT activity in seven neoplastic cells. The reduced activity of CAT in tumor bearing mice may be attributed to its inactivation by superoxide radical through converting it to the ferroxy and ferryl states of the enzyme [46]. Iron has an important role in catalase activity. There is number of report of an association between the increase body iron during tumor growth. Mono AM [47] reported that serum iron level was increased and decreased total iron binding capacity and transferrin level in tumor bearing animal there total iron binding capacity and transferrin are responsible for the formation of catalase [48]. By the treatment with extract CAT level was increased it may be due to scavenge the superoxide radical and the conversion of its ferroxy and ferryl states or increase the total iron binding capacity and transferrin.

In the haemopoetis system, iron plays pivotal role so the same hypothesis replied in hematological parameters. The reduced Hb content may be due to reduced iron binding capacity. The Hb content was restored by extract and fractions of MEPO. It may be due to the balance the iron states by scavenging the superoxide ions and this may prevent the inactivation of catalase enzyme.

From the above carried experiment it may be concluded that MEPO (400 mg/kg), R-H_2O Fr and EtOAc Fr were effective antitumor agent by preventing the tumor growth, possessing cytotoxicity property and acts as potent antioxidant. The further studies are carried out in our research laboratory to find the bioactive molecules from R-H_2O Fr and EtOAc Fr and it can be a good weapon for the treatment of cancer.

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