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Antitumor and Antioxidant Activities of *Triumfetta rhomboidea* against Ehrlich Ascites Carcinoma bearing Swiss Albino mice

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ABSTRACT

The purpose of this study was investigating experimentally the possible antitumor effect and antioxidant role of methanol extract of *Triumfetta rhomboidea* (METR) leaves against Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The METR administered at the doses of 100, 200 mg/kg, in mice for 14 days after 24 hours of tumor inoculation. The effects of METR on the growth of murine tumor, life span of EAC bearing mice were studied. Hematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were also estimated. Treatment with METR decreased the tumor volume and viable cell count there by increasing the life span of EAC bearing mice. METR brought back the hematological parameter more or less normal level. The effect of METR also decreases the levels of lipid peroxidation and increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The present work indicates that the methanol extract of *Triumfetta rhomboidea* exhibited significant antitumor and antioxidant activity *in vivo*.
Key words: *Triumfetta rhomboidea*; Antitumor activity; Lipid peroxidation; Antioxidants.

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INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. The plant *Triumfetta rhomboidea* Jacq (Family: Tiliaceae) is an under shrub, widely distributed in tropical and subtropical India, Ceylon, Malay Peninsula, China, Africa and in America. In Ayurveda the root is bitter and acrid; aphrodisiac, tonic, cooling; useful in dysentery. The leaves and stem are used as a poultice on tumors[1]. Powdered leaf infusions of *Triumfetta rhomboidea* are drunk represents for the treatment of anemia in different regions of East Africa [2]. In folklore remedy the plant was used in the treatment of cancer among the tribal population in Kolli Hills, South India. However a fewer reports are available with respect to the pharmacological properties of the plant.

Reactive oxygen species such as superoxide, hydroxyl radical, iron-oxygen complexes, hydrogenperoxide and lipid peroxides are generated by several reactions. These are metabolisms of triplet oxygen molecule; one electron reduction of oxygen; catalytic decomposition of hydrogen peroxide and lipid peroxides by metal ions; attack of metal and/or metal oxygen complex; irradiation of visible light and X-ray, and intake of exogenous radicals [3]. These radicals react with biological molecules such as DNA, proteins and phospholipids and eventually destroy the structure of these membranes and tissues [4, 5].

At present, the scientific community is interested in elucidating the role of several therapeutic modalities, currently considered as elements of complementary and alternative medicine, on the control of certain diseases. Plant derived natural products such as terpinoids and steroids etc. have received considerable attention in resent years due to their diverse pharmacological activity [6,7]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infection and degenerative diseases. *Triumfetta rhomboidea* have been indicated for the treatment of several diseases, one among which is cancer. The present study was carried out to evaluate the antitumor activity and antioxidant status of methanol extract of *Triumfetta rhomboidea* (METR) against EAC bearing mice.

MATERIALS AND METHODS

Plant material and extraction

The leaves of *Triumfetta rhomboidea* (Family: Tiliaceae) were collected in the month of October 2005 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by Dr. G.V.S. Murthy, Botanical survey of India, Coimbatore, Tamil Nadu, India and the voucher specimen (No; BSI/SC/5/23/05-06/Tech-132) was retained in our laboratory for future reference. The dried powdered leaves were extracted by methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semisolid mass was obtained and vacuum dried to yield a solid residue (4.65% w/w). The extract showed positive test for steroids, triterpinoids and flavonoids. The extract at the doses of 100, 200 mg/kg and Vincristine 0.8mg/kg used as standard in saline were used for the present study

Chemicals and reagents

Chloro-2-4-nitrobenzene (CDNB) was purchased from Sigma chemicals, USA, Thiobarbituric acid (Loba Chemicals, Mumbai, India) 5,5'-Dithio-bis-2-nitrobenzoic acid (DTNB) (Sisco research laboratory Mumbai) Nitroblue tetrazolium chloride (NBT) (Sigma chemicals USA) Tryphan blue (Otto kemi Mumbai) and other solvent and /or reagent obtained was used as received. The EAC cells were obtained from Amala Cancer Research Center. Thrissur, Kerela, India. The EAC cells were maintained by intraperitoneal innoculation of 2 X10^6 cells/mouse. Studies were carried out using male Swiss albino mice weighing 22 ± 2 g were obtained from Perundurai Medical College, Perundurai, Tamil Nadu, India. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Animals

Male Swiss albino mice weighing between 18-22 g were used for the present study; they were maintained under standard environmental conditions and were fed with standard laboratory diet and water *ad libitum*.

