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Anticancer Evaluation of *Artemisia nilagirica*

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ABSTRACT : Cancer is still considered as one of the most dreadful disease despite the current improved method of precaution, surgery and therapy. Plants have provided to mankind a large variety of potent drugs to alleviate suffering from diseases. In spite of spectacular advances in synthetic drugs in recent years, some of the drugs of plant origin have still retained their importance. Phytochemical Screening of *Artemisia Nilagirica* (Clarke) was carried out to investigate its active constituents. Methanolic extract of *Artemisia Nilagirica* (Clarke) was evaluated for its anticancer activity in Swiss albino mice. A result indicates that the plant has good anticacancer potential and was comparable with standard.
Key words: *Artemisia Nilagirica,* Phytochemical Screening, Methanolic extract, Anticancer potential.

INTRODUCTION

In recent year, much effort has been applied to the synthesis of potential anticancer drug. However, a vast amount of synthetic work has given relatively small improvements over the prototype drug. There is a continued need for new prototypes new templates to use in the design of potential chemotherapeutic agent. Natural products are providing such templates. Recent studies of tumor inhibiting compound of plant origin have yielded an impressive array of novel structure. So, in present study anticancer activity of *Artemisia Nilagirica* was evaluated. *Artemisia Nilagirica (*Asteraceae) is the aromatic shrub found throughout the mountains districts of India. *A. Nilagirica (Clarke)* wile shows reasonably high potency against plasmodium falciparum.[1,2] It is also said to be anthelmintic, antiseptic and expectorant, leaves & flowering tops are bitter, astringent, aromatic, anti inflammatory, appetizer, digestive and diuretic. Also use in cough, asthma, nervous and leprosy.[3, 4]

MATERIAL AND METHOD

Plant *Artemisia Nilagirica* (Clarke) pamp belonging to the family *Asteraceae*, is an herb commonly found in hilly districts.

Extraction

The leaves and flowering top of *Artemisia nilagirica (Clarke)* was dried under shade and then crushed into powder with a mechanical pulveriser and passed through sieve No. 8 and then sieve No-16.The dried powder material of plant was extracted with 95% methanol in soxhlet apparatus about 85 hours. The solvent was removed by vacuum distillation under reduced pressure .

Phytochemical Screening

The methanol extract of *Artemisia nilagirica (Clarke)* subjected to various color reaction to identify the nature of the components.[5-12]

Test for Alkaloids

The small portion of solvent free methanol extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) Dragendorffs reagent (orange brown precipitate) Hager's reagent yellow precipitate and Wagner's reagent (reddish-brown precipitate).

Test for Carbohydrates and Glycosides

Small quantities of methanolic extract were dissolved separately in 5 ml of distilled water and filtered. The filtrate may be subjected to Molisch's test to defect the absence of carbohydrates.

Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in water-bath and was subjected to Liebermann- Burchard's, legal and Borntrager's test to defect absence of different

glycosides. (Pink to red color indicates presence of glycosides)

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4. A yellow coloration absorbed in extract indicated presence of flavonoids. The yellow coloration disappeared on standing.

Test for Steroids

(2ml) Two ml of acetic anhydride was added to 0.5 g methanolic extract with 2 ml H_2SO_4. The color changed from violet to blue or green in samples indicated presence of steroid.

Test for Terpenoids (Salkowski Test)

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml), was carefully added to form a layer. A reddish brown coloration of the interface was formed indicated presence of terpenoids.

Test for Saponin

About 1 ml of alcoholic extract was diluted with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.

Test For Tannin

Vanillin-hydrochloric acid test (Vanillin 1 g, alcohol 10 ml, concentrated hydrochloric acid 10 ml). When a drug is treated with vanillin-hydrochloric acid reagent, pink or red color is formed due to formation of phloroglucinol.

Test for Protein

Mellon's reaction: Million's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution which turns red on heating. This reaction is characteristic of phenols (e.g. the phenolic amino acid tyrosine).

Test for Volatile Oil or Essential Oil

Place a thick section of drug on glass slide. Add a drop of Sudan red 3rd reagent and after two minute wash with 50% alcohol mount in glycerin. In microscope oil globule appearance red colors.

