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Research Article

Pharmacological Evaluation of *S.trifoliatus* fruit extract of the plant *S.trifoliatus* in Ehrlich Ascites Carcinoma (EAC) tumor bearing mice

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ABSTRACT

Evaluation of fruit of the plant *S.trifoliatus* in ehrlich ascites carcinoma (eac) tumor bearing mice was established. *S.trifoliatus* fruit extract extract 100 mg/kg, 200mg/kg body weight dose significantly reduced ascitic fluid volume. 300 mg/kg, 400 mg/kg reduced the percentage of viable ascitic cells to 47 and 48% respectively in the treated groups as compared to 93% in the EAC control. 500mg/kg increase the life span of the EAC treated mice by 112% and 100% respectively. All the doses of leaf extracts of the plant *S.trifoliatus* increased RBC count and hemoglobin content and decreased WBC count to near normal values, in EAC bearing mice. *S.trifoliatus* extract extracts marginally altered SGOT, SGPT values and significantly increased alkaline phosphatase. In creased urea and creatinine content in blood have been observed in 100 mg and 200 mg/Kg body wt dose. The Results of the present study clearly demonstrates the tumor inhibitory activity of the plant extracts against transplantable murine tumor cell line. The mechanism by which these compounds mediate is antitumor effect is still to be elucidated.

Key words: *S.trifoliatus*, murine tumor cell line, ehrlich ascites carcinoma, tumor.

INTRODUCTION

Breast cancer therapy which is usually multimodality treatment is in advance, cytotoxic drugs still play the important roles for the increasing survival rate together with good quality of life (P. Fargeot *et al*, 2004). Therefore, the development and search for novel and effective anticancer agents have become very important issues (D. Cameron and R. Bell *et al*, 2004). To date, many cytotoxic agents including natural products isolated from plant sources have been investigated for the discovery of the potential novel anticancer drugs (Merkel DE and Mcguire WL *et al*, 1990). Higher plants have long been shown to be excellent and reliable sources for the development of novel anticancer drugs. In India, many plants have been used for treatment of various malignancies over centuries. *Sapindus trifoliatus* (Family: Sapindaceae) is an India traditional herbal medicine that has long been used for many decades to relieve symptoms from fever caused by infection, inflammation and prescribed in the mixture of traditional medicine for treating of various malignancies (Hardman JG *et al*, 1996), (Rang HP *et al*, *1999*). The plant is locally known as ritha. It also called as soap nut tree**.** The plant has been reported for its high content of saponin and sugars. The saponin moiety is characterized as the hederagenin group of glycosides. (Arulmozhi DK *et al*, 2005). The pericarp of the fruit of this plant is reported for its various medicinal properties like tonic, stomachic, spermicidal, and also used in the treatment of hemicranias, migraine, hysteria etc. (Arulmozhi DK *et al*, 2005). However, the property of this plant, especially its anticancer activity, has not yet been investigated. Therefore, this prompted us to investigate the inhibitory growth effect of this plant on two different breast cancer cell lines, SKBR3 and MDA-MB435.

S.trifoliatus (Linn), family - Sapindaceae, is extensively used in indigenous system of medicine for gravel and other urinary complaints[2, 3]. It is also used in the treatment of menorrhagea, leucorrhoea, piles and fistula[4]

