Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
3(3), 2014, 91-100

ISSN 2321 – 0958

Original Research Article

ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF *SONERILA TINNEVELLIENSIS* FISCHER WHOLE PLANT AGAINST DALTON ASCITES LYMPHOMA

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Abstract

The ethanol extract of whole plant of *S. tinnevelliensis* was evaluated for antitumor activity against Dalton's ascites lymphoma (DAL)-bearing swiss albino mice. The extract was administered at the doses of 200 and 400 mg/kg body weight per day for 14 days after 24h of tumor inoculation. Evaluation of the antitumor effect of ethanol extract of whole plant of *S. tinnevelliensis* on tumor growth and host survival time was made by the study of the following parameters: tumor volume, viable and non viable cell count and life span of host. The results showed decrease in tumor volume and viable cell count. Haematological studies revealed that, the Hb count decreased in DAL treated mice, whereas it was induced by the drug treated animals and showed an increase in Hb near to normal levels. The results suggested that the extract of whole plant of *S. tinnevelliensis* exhibited significant antitumor activity on DAL bearing mice.

Keywords: *S. tinnevelliensis*, Antitumor, DAL, tumor volume, HB, haematological profile.

Introduction

Cancer is the uncontrolled growth of abnormal cells in the body. This results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal systems that prevent cell overgrowth and the invasion of other tissues are disabled. These altered cells divide and grow, display uncontrolled growth, invasion and sometimes metastasis. According to a study by the World Health Organization, one in 12 women in urban India will develop cancer in their lifetime. Approximately 40 per cent of new cases of cancer in India afflict women. Cancer is one of the leading causes to death in the developed and developing countries[1]. Cancer accounted for 7.1 million deaths in 2003 and it is estimated the overall number of new cases will rise by 50% in the next 20 years [2].

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Received on: July 2014
Accepted after revision: August 2014

Experimental tumor models have a wide role in anticancer drug discovery. A Dalton's Ascites Lymphoma (DAL) tumorigenesis model in Balb/C/Swiss albino mice provides a convenient model system to study antitumor activity within a short time [3]. Following transplantation of DAL cells into the abdominal cavity of healthy recipient mice, tumorigenesis begins immediately and aggressively [4-5].

Within the scientific community, interest in natural compounds is increasing now a day, which is fuelled partly by well-documented limits and adverse effects of current chemotherapy drugs, as well as the ongoing search for better ways to fight the disease. Scientists are now developing newer drugs by using the natural basic skeleton of an isolated component that targets the unique makeup mechanism of cancer cells. A number of natural products have been studied still now for anticancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs [6] that is explored.

Plants are the rich source of medicines from ages. They produce bioactive molecules which can used to treat various types of disorders. Over the last few decades there has been increased interest by pharmaceutical industries to discover the new drugs from the ethnobotanicals to provide new and alternative drugs to synthetic drugs for treatment of dreadful diseases. Potent anticancer drugs like taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from plant sources and they are in efficient clinical use. Irinotecan[7] and topotecan[8] are two water-soluble derivatives of camptothecin, have been approved by the Food and Drug Administration (FDA) of the United States of America for treating colorectal and ovarian cancer [9-10].

Sonerila, with between 100 and 175 species [11], is the largest genus in the Sonierileae. It is the only consistently trimerous genus in the family and as such easily diagnosed. Leaf extract of *Sonerila tinnevelliensis* was orally administered to cure body swelling by kanikaran[12]. A handful of leaves consumed on an empty stomach once in a day for 12 to 15 days to get relief from rheumatic complaints[13-14]. Taking into consideration of the medicinal importance of *Sonerila tinnevelliensis*, the ethanol extract of the whole plant of *Sonerila tinnevelliensis* were analyzed for their anticancer activity against Dalton Ascites Lymphoma (DAL) tumor model

Materials And Methods

Collection of plant sample

Whole plant of *Sonerila tinnevelliensis* (STW) was collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu with the help of local flora. The specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for anticancer activity

The whole plants of *S. tinnevelliensis* were cut into small pieces, washed, dried at room temperature; the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered whole plant was separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of whole plant was used for anticancer activity.