Treatment schedule

Tumor was induced by injecting 0.2ml of 2 X 10^6 cells/ml of EAC in to peritoneal cavity of mice. The animal were divided in to five groups (n=12). All the groups were injected with EAC cells (2 X 10^6 cells/mouse) intra peritoneally except normal group. This was taken as day 0. On the first day normal saline (0.9%w/v, NaCl, 5ml/kg/mouse/day) administered into normal group (group 1). EAC control mice were received only vehicle (Propylene glycol 5ml/kg/day/mouse) as groups 2. The different doses ethanol extract of *Triumfetta rhomboidea* (100 and 200mg/kg/day/mouse) and standard drug Vincristine (0.8 mg/kg) were subsequently administered in groups 3, 4, and 5 respectively for 14 days intraperitoneally. On 15th day, after the last dose and 18 hours fasting six mice from each group were sacrificed for the study of antitumor activity, hematological, and antioxidant enzyme estimation and rest of the animal of each group were kept to check the Mean Survival Time (MST) and percentage increase in the life span (%ILS) of the tumor bearing mice.

Tumor growth response

Antitumor effect of METR was assessed by observation of changes with respect to body weight, Ascetic tumor volume, packed cell volume, viable and non viable tumor cell count. MST and %ILS were also calculated. Transplantable murain tumor was carefully collected with the help of a sterile 3ml syringe and measured the tumor volume and the ascetic fluid was with draw in a graduated centrifuge tube and packed cell volume was determined by centrifuge tube at 1000 rpm for 5min, Viable and nonviable cell count of ascetic cell were stained by the tryphan blue (0.4% in normal saline) dye exclusion test and count was determined in Neubour counting chamber. The effect of METR on tumor growth was monitored daily by recording the mortality and %ILS was calculated using following formula

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$$\text{ILS (\%)} = \frac{\text{Mean survival of treated group}}{\text{Mean survival of control group-1}} \times 100.$$

Hematological studies

Blood was obtaining from the tail vein, blood was drawn into RBC or WBC pipettes, diluted and counted in a Neubauer counting chamber Sahli's Hemaoglobinometer determined of hemoglobin concentration. Differential count of leukocytes was done on a freshly drawn blood film using Leishman's stain. Hemoglobin content [8] , RBC, WBC count [9] and differential leukocyte count [10] was estimated from the peripheral blood of normal, EAC control and extract treated animal groups.

Biochemical assay

The liver was excised, rinsed in ice cold normal saline followed by cold 0.15M Tris-Hcl (pH 7.4), blotted and weighed. The homogenate was processed for estimation of lipid peroxidation, GSH, SOD and CAT. Assay for microsomal lipid peroxidation was carried out by the measurement of thiobarbituric acid reactive substances (TBARS) in the tissues [11] the pink chromaogen produced by the reaction of malondialdehyde, which is a secondary product of lipid peroxidation reaction with thiobarbituric acid was estimated at 532nm. Reduced glutathion (GSH) in the tissues was assayed by the method of Ellman [12]. GSH estimation is based on the development of yellow color when 5, 5'-dithiobis (2-nitro benzoic acid) dinitrobis benzoic acid was added to compounds containing sulphydryl group. SOD was assayed by the method of Kakkar et al [13]. The assay was based on the 50 % inhibition of formation of NADH-Phenazine methosulphate Nitroblue tetrazolium at 520 nm. The activity of CAT was assayed by the method of Abei[14]. Proteins were estimated by the method of Lowry et al [15] using bovine serum albumin as the standard.

Statistical analysis

Total variation present in set of data was performed by using one way analysis of variance (ANOVA) and the results are expressed as mean ± SEM.

RESULTS

The present investigation indicates that the METR showed significant antitumor and antioxidant activity in EAC bearing mice. The effects of METR (100, 200 mg/kg) at different doses on tumor volume, viable and nonviable cell count, survival time and ILS, were shown in Table 1 and 2. Administration of METR reduces the tumor volume, packed cell volume and viable tumor cell count in a dose dependent manner when compared to EAC control mice. In EAC control mice the mean survival time was 22.21±0.25 days. Whereas, it was significantly increased mean survival time (26.32 ± 1.20, 30.65 ± 0.66 days) with different doses (100 and 200 mg/kg) of METR and standard drug (31.25 + 0.55 days) respectively.

As shown in the Table 3, the hemoglobin content in the EAC control mice (9.8g %) was significantly decreased when compared with normal mice (12.18 g %) METR at the dose of 100 and 200 mg/kg the hemoglobin content in EAC treated mice were increase to 10.55± 0.12 g % and 11.67± 0.13 g % moderate changes in the RBC count were also observed in the extract treated mice. The total WBC counts were significantly higher in the EAC control mice when compared with normal mice. Whereas, METR treated mice significantly reduced the WBC counts as compared to that of control mice. The different leukocyte count, the percentage of neutrophils was increased while the lymphocyte count was decreased in the extract treated mice when compared with EAC control mice.

