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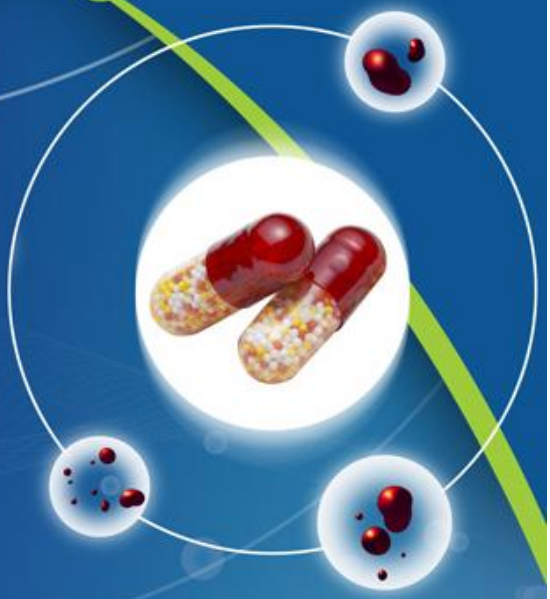

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Research Article

ANTITUMOR ACTIVITY OF METHANOL EXTRACT OF *TEDANIAANHELANS* AGAINST EHRLICH ASCITES CARCINOMA IN SWISS ALBINO MICE.

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ABSTRACT.

The study was aimed to evaluate antitumor activity of the methanol extract of Tedaniaanhelans against Ehrlich ascites carcinoma in swiss albino mice. In the present study, the methanolic extract of Tedaniaanhelans (MET) was screened for in-vivo antitumor properties against Ehrlich ascites carcinoma (EAC) tumor bearing mice at 250 and 500 mg/kg body weight doses given orally once daily for 14 days. Results indicated that the administration of extract not only increased the survival of mice with ascites tumor, but also decreased the body weight induced by the tumor burden, viable tissue cell count and reduced packed cell volume. It also altered many hematological parameters during tumor progression indicating the potent antitumor nature of the extract. Among the two doses tested, the 250 mg/kg body weight dose was found to be most potent.

Keywords: *Tedaniaanhelans, antitumor, Ehrlich ascites carcinoma.*

INTRODUCTION

Over the decades, cancer is the leading cause of death in both economically developed and developing countries and the number of persons living with cancer is continuing to increase [1]. The progression of resistance to chemotherapy is a major hindrance to treatment of various cancers ultimately resulting in multidrug resistance following exposure to multiple anticancer drugs with diverse structure and mechanisms of action [2]. Hence, discovery of new natural products and metabolites isolated from microorganisms, animals, and plants possessing high efficacy against tumor cells without any toxicity on normal cells will be a major breakthrough in scientific researches.

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Sponges produce biomolecules to defend against predators, and regulate symbiotic bacteria or contend with other sessile species. Accordingly, they are a rich source of bioactive molecules, a number of these compounds and analogues thereof, such as eribulinmesylate and α-galactosylceramide have been isolated and revealed a potential therapeutic effect against various cancers in clinical trials [3]. The sponge of genus Tedania, is reported to possess strong cytotoxic and anticancer properties. Several constituents including tedanin and tedanolide, isolated from T. ignis, have been studied in detail for their potent cytotoxic properties [4;5]. Secondary metabolites of genus Tedania were also reported to possess anti-inflammatory [6], phytotoxic [7], plant growth regulator [8], signal transduction agent, and cell adhesion inhibitor [9] properties.

The versatility of the functions of sponge may be derived from their ample bioactive metabolites with antitumor and cytotoxic activities [10]. Therefore, based on the potent

cytotoxic properties [11; 12] and genus Tedania possessing a number of anticancer metabolites, we studied the MET for its in-vivo anticancer activity against the EAC tumor model.

MATERIALS AND METHODS

Sponge material and extraction

The sponge material Tedaniaanhelans (Family: Tedaniidae) was collected at the Devgad Island, Karwar in the month of April 2004 and was authenticated by Dr. P.A. Thomas, Scientist, Central Marine Fisheries Research Institute, Vizhinjam, India. A specimen voucher is deposited for future reference (Voucher No BLDE-2004-002). The sponge (900g) was extracted with methanol (2.5 l) at room temperature and the solvent was removed at reduced pressure. The crude methanol extract was a dark brown solid weighing 40g (yield, 15.75%). The extract was preserved in a refrigerator at 4°C until further use.