Animals

Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microlon boxes in a controlled environment (temperature 25±20°c) and 12 hr dark/light cycle) with standard laboratory diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum*. The mice well segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Tumor cells

Dalton Ascites Lymphoma (DAL) cells were obtained from Division of Oncology Department of Biotechnology, Tamil Nadu, Veterinary and Animal Husbandry, Chennai, Tamil Nadu, India. The DAL cells were

maintained *in vivo* in Swiss albino mice by weekly intraperitoneal (i. p) inoculation of 10^6cells / mouse after every ten days. DAL cells 9 days old were used for the screening of the anticancer activity.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline – 420[15] fixed dose procedure for ethanol extract of whole plant of *S. tinnevelliensis* and it was found that dose increasing up to 2000 mg / kg body weight, shown no toxicity or mortality in experimental mice.

Antitumor activity

Healthy Swiss albino mice were divided into five groups of five animals (n=5) each. The test samples were dissolved in isotonic saline (0.9% NaCl W/V) and used directly in the assay. DAL cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable DAL cells were counted (Trypan blue indicator) under the microscope and were adjusted at 1 X 10^6 cells/ ml. 0.1 ml of DAL cells per 10g body weight of the animals were injected (i. p) to each mouse of each group except normal saline group (Group I). This was taken as Day 0. Group I served as a normal saline control (1mL/kg, p.o) and group II served as DAL bearing control. On day 1, the ethanol extract of *S. tinnevelliensis* (STW) at a dose of 200 and 400mg/kg each of the Group III, IV were administrated orally and continued for 14 consecutive days respectively. Group V served as tumor induced animal administrated with vincristine (80mg/kg body weight) for 14 consecutive days. On day15, half of the animals (n=3) in each case were sacrificed and the remaining animals were kept to observe the life span study of the tumor hosts. The effect of ethanol extract of *S. tinnevelliensis* on tumor growth and host's survival time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span [16-17].

Determination of tumor volume

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5min.

Determination of tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension as placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Estimation of viable and non viable tumor cell count (Tryphan blue dye assay)

The cells were then stained with tryphan blue (0.4% normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non viable. These viable and non viable cells were counted.

Percentage increase of life span (% ILS)

The percentage increase in life span (% ILS) was calculated from the following equation.

$$\textbf{Increase in life span} = \frac{T - C}{C} \times 100$$

Body weight

Body weights of the experimental mice we rerecorded both in the treated and control group at the beginning of the experiment (zero day) and sequentially on every 5th day during the treatment period.

Haematological studies

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Haemoglobin content(Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears[18].

Statistical analysis

The data were analyzed using student's t- test statistical methods. For the statistical tests, *p* values of less than 0.01, 0.05 and 0.001were taken as significant.

Result and Discussion

In acute toxicity studies animals treated with ethanol extract of *S. tinnevelliensis* did not show any toxic symptoms or mortality when dosed up to 2000 mg/kg body weight by oral route. This indicated that the extract was found to be safe at the tested dose level. Hence 200

mg/kg and 400mg/kg of this dose were selected for the *in vivo* studies. The present investigation indicates that ethanol extract of whole plant of *S. tinnevelliensis* showed significant antitumor activity in DAL bearing mice. Table-1 shows administration of ethanol extract of whole plant of *S. tinnevelliensis* to DAL bearing mice showed reduction in bodyweight (g), spleen, thymus, liver, kidney and lungs. The effects of ethanol extract of *S. tinnevelliensis* whole plant at the doses of 200 and 400mg/kg on solid tumor volume is shown in Table-2. Treatment with ethanol extract of whole plant of *S. tinneveliensis* and vincristine significantly ($P<0.01$) reduces the solid tumor volume in a dose dependent manner as compared to that of the DAL control group.

The effect of ethanol extract of *S. tinnevelliensis* whole plant at the doses of 200 and 400mg/kg on survival time (days), life span (%), packed cell volume, tumor cell count (viable and non viable cell) is shown in Table-3. In the DAL control group, the mean survival time was 19.80±0.11days, while it in increased upto 27.18±0.25 (200mg/kg) and 34.61±0.15 (400mg/kg) days respectively, in the ethanol extract of *S. tinnevelliensis* treated groups, whereas the standard drug vincristine (80mg/kg) treated group had a mean survival time of 34.90±0.55days. The percentage increase in life span, it was found to be 37.27%, 74.79% and 76.26% respectively as compared to DAL control group. Treatment with ethanol extract of *S. tinnevelliensis* whole plant significantly ($p<0.01$) reduced the packed cell volume and viable tumor cell count in a dose dependent manner as compared to that of the DAL control group. Furthermore, nonviable cell count at different doses of ethanol extract of *S. tinnevelliensis* was increased in a dose dependent manner.