ANTICANCER ACTIVITY

Materials and Method

Animals

Swiss albino mice weighing between 20-25 gm were were fed with standard pellet diet of water and *ad-libitum*. The mice were acclimatized and laboratory condition for 10 days before commencement of experiment.

Plant Material

Artemisia nilagirica procured commercially and authenticate by Medicinal Plants Authentication Committee of RBPMPC, Atkot.

Cancer Cell line and Tumor Transplantation

EAC cells were maintained by weekly intraperitoneal inoculation of 10^6 cells / mouse. Ehrlich's Ascitic carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. Ascitic fluid was drawn out from tumor bearing mice at the log phase (day 7-8 of tumor bearing) of the tumor cells. The freshly drawn fluid was diluted with ice cold normal saline (0.9%) and the tumor cell number was adjusted to $2X10^6$ tumor cells / ml. Each animal received 0.3 ml of tumor cell suspension containing 2 X 10^6 cells / ml intrperitoneally.[13]

Drug Treatment Schedule

Swiss albino mice were divided into 5 groups (n = 6). First normal group, control group, third standard group and fourth and fifth group of MEAN with doses 150 mg/kg and 250 mg/kg respectively. All the groups were injected with EAC cells (0.3 ml of 2 X 10^6 cells / mouse) intraperitoneally except the normal group. This was taken as day zero. From the first day normal saline 5 ml / kg / mouse / day and propylene glycol 5 ml / kg / mouse / day was administered to normal and EAC control groups respectively for 16 days orally. Standard drug vincristine (30 mg/kg) administered in third group. Similarly MEAN at different doses (150 mg and 250 mg / kg / mouse / day) were administered in groups fourth and fifth respectively, after the administration of last dose followed by 18 hrs, fasting three mice form each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival time (MST) and percent increase in life span of the tumor bearing hosts.[15-18] Various parameters like Body weight of animals, Life span of animals, Cytological studies on cell lines, Hematological parameter, Biochemical parameters.

Tumor growth response

Anticancer effect of MEAN was assessed observation of change with respect of body weight, Ascitic tumor volume, packed cell volume, viable and non viable tumor cell count, mean survival time (MST) and percentage increase in life span (%ILS).[14,15,16]

Tumor cell volume and packed cell volume

The mice were dissected for collecting ascetic fluid from peritoneal cavity. The transplantable murrain tumor was carefully collected with the help of 5 ml sterile syringe and measured the tumor volume and the ascitic fluid was withdraw in graduated glass centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.[14,15,16]

Viable and non viable cell count

For viable and non viable cell counting the ascitic cell were stained by the tryphan blue (0.4% in normal saline), dye exclusion test and count was determined in

a neubauer counting chamber. The cells that did not take up they dye were viable and those that took the stain were non viable. [14,15,16]

Mean survival time and percent increased in life span

The effect of MEAN on tumor growth was observed by MST and % ILS. MST of each group containing 5 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation. [14, 15, 16]

MST = (Day of first death & Day of last death)/2

$$\% \text{ ILS} = \left\{ \frac{\text{MST of treated group}}{\text{MST of control group}} \right\} - 1 \text{ X } 100$$

Effect of MEAN on Hematological parameters

In order to detect the influence of MEAN on the hematological status of EAC bearing mice, or comparison was made among five groups each of three mice on the 15th day after inoculation. Blood was collected from each mice by intracardial puncture with blood anticoagulant (EDTA) and white blood cells (WBC), red blood cells (RBC); Hemoglobin and differential count were determined. [17, 18]

RESULT AND DISCUSSION

The phytochemical studies revealed the presence of flavonoids, steroids, terpenoid, saponins, tannins, proteins and essential oil. (Table 1)

Anticancer Activity

A. Nilagirica increases Mean survival time and ILS% (increase life span) of test animals when administered at different doses (150 mg/kg, 250 mg/kg) were shown in table 2. Administration of methanolic extract of *Artemisia nilagirica (Clarke)* (MEAN) reduces the tumor volume, packed cell volume and viable tumor cell count in a dose dependant manner when compared to EAC control mice (Table 3). In EAC control mice the median survival time was 22±0.25 days, whereas, there was significant increased in median survival time (24±0.33, 29±0.49, 31±0.55) with different doses 150 mg/kg, 250 mg/kg of MEAN and standard drug vincristine 30 mg/kg respectively.