Chemicals

5-Flurouracil (5-FU) was obtained from Hi-Media, Ltd., India. Tryphan blue was obtained from s.d. Fine chemicals, Mumbai, India. All the chemicals used were of analytical grade.

Tumor cells

Ehrlich ascites carcinoma (EAC) cells were obtained from Life Sciences, Manipal University, Manipal, India. The cells were maintained in-vivo in Swiss albino mice by intraperitoneal transplantation. EAC cells, aspirated from the peritoneal cavity of mice, were washed with saline and given intraperitoneally to develop ascitic tumor.

Preparation of suspensions and solutions

For the short term cytotoxicity assay against the EAC cell line, MET was dissolved in dimethyl sulphoxide (DMSO) and the volume made up to 10 ml with DMEM to obtain a 1000 µg/ml stock solution, which was stored at −20°C until further use. Serial two-fold dilutions were made using maintenance medium from the stock solution. The MET and standard 5-FU were suspended in distilled water using sodium carboxy methyl cellulose (CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter to study in-vivo antitumor activity.

Animals

Healthy swiss albino mice weighing 20-25g were obtained from the animal house, BLDEA's. College of Pharmacy, Vijayapur, Bijapur, India. The mice were grouped and housed in polyacrylic cages and maintained under standard conditions (temperature 25±2 0C, relative humidity 65±10% and in 12 h dark/light cycles). The animals were fed with rat pellet feed supplied by Pranav Agro Industries, Sangli, India and water ad libitum. All the procedures were reviewed and approved by CPCSEA B.L.D.E.A's College of Pharmacy, Bijapur, India (B.L.D.E.A' COP/ IAEC, Clear/ BPC/1109/68/10-11).

Short-term cytotoxic activity

Short-term cytotoxic activity of the MET was determined to find the percentage viability of EAC cells using the trypan blue dye exclusion technique [13]. EAC cells were cultured in the peritoneal cavity of healthy albino mice weighing between 25 to 30g by injecting a suspension of EAC cells (1 × 106 cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of mice on day 15 and washed with Hank's balanced salt solution (HBSS) and centrifuged for 15 min at 1,500 rpm in a cooling centrifuge. The pellet was re-suspended with HBSS and the process was repeated three times. Finally, the cells were suspended in a known quantity of HBSS and the cell count was adjusted to 2 × 106 cells/ml. Then, 0.1 ml of this diluted cell suspension was distributed into eppendorf tubes and exposed to 0.1 ml each of the different concentrations of the MET and incubated at 37°C for 3 h. After 3 h, the trypan blue dye exclusion test was performed to determine the percentage viability and the GI50 value was calculated.

Antitumor activity

Swiss albino mice were divided into five groups (n = 12). All the animals were injected

with EAC cells (2 × 106 cells/mouse) intraperitoneally except for the normal group. This was considered as day zero. Group I served as the normal control and group II served as the tumor control. These two groups received normal saline. Group III, which served as the positive control, was treated with the suspension of 5-FU at 20 mg/kg body weight. Groups IV and V were treated with the MET at 250 and 500 mg/kg body weight doses, respectively. All these treatments were given 24 h after the tumor inoculation, once daily for 14 days. After the last dose and 24 h fasting, six mice from each group were sacrificed. The blood was collected from the animals by retro-orbital puncture under slight anesthesia (diethyl ether) condition; and the hematological parameters such as red blood cells (RBC), white blood cells (WBC), differential count, and hemoglobin content were determined [14]. The ascitic fluid was collected from the peritoneal cavity of the animals and divided into two parts. One part was centrifuged in a graduated centrifuge tube at 1,000 rpm for 10 min and the packed cell volume was measured. The cells in the other part of the ascitic fluid were separated by centrifugation and stained with trypan blue (0.4% in normal saline). The number of viable cells was counted. The rest of the animals were kept to check the average life span and change in body weight for 6 weeks. Percentage increase in life span (ILS) was calculated by the following formula: % ILS = [(Life span of treated group / Life span of control group) – 1] × 100.