As shown in the Table 4, the haemoglobin content in the DAL control mice (8.12g %) was significantly decreased when compared with normal mice (11.96 g %) ethanol extract of whole plant of *S. tinnevelliensis* at the doses of 200 and 400 mg/kg the haemoglobin content in DAL treated mice were increased to 12.56±0.23 and 13.84±0.18 moderate changes in the RBC count were also observed in the extract treated mice. The total WBC counts were significantly higher in the DAL control mice when compared with normal mice. Whereas, ethanol extract of whole plant of *S. tinnevelliensis* treated mice significantly reduced the WBC counts as compared to that of control mice. All these results suggest the anticancer nature of the extract. However, the standard vincristine at the dose of 80mg/kg body weight produced better result in all these parameters.

Cancer is a disease characterized by uncontrolled cellular growth, local tissue invasion and distant metastases and the free radicals have been implicated in carcinogenesis [18]. Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumor activity. The present study was carried out to evaluate the antitumor effect of ethanol extract of whole plant of *S. tinnevelliensis* in DAL bearing mice. The ethanol extract of whole plant of *S. tinnevelliensis* treated animals at the doses of 200 and 400 mg/kg significantly inhibited the tumor volume, packed cell volume, tumor cell count and brought back the haematological parameters to more or less normal levels. In DAL bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascites fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells [19]. Treatment with ethanol extract of whole plant of *S. tinnevelliensis* increased the percentage of tryphan blue positive stained dead cells in tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals [20]. The ethanol extract of whole plant of *S. tinnevelliensis* decreased the ascites fluid volume, viable cell count and increased the percentage of life span. It may be concluded that ethanol extract of whole plant of *S. tinnevelliensis* by decreasing the nutritional fluid volume and arresting the tumor growth,

this could be the reason for the increase life span of DAL bearing mice. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia [21]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions [22]. After the repeated treatment, ethanol extract of whole plant of *S. tinnevelliensis* able to reverse the changes in haematological parameters haemoglobin content, RBC and WBC counts near to normal levels. This indicates that ethanol extract of whole plant of *S. tinnevelliensis* is showing protective action on the haemopoietic system.

Plant derived compounds have played an important role in the development of several clinical useful anticancer agents [23]. Tetrahydrospirilloxanthin, Linolelaidic acid and methyl ester were reported in the ethanol extract of whole plant of *S. tinnevelliensis* by GC-MS analysis. These compounds may play a role in anticancer activity [24].

Therefore in conclusion the present investigation showed a decrease in cancer cell count, tumour volume, RBC counts as a confirmatory evidence for protection against DAL. Consequently increased WBC counts, life span, haemoglobin content were observed the extracts of whole plant of *S. tinnevelliensis* extract treated mice. Further studies to characterize the active principle and elucidate the mechanism of action of ethanol extract of whole plant of *S. tinnevelliensis* in progress using different cell lines. All these data point out the possibility of developing an ethanol extract of whole plant of *S. tinnevelliensis* as a novel, potential phytochemical in the field of cancer management.

Acknowledgement

The Authors wish to thank Dr. R. Sampatharaj, Honorary Advisor, Samsun Clinical Research Laboratory, Tirupur, for their assistance in animal studies.

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Table 1. Effect of *S. tinnevelliensis* whole plant on relative organ weights of tumor induced (DAL) and drug treated mice

Treatment	Relative Organ Weight (g/100g body wt.)					
	Body weight (g)	Spleen	Thymus	Liver	Kidney	Lungs
Normalcontrol(GroupI) (Saline)	19.28±0.56	0.39±0.014	0.21±0.013	3.18±0.64	0.91±0.04	0.54±0.011
Tumor induced (GroupII) control(Saline)	41.64±1.93**	0.58±0.026*	0.39±0.026*	4.93±0.29^{ns}	1.74±0.05*	0.87±0.053^{ns}
STW extract (200 mg/kg)+(GroupII) DAL	24.37±0.73^{nsa}	0.32±0.017^{a}	0.26±0.018^{a}	4.02±0.34	1.13±0.08	0.64±0.013
STW extract(400mg/kg)+(GroupIV)DAL	21.63±0.69^{aa}	0.38±0.013^{a}	0.28±0 .031^{a}	3.68±0.24	1.38±0.05	0.53±0.018
Vincristine (80 mg/kg)+ (GroupV)DAL	20.43±0.22^{aa}	0.28±0.019	0.23±0.014a	3.81±0.18	0.98±0.07	0.54±0.027^{a}