Table 1:

Phytoconstituent	Steroids	Flavonoids	terpenoids	Saponin	Tannin	Protein	Essential oil
Ethanol Extract	+	+	+	+	+	+	+

Table 2: Effect of Ethanol Extract of *A.Nilagirica* on survival time on EAC bearing mice

S. No.	Experimental groups	Mean survival time (MST) days	% increase in life span
1	Normal control (normal saline 5 ml/kg b.w.)	-	-
2	EAC control	22±0.25	-
3	EAC + EEAV (150 mg /kg)	24±0.33	9.09
4	EAC + EEAV (250 mg / kg)	29±0.49	31.81
5	EAC + Vincristine	31±0.55	40.90

Values are mean ±SEM , (n =4), EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.01$, $^*P < 0.05$

Table 3: Effect of Ethanol extract of *A. Nilagirica* on tumor volume, packed cell volume, viable and non viable tumor cell count of EAC bearing mice.

Parameters	EAC control	MEAN 150 mg / kg	MEAN 250 mg/kg	Standard vincristine
Body weight	26.11±0.12	24.34±0.16	23.28±0.13	23.9±0.02
Tumor volume (ml)	5.82±0.042	4.22±0.051	3.42±0.082	2.42±0.13
Packed cell volume (ml)	2.12±0.104	1.75±0.043	1.05±0.092	1.15±0.03
Viable tumor cell count % 10^7 cells /ml	11.25±0.098	7.78±0.18	4.85±0.23	4.90±0.015
Non viable tumor cell count X 10^7 cells / ml	0.5±0.017	0.92±0.023	1.47±0.021	1.23±0.81

Values are mean ±SEM , (n =4), EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.01$, $^*P < 0.05$

Table 4: Effect of Ethanol Extract of *A.Nilagirica*on hematological parameters of EAC treated mice

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + MEAN 150 mg/kg	EAC + MEAN 250 mg / kg	EAC Cell + Vincristine
Haemoglobin (gm)	12.85±0.25	9.8±0.02	10.6±0.057	11.45±0.18*	11.7±0.045*
Total RBC million/mmcu	6.65±0.18	3.8±0.035	4.75±0.032	5.42±0.22*	5.8±0.054
Total WBC Million/mmcu	7.8±0.045	20.07±0.068*	11.92±0.042	8.85±0.059	9.12±0.055
Lymphocyte	77.75±0.19	33.37±0.56*	52.7±0.50*	60.72±0.36*	59.12±0.30
Monocyte	1.7±0.035	0.82±0.024	1.15±0.014*	1.2±0.045	1.32±0.024
Granulocyte	29.97±0.46	52.6±0.37*	40.87±0.2	31.72±0.63*	41.65±0.29

Values are mean ±SEM , (n =4), EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.01$, $^*P < 0.05$

As shown in table 4 the hemoglobin contents in the EAC control mice (9.8±0.02) was significantly decreased when compared with normal mice (12.85±0.25) MEAN at the dose of 150 mg/kg and 250 mg/kg, the hemoglobin content in EAC bearing mice were increased to 10.6±0.057 and 11.45±0.18. Moderate changes in RBC count were also observed in extract treated mice. The total WBC count was significantly higher in the EAC treated mice when compared with normal mice. Whereas MEAN treated mice significantly reduced the WBC count as compared to that of control mice. Significant changes were observed on biochemical parameter when extract treated mice compared with EAC control mice. (Table 5) Hence, it is evident that methanolic extract has very good anticancer activity.

Table 5 : Effect of different doses of methanolic extract of A.Nilagiricaon different biochemical parameter in EAC bearing mice.

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + MEAN 150 mg/kg	EAC + MEAN 250 mg / kg
Lipid peroxidation (mole MDA/gm of tissue)	0.92±0.02	1.36±0.09*	1.27±0.04*	1.13±0.02
Catalase (units /mg tissues)	2.51±0.72	1.71±0.15*	1.75±0.13	2.34±0.23*
Protein content (gm / 100 ml)	12.66±0.69*	17.25±0.76	16.50±0.70	16.10±0.55

Values are mean ±SEM , (n =4)
EAC control group compared with normal group, Experimental group compared with EAC control. $P < 0.05$, *P< 0.05

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