Statistical analysis

The significance of the in-vivo data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. P<0.001 was regarded as significant.

RESULTS

Short term cytotoxicity

The GI50 value of MET against EAC was found to be 48.60µg/ml.

Effect of MET on antitumor parameters

The mean survival time of animals in tumor control group inoculated with EAC was for period of 17.5 days. The treatment with the MET at 250 and 500 mg/kg body weight increased the average life span of animals by 34.28 and 68.57 days, respectively (Fig. 2). Both 250 and 500 mg/kg body weight was found to be significant in increasing the life span. The MET at the 500 mg/kg body weight dose was found to be more potent in inhibiting the proliferation of EAC with the percentage increase in life span of 68.57%. The percent increase in body weight of the EAC tumor control group was found to be 45.09 ± 2.054%. The MET treatment at 250 and 500 mg/kg doses significantly inhibited the percent increase in body weight when compared to the tumor control ($P < 0.001$) The packed cell volume (ml), viable tumor cell count (×107 cells/ml), and total WBC (×103 /mm3) were found to decrease significantly in animals treated with MET at almost all the doses tested when compared to the EAC tumor control indicating the antitumor nature of the extract. Similarly, red blood cells (RBC) count, hemoglobin (HGB) content, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and platelets (PLT), and lymphocytes count which were decreased after EAC inoculation, were found to be significantly restored to the normal levels in the animals treated with the MET at all both doses (Table 1). The neutrophils count, which was increased in EAC tumor control animals was found to be decreased towards normal by MET significantly ($P < 0.001$) at all doses; which is well within the reference intervals [15]. All these results suggest the potent antitumor properties of the MET. However, the standard 5-FU treatment at 20 mg/kg body weight produced better results than the extract treatment in all these parameters.

Table 1. Effect of the MET on hematological parameters of EAC-bearing mice on day 15 of the experiment

Treatment	*Hb content*	*Total RBC (cells/ml×10^6)*	*Total WBC (cells/ml×10^3)*	*Differential count*		
				Lymphocyte (%)	*Neutrophil (%)*	*Eosinophils (%)*
Normal (0.9%Saline)	15.72 ± 0.302	11.255± 0.202	5.150 ± 0.295	80.05 ± 2.438	28.00 ± 0.51	4.75 ± 0.47
Control (2 × 10^6 cells/ml per mice	6.780 ± 0.694a	7.445±0.39a	20.12 ± 4.615a	35.75 ± 0.70a	64.83 ± 0.90a	5.75 ± 0.62
5-flurouracil (20mg/kg)	13.03 ± 0.368c	10.813± 1.158c	7.567± 3.316d	67.28 ± 3.020d	33.83 ± 2.18d	5.50 ± 0.64
MET (250mg/kg)	12.22 ± 1.680	10.564± 0.9321d	9.64±1.336d	62.71 ± 6.055d	35.25 ± 1.37d	5.57 ± 0.62
MET (500mg/kg)	11.09 ± 0.207b	9.701± 0.136647c	10.800 ± 0.558d	61.341± 5.180d	34.16 ± 1.44d	4.75 ± 0.47

Values are expressed as the mean ± S.E.M. for six animals in each group. a P<0.001: between normal and EAC tumor group values; b P<0.05, c P<0.01, and d P<0.001: between tumor control and treated groups.

DISCUSSION

Cancer is the second leading cause of death worldwide. There is an increase need for more effective and less toxic therapeutic and preventive strategies. Natural products are becoming an important research area for novel and bioactive molecules for drug discovery. Phytochemicals have been used for the treatment of cancer throughout history due to their safety, low toxicity, and general availability. Many active phytochemicals are in human clinical trials [16]. Of all the available anticancer drugs during 1940 – 2008, 40% were natural products or related to them, with another 28% being synthetic with natural product pharmacophores or natural product mimics [17;18]. Among all marine organisms, sponges represent one of the most promising sources of leads in the research of novel cancer drugs [19]. The greatest recent impact is observed in the area of antitumor research, where compounds cytarabine and eribulin have been approved for use in humans has dramatically improved the effectiveness of chemotherapy against some of the dreaded cancers [3]. Hence, there is a huge potential for the development of anticancer drugs from the essentially untapped reservoir of the Phylum Porifera. Sponges belonging to the genus Tedania and their constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world.