The levels of LPO, GSH, SOD, catalase and protein content were summarized in Table 4, the levels of lipid peroxidation in liver tissue significantly increased in EAC control mice (1.36 n moles MDA/g of tissue) as compared to the normal mice (0.92 n moles MDA/g of tissue) Treatment with METR (100 and 200 mg/kg) were significantly decrease the LPO levels 1.15 and 0.99 n moles MDA /g of tissue in a dose dependent manner. The GSH count in liver tissues of normal mice was found to be 2.36 mg/kg wet tissue. Inoculation of EAC drastically decreased the GSH content to 1.69 mg/g-wet tissue. Whereas treatment with different doses of METR, the GSH levels were reverse to normal level (2.75 and 2.14 mg/g wet tissue) respectively.

As shown in table 4, SOD level of lower of EAC bearing mice was significantly decreased 3.29 units/mg proteins when compared with normal mice (4.38 units/mg protein). Administration of the METR significantly increased the SOD levels (3.42 and 3.87 units/mg of protein in tissues) at the doses of 100 and 200 mg/kg respectively.

The catalase levels were decreased in EAC control mice (1.71 unit/mg protein) when compared with normal mice (2.51 unit/mg of protein in tissues) treatment with METR at the doses at 100 and 200 mg/kg it brought back to normal level (1.99 and 2.05 unit/kg of protein in tissues).

DISCUSSION

The present study was carried out to evaluate the effect of METR on EAC bearing mice. The METR were showed significant antitumor activity against the transplantable murain tumor. The reliable criteria for judging the value of any anticancer drug are the prolongation of life span of animals [16]. A reduction in the number of ascitic tumor cells may indicate either an effect of METR on peritoneal macrophages or other components of the immune system[18], therefore increasing their capacity of killing the tumor cells, or a direct effect on tumor cell growth. METR inhibited significantly the tumor volume, viable cell count and enhancement in survival time of EAC bearing mice and thereby acts as anti-neoplastic agent.

Myelo supression is a frequent and major complication of cancer chemotherapy. METR treated and subsequent tumor inhibition resulted in appreciable improvements in hemoglobin content, RBC and WBC counts (Table 3). These observations assume great significance, as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive

effects on erythropoiesis [19,20] and thereby limiting the use of these drugs. The improvement in hematological profile of the tumor bearing mice following the treatment with extract could be due to the action of the different phytoconstituents present in the extract.

Lipid peroxidation mediated by free radicals considered being a primary mechanism of cell membrane destruction and cell damage. The oxidation of unsaturated fatty acids in biological membrane leads to a reduction in membrane fluidity and disruption of membrane structure and function [21]. MDA, the end product of lipid peroxidation was also reported to be higher in carcinomatous tissue than in non-diseased organs [22]. Increase in the level of TBARS indicated enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals. The active role of GSH against cellular lipid peroxidation has been well recognized and thereby reduces the glutathione (GSH) activity. GSH can act either to detoxify activate oxygen species such as H_2O_2 or reduce lipid peroxides themselves. In the present study indicated that METR significantly reduced the elevated levels of lipid peroxidation and increased the levels of glutathione content and thereby it may act as an antitumor agent.

On the other hand, SOD is a ubiquitous chain breaking antioxidant and is found in all aerobic organisms. It is a metelloprotein widely distributed in all cells and plays an important protective role against ROS-induced oxidative damage. The free radical scavenging system catalase, which are present in all major organs in the body of animals and human beings and is especially concentrated in liver and erythrocytes. Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotic in liver tissues [23, 24]. It was suggested that catalase and SOD are easily inactivated by lipid peroxides or ROS [25]. In correlation, it has been reported that EAC bearing mice showed decreased levels of SOD activity and this may be due to loss of Mn^{++} SOD activity in, liver [26]. Inhibition of catalase activity in tumor cell line was also reported [27]. In this study, Catalase and SOD were appreciably elevated by administration of METR, suggesting that it can restore the levels of SOD and catalase enzymes.

The present study demonstrated that METR increased the life span of EAC tumor bearing mice and decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. The above parameters are responsible for the antitumor and antioxidant activities of *Triumfetta rhomboidea*.

Further investigations are in progress in the laboratory to identify the active principles involved in this antitumor and antioxidant activity and investigate their mechanism.