Each Value is SEM of 6 animals Significance between normal control, tumor induced control vs drug treated group

* p <0.05 ; ** p < 0.01 ; NS :Not significant : a p <0.05 ; aa p < 0.01- Significance between tumor induced control vs drug treated group

Table 2: Antitumor activity of *S. tinnevelliensis* whole plant extracts on solid tumor volume in tumor (DAL)induced mice

Treatment	Solid Tumor Volume			
	15th day	20th day	25th day	30th day
Normal control(GroupI)(Saline)	-	-	-	-
Tumor induced (GroupII) control(Saline)	4.22±0.15	4.81±0.28	5.64±0.20	6.93±0.15
STW extract (200 mg/kg)+(GroupIII) DAL	3.64±0.24^{ns}	3.06±0.18**	2.90±0.12**	2.54±0.23***
STW extract(400mg/kg)+(GroupIV)DAL	3.06±0.18	2.84±0.13*	2.61±0.34**	2.32±0.37***
Vincristine (80 mg/kg)+ (GroupV)DAL	2.73±0.15**	2.57±0.16**	2.26±0.13***	2.07±0.24***

Each Value is SEM of 6 animals. Significance between tumor induced control vs drug treated group * $p < 0.05$;** $p < 0.01$;* ** $p < 0.001$.NS :Not significant

Table 3 .Antitumor activities of *S. tinnevelliensis* whole plant on the survival time, life span, tumor volume and viable and non-viable cell count in tumor Induced mice

Treatment	Mean Survival time (Days)	Increase of life span(%)	Packed cell volume	Viable cell count X 10^6 cells/ml	Non-viable tumor cells count X 10^6cells/ ml
Normalcontrol(GroupI)(Saline)	-	-	-	-	-
Tumor induced (GroupII) control(Saline)	19.80±0.11	-	3.83±0.016	16.91±0.93	0.93±0.016
STW extract (200 mg/kg)+(GroupIII) DAL	27.18±0.25*	37.27	2.51±0.035^{ns}	8.65±0.14*	1.28±0.054ns
STW extract(400mg/kg)+(GroupIV)DAL	34.61±0.15**	74.79	1.58±0.013**	7.22±0.29*	1.93±0.016*
Vincristine (80mg/kg)+(GroupV)DAL	34.90±0.55**	76.26	1.64±0.018**	6.26±0.13**	2.26±0.011**

Each Value is SEM of 6 animals Significance between normal control, tumor induced control vs drug treated group

* p <0.05 ; ** p < 0.01 ; NS :Not significant : a p <0.05 ; aa p < 0.01- Significance between tumor induced control vs drug treated group

Table –4 Anticancer activity of *S. tinnevelliensis* whole plant extracts on hematological parameters in tumor(DAL)bearing mice

Parameter	Hb (gm%)	RBC (million/mm^3)	WBC $.0^3$ cells/ mm^3)	Differential count		
				Lymphocytes	Neutrophils	Eosinophil
Normal control(GroupI)(Saline)	11.96±0.12	3.13±0.27	9.16±0.88	52.12±2.11	43.16±1.13	4.72±0.14
Tumor induced (GroupII)control(Saline)	8.12±0.16*	2.57±0.12*	13.86±0.36*	43.84±1.26	50.18±1.12*	5.98±0.26
STWextract(200mg/kg)+(GroupIII)DAL	$12.56±0.23^{ns}$	3.66±0.63	10.15±0.25	51.13±1.84	42.27±1.03	6.60±0.17*
STWextract(400mg/kg)+(GroupIV)DAL	$13.84±0.18^{a}$	$4.26±0.76^{a}$	$9.63±0.18^{a}$	$53.26±1.36^{ns}$	$40.16±0.93^{a}$	$6.98±0.11^{ns}$
Vincristine (80 mg/kg)+ (GroupV)DAL	$13.76±0.36^{a}$	$4.05±0.74^{a}$	$7.84±0.17^{a}$	$53.13±0.91^{ns}$	44.28±0.76	$2.59±0.15^{a}$

Each Value is SEM of 6 animals Significance between normal control, tumor induced control vs drug treated group * $p < 0.05$;** $p < 0.01$; NS :Not significant : a $p < 0.05$; aa $p < 0.01$- Significance between tumor induced control vs drug treated group