Ascitic tumor implantation stimulates local inflammatory reactions resulting in increase in vascular permeability, and results in intense edema formation, cellular migration and progressive ascitic fluid formation. Ascitic fluid is essential to tumor growth, since it constitutes the direct nutritional source for tumor cells. Decrease in viable cell count and increase in non-viable cell count in tumor bearing mice suggests antitumor activity against EAC cells in mice [20;21]. The reduction in packed cell volume and the number of viable EAC tumor cells in peritoneum in mice treated with MET when compared to the tumor control group suggested that extracts stimulate the growth and activity of immune cells by the production of Interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, increase in survival time and decreased volume of EAC could indicate either a direct cytotoxic effect of MET on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition [22].

The reliable criterion for judging the value of an anticancer drug is the prolongation of life

span of the animals and the decrease in leucocytes from blood [23]. The MET treatment decreased packed cell volume and viable tumor cell count. It also inhibited the increase in body weight due to the tumor burden and increased the average life span of animals when compared to the EAC control. Hence, it may be concluded that the MET with a direct cytotoxic effect or by decreasing the nutritional fluid volume and arresting the tumor growth, has increased the life span of EAC-bearing mice. The percentage increase in the life span at the 250 mg/kg body weight dose of the MET was found to be the highest among two doses tested indicating its potent anticancer nature. However, no toxic symptoms occurred in selected two doses during the period of study.

Usually in cancer chemotherapy, the major problems encountered are myeloid-suppressor and anemia due to reduction in RBC or HB content [24].Treatment with MET brought back the HB content, RBC and WBC count more or less to normal levels in the present study in animals of the EAC tumor control group (Table 1). This reversal of haematological parameters suggests that the MET possesses protective action on the hemopoietic system.

Preliminary phytochemical studies indicated the presence of steroids, fatty acids and tannins in the MET. Many such compounds are deemed to possess potent antitumor properties (13). The potent antitumor properties of the MET may be due to the presence of any of these phytoconstituents.

Table 2. Effect of the MET on tumor volume, tumor weight, mean survival time (MST), percentage increase life span (%ILS) in EAC-bearing mice

Group	Tumor Volume (ml)	Tumor weight (gm)	MST days	%ILS
EAC control	2.92 ±0.17	3.40±0.24	17.5	00.00
250 mg/kg	1.48 ±0.22 **	1.40±0.24 **	23.5	34.28
500 mg/kg	0.98 ±0.24 *	0.98±0.27 *	29.5	68.57
5-FU	0.52±0.21 **	0.49±0.12 **	34.5	97.14

n= 12 *P < 0.05, **P < 0.01,

CONCLUSION

The present study demonstrates the potent antitumor properties of the MET. Further investigation to evaluate the active ingredients and understating the mechanism of action will provide new scope of use of marine organism as a potent source of anticancer drugs.