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Table 1. Effect of methanol extract of *Triumfetta rhomboidea* (METR) on tumor volume, packed cell volume, viable and non-viable tumor cell count of EAC bearing mice.

Parameter	EAC control ($2x10^6$ cells/ mouse/ml)	METR (100 mg/kg) +EAC	METR (200 mg/kg) + EAC	Standard Vincristine (0.08 mg/kg) + EAC
Body weight (g)	26.11± 0.12	23.80±0.58	22.00±0.44	23.9±0.02
Tumor volume (ml)	5.82 ± 0.042	3.45± 0.04	2.65 ±0.04	2.42±0.13
Packed cell volume (ml)	2.12 ±0.104	1.37 ±0.03	0.80 ±0.03	1.15±0.03
Viable tumor count x 10^7cells/ml	11.25±0.098	7.96 ± 0.02	4.12± 0.06	4.90±0.015
Non-viable tumor cells count x 10^7 cells/ml	0.5 ±0.017	0.66 ± 0.10	1.11 ± 0.01	1.23±0.81

Value are mean ± SEM. Number of mice in each group (n=6). P<0.01, Experimental groups was compared with EAC control. (Weight of normal mice 20 ± 0.15)

Table 2. Effect of the methanol extract of *Triumfetta rhomboidea* (METR) on survival time on EAC bearing mice.

Groups	Experiment	Mean Survival (days)	% Increase in life span (% ILS)
1.	Normal control (Normal saline 5 ml/kg b.w.)	-	-
2.	EACcontrol($2x10^6$cells)+Propylene glycol (5ml/kg b.w.)	22.21 ± 0.25	-
3.	METR(100mg/kg)+EAC ($2x10^6$cells)	26.32 ± 1.20	8.33 %
4.	METR(200mg/kg)+EAC ($2x10^6$cells)	30.65 ± 0.66	25.00 %
5.	Vincristine (0.8mg/kg) +EAC ($2x10^6$cells)	31.25 ± 0.55	40.90 %

Value are mean ± SEM. Number of mice in each groups (n=6) P<0.01, Experimental groups compared with control.

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Table 3.Effect of the methanol extract of *Triumfetta rhomboidea* (METR) on hematological parameter of EAC treated mice

Parameters	Normal saline (0.5 ml/kg)	EAC ($2x10^6$ cells) Control +vehicles	EAC ($2x10^6$ cells) + METR 100mg/kg	EAC ($2x10^6$ cells)+ METR 200mg/kg
Hemoglobin (g %)	12.85± 0.25	9.8 ± 0.02	10.55 ± 0.12	11.67 ± 0.13
Total RBC (cells/ml x 10^9)	6.65 ± 0.18	3.8± 0.035	4.65 ± 0.12	5.62 ± 0.12*
Total WBC (cells/ml x 10^9.)	7.8 ± 0.045	20.07± 0.068*	15.95 ± 0.08	12.19 ± 0.07
Differential count (%)				
Lympocytes	77.75± 0.19	33.37± 0.56*	48.56 ± 0.12*	62.71 ± 0.10
Neutrophils	1.7 ± 0.035	0.82± 0.024	51.65± 0.06	41.59 ± 0.11
Monocytes	29.97± 0.46	52.6 ± 0.03*	1.16 ± 0.07*	1.32 ± 0.04

Value are mean ± SEM. Number of mice in each groups (n=5) EAC compared with normal group*P<0.01. Experimental groups compared with EAC control, *P<0.05.control group

Table 4. Effect of methanol extract of Triumfetta *rhomboidea* (METR) on biochemical parameter, in EAC bearing mice.

Parameters	Normal (Saline 0.5ml/kg)	EAC control ($2x10^6$ cell/ml)	EAC control ($2x10^6$ cell/ml) + METR (100 mg/kg)	EAC control ($2x10^6$ cell/ml) + METR (200 mg/kg)
Lipid Peroxidation n moles MDA/gm of tissue	0.92± 0.02	1.35±0.12*	1.15 ±0.20*	0.99±0.01
GSH(mg/g of tissue)	2.36±0.03	2.49±0.08	3.32± 0.08	3.81± 0.09
SOD(unit/mg prtein)	4.38 ± 0.43	3.27± 0.09	3.83±0.09	5.15± 0.09
Catalase (units/mg tissues)	2.51±0.72	1.64±0.15*	1.99± 1.20	2.05±0.04*
Protein content (gm/100 ml)	12.66± 0.69*	18.17±1.15	16.88±0.96	15.66+0.12

Value are mean ± SEM (n=5) EAC control group was compared with normal group. Experimental groups were compared with EAC control P<0.01, *P<0.05.

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