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MATERIALS & METHODS

The dried pericarps of the leaves of *S.trifoliatus* (Family: sapindaceae) were collected from the local market and were authenticated by Dr. P.K.Sahu Botanist, and Depts. Of Botany, Utkal University, Bhubaneswar, matched with the specimen with the existing herbarium No.109. Aqueous extraction was done with polarity order following continuous/successive extraction method. The method of extraction followed by Arulomozhi Dk, J-etal2004 is adopted to obtain the extracts of leaves of *S.trifoliatus* [3,4].Briefly one hundred gram of the pericarp is soaked in 400ml of distilled water for 16h. The percolate was then decanted, centrifuged and filtered through Whattman (No.1) filter paper to obtain clear extract (300ml). This process of extraction was repeated again with the same volume of distilled water. The percolates were pooled and lyophilized which yielded a brown colored powder (70% yield). Acid hydrolysis of the extract yielded only one glycone, which was identified as hederagenin. Therefore estimation of this saponin present with extract was calculated as hederagenin. The content of hederagenin was estimated in the extract by boiling it with 50% methanolic hydrochloric acid. The entire mixture was evaporated to dryness and reconstituted in methanol. The concentration of hederagenin was found to be between 5.65-6.5% by weight of the extract. The whole extract had been taken for phytochemical evaluation and

confirmed the presence of compound as saponin, flavonoids and alkaloids.

Treatment Protocol

Albino Swiss mice (18-20 g body weight) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever) and water *ad libitum*. LD_{50}[7] values of *S.trifoliatus* are 100 mg/kg, 200mg/kg, 300 mg/kg, 400 mg/kg & 500mg/kg body weight respectively. The animals were divided into 8 groups each containing 10 mice. Animals of groups 1-3 were kept as saline control (5 ml/kg body weight i.p). Ehrlich Ascites Carcinoma (EAC) control ($2x10^A$ EAC cells/mouse i.p.) and EAC ($2x10^6$ EAC cells/ mouse i.p.) + propylene glycol respectively. 100mg/kg dose was dissolved in water and 100mg/kg, 200mg/kg, 300mg/kg,400mg/kg & 500mg/kg were dissolved in propylene glycol and administered (i.p) at a does of 2 mg/kg body weight in group 4, 5, 6, 7 and 8 respectively. All compounds were administered daily for 9 days starting 24 h after tumor transplantation. Five animals from each group were sacrificed 24 h after the last does and the ascitic fluid volume, ascitic cell count and hematological parameters were noted. Mean survival time (MST) for remaining 5 mice of each group was noted for 6 w.

Ascites volume was noted by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuge at 1000 g for 5 min. Viability of ascitic cells were checked by trypan blue (0.4% in normal saline) dye exclusion test and the count was taken in Neubauer counting chamber. The effect of the *S.trifoliatus* fruit extract on tumor growth was monitored by recording the mortality daily for 6 w and percentage increase in life span (%ILS) was calculated. ILS(%) = [(Mean survival of treated group) (Mean survival of control group) –1] x 100.

Hematological Studies

Blood was obtained from tall vein, 24 h after the last dose. For total count, blood was drawn into RBC to WBC pipettes in proper dilution and counted in Neubauer counting chamber. Hemoglobin concentration was determined by Sahlis Hemoglobinometer method. Differential count of leukocytes was done on freshly drawn blood film using Leishman's stain.

Chronic Toxicity Study

For chronic toxicity studies, the mice were divided into five groups containing 10 mice in each group. Compounds were injected (i.p.) once a week for 4 w at a dose level of mg/kg body weight. Group I received normal saline (0.9% NaCI, w/v; 5 mI/kg) once in a week for 4 w. Animals from each group were decapitates after 24 h of the last dose. *S.trifoliatus* fruit extract was separated from clotted blood for the estimation of SGOT, SGPT and Alkaline phosphatase. Heparinised whole blood was taken for estimation of urea[10], creatinine[10] and cholesterol[10]. The data was statistically analyzed by students 't' test.

RESULTS AND DISCUSSION

Results are summarized in and were analyzed statistically by student's unpaired 't' test and statistical significance were considered only when $p<0.05$.