REFERENCE

1. *Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.*
2. *Perez EA. Impact, mechanisms, and novel chemotherapy strategies for overcoming resistance to anthracyclines and taxanes in metastatic breast cancer. Breast Cancer Res Treat 2008;114(2):195-201.*
3. *Abolfazl Shakeri and Amirhossein Sahebkar. Anti-Cancer Products from Marine Sponges: Progress and Promise. Recent Patents on Drug Delivery & Formulation 2015;9(3):187-8.*
4. *Costantino V, Fattorusso E, Mangoni A, Perinu C, Cirino G, De Gruttola L, et al. Tedanol: A potent anti-inflammatory ent-pimarane diterpene from the Caribbean Sponge Tedania ignis. Bioorg Med Chem 2009;17(21):7542-7.*
5. *Schmitz FJ, Gunasekera SP, Yalamanchili G, Hossain MB, Van der Helm D. Tedanolide: a potent cytotoxic macrolide from the Caribbean sponge Tedania ignis. J Am Chem Soc 1984;106(23):7251-2.*
6. *Kim YH, Nachman RJ, Pavelka L, Mosher HS, Fuhrman FA, Fuhrman GJ. Doridosine, 1-methylisoguanosine, from Anisodoris nobilis; structure, pharmacological properties and synthesis. J Nat Prod 1981;44(2):206-14.*
7. *Schmidtz FJ, Vanderah DJ, Hollenbeak KH, Enwall CEL, Gopichand Y, SenGupta PK, et al. Metabolites from the marine sponge Tedania ignis. A new atisanediol and several known diketopiperazines. J Org Chem 1983;48(22):3941-5.*
8. *Dillman RL, Cardellina JH. Aromatic secondary metabolites from the sponge Tedania ignis. J Nat Prod 1991;54(4):1056-61.*
9. *Wright AE, Chen Y, Winder PL, Pitts TP, Pomponi SA, Longley RE. Lasonolides C-G, Five new lasonolide compounds from the sponge Forcepia sp. J Nat Prod 2004;67(8):1351-5.*
10. *Zeng Z, Zhao J, Ke C, Wang D. Antimicrobial activities of novel cultivable bacteria isolated from marine sponge Tedania anhelans. Chinese J Oceanol Limnol 2013;31(3):581-90.*

11. *Zeng Z, Zhao J, Ke C, Wang D. Antimicrobial activities of novel cultivable bacteria isolated from marine sponge Tedania anhelans. Chinese J Oceanol Limnol 2013;31(3):581-90.*
12. *Iwata Y, Tanino K, Miyashita M. Synthetic Studies of Tedanolide, a Marine Macrolide Displaying Potent Antitumor Activity. Stereoselective Synthesis of the C13-C23 Segment. Org Lett 2005;7(12):2341-4.*
13. *Macleod KG, Langdon SP. Essential techniques of Cancer Cell Culture. In: Langdon SP, (eds.),Cancer Cell Culture: Methods and Protocols, Humana Press, New Jersey, 2004; 17-29.*
14. *Briggs C, Bain BJ. Basic haematological techniques. In: Bain BJ, Bates I, Laffan MA, Lewis SM, (eds.),Dacie and Lewis Practical Haematology: Expert Consult: Online and Print. Elsevier Health Sciences, UK; 2011; 27-47.*
15. *Everds NE. The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models. In: Fox JG, Barthold S, Davisson M, Newcomer CE, Quimby FW, Smith A, (eds.),The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models. 2 ed. Elsevier Science, 2006; 133-70.*
16. *Poyil P, Chakkenchath S, Zhuo Z, Amit B, Songze D, Young-Ok S, et al. Cancer prevention with promising natural products: Mechanisms of action and molecular targets. Anticancer Agents Med Chem 2012;12(10):1159-84.*
17. *Demain AL, Vaishnav P. Natural products for cancer chemotherapy. Microb Biotechnol 2011;4(6):687-99.*
18. *Bala A, Kar B, Haldar PK, Mazumder UK, Bera S. Evaluation of anticancer activity of Cleome gynandra on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol 2010;129(1):131-4.*
19. *Thomas TR, Kavlekar DP, LokaBharathi PA. Marine drugs from sponge-microbe association- A review. Mar Drugs 2010;8(4).*
20. *Dongre SH, Badami S, Godavarthi A. Antitumor activity of Hypericum hookerianum against DLA induced tumor in mice and its possible mechanism of action. Phytother Res 2008;22(1):23-9.*
21. *Bisht M, Bist S, Dhasmana D. Biological response modifiers: Current use and future prospects in cancer therapy. Indian J Cancer 2010;47(4):443-51.*
22. *Clarkson BD, Burchenal JH. Progress in leukemias. Prog Clin Cancer 1965;10:625-63.*
23. *Gupta M, Mazumder UK, Kumar RS, Sivakumar T, Vamsi MLM. Antitumor activity and antioxidant status of Caesalpinia bonducella against Ehrlich ascites carcinoma in swiss albino mice. J Pharmacol Sci 2004;94(2):177-84.*