S.trifoliatus fruit extract 100 mg/kg, 200mg/kg body weight dose significantly reduced ascitic fluid volume. 300 mg/kg, 400 mg/kg reduced the percentage of viable ascitic cells to 47 and 48% respectively in the treated groups as compared to 93% in the EAC control. 500mg/kg increase the life span of the EAC treated mice by 112% and 100% respectively. All the doses of leaf extracts of the plant *S trifoliatus* increased RBC count and hemoglobin content and decreased WBC count to near normal values, in EAC bearing mice. *S.trifoliatus* fruit extract marginally altered SGOT, SGPT

Table 1: Physical Properties Of *S.trifoliatus* Fruit Extracts at different doses

Dose	IB absorption (cm^{-1})	Element analysis nitrogen		Solubility
		Theoretical (%)	Experimental (%)	
S.trifoliatus 25 mg/kg	1410 (C-N)	7.31	7.30	Water
S.trifoliatus 50mg/kg	3050 (C-H)	7.23	6.52	Propylene glycol
S.trifoliatus100 mg/kg	1150 (C-N)	--------	--------	Propylene glycol
S.trifoliatus 150mg/kg	1590 (N-H)	6.32	6.30	Propylene glycol
S.trifoliatus 200mg/kg	1500	--------	---------	Propylene glycol
S.trifoliatus250mg/kg	1210(C-N)	--------	---------	Propylene glycol
S.trifoliatus 300mg/kg	1030	--------	---------	Propylene glycol
S.trifoliatus 350mg/kg	1580 (N-H)	10.5	11.12	Propylene glycol
S.trifoliatus400mg/kg	1280 (C-N)	6.52	6.52	Propylene glycol
S.trifoliatus 500mg/kg	3320(N-H)	--------	--------	Propylene glycol

Table 2: Antineoplastic Activity Of The *S.trifoliatus* Fruit Extract doses Against EAC Bearing Mice

Parameter	NS (5ml/kg)	EAC only ($2x10^6$ / mouse)	Vehicle control (5ml/kg)	S.T- 25 mg/kg	S.T-50 mg/kg	S.T-100 mg/kg	S.T-150mg/kg	S.T-200mg/kg
TBW (gm)	18.0	22.0	21.9	19.2	18	21.0	22	22.2
MST (days)	-	15.5	15	33	30	29	27	31
%ILS	-	-	-	122	93	87	74	100
Tumor volume (ml)	-	5.26±0.2	4.76±0.4	27+.02	2.5±.8	2.8±.02	3.75±.3	3.0±.09
VCA	-	93.6	93.8	51.1	47.3	48.6	51.5	67.5

Significant at $p<0.05$, All p values are calculated with vehicle control.
NS-Normal saline,VC-Vehicle control,
MST-Mean Survival Time, VCA-Viable cells in Ascites

values but at the same time significantly increased alkaline phosphatase. In creased urea and creatinine content in blood have been observed in *S.trifoliatus* fruit extract.

The Results of the present study clearly demonstrates the tumor inhibitory activity of the plant extracts against transplantable murine tumor cell line. The mechanism by which these compounds mediate is antitumor effect is still to be elucidated. In the EAC bearing mice cells are present in the peritoneal cavity and the compounds were administered directly into the peritoneum. Thus tumor inhibition might be due to direct effect of the compounds of tumor cells. Likewise the action of the synthesized compounds could also be mediated via its effect, if any on the DNA. Myelosupression is a frequent and major complication of cancer chemotherapy. Compared to the pretreatment values in EAC. These doses of the plant extract treatment and subsequent tumor inhibitor resulted in appreciable improvements in hemoglobin content RBC and WBC counts. These observations assume great significance as anemia is a common complication in cancer and the situation aggravates further during chemotherapy since a majority in antineoplastic agents exert suppressive effects on erythropoiesis and thereby limiting the use of these *S.trifoliatus* fruit extract. The improvements in hematologic profile of the tumor bearing mice following the treatment with *S.trifoliatus* doses could be secondary to tumor regression or due to the action of the compounds itself. In any case, the results of the present study are encouraging as these compounds exhibit significant reduction in the tumor burden and caused prolongation of lifespan of the hosts. Improvements, rather than aggravation of tumor associated hematological complications such as anemia and bone marrow suppression was also noticed.

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