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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTICANCER, ANTIOXIDANT ACTIVITY OF CRUDE LEAF EXTRACT OF VITEX NEGUNDO LINNIN EXPERIMENTAL ANIMAL

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ABSTRACT

The anticancer and antioxidant activity crude leaf extract of vitex negundo linn evaluated against Dalton's ascetic lymphoma cell line and antioxidant activity for measurement of free radical scavenging activity (RSA) [reactions with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and assessment of the influence of extracts on the enzyme xanthine oxidase (XO), and Fe3+ reducing ability. Anticancer activity was performed on swiss albino mice at the dose of 250, 500 and 1000 mg/kg. body weight. The experimental parameters used were tumour volume, tumour cell count, viable tumour cell count, mean survival time and increase in life span to assess antitumour activity. The extract administered orally for 14 consecutive days to tumor bearing group of animals. The extract increase the life span of DAL treated mice and restore the hematological parameters as compared with the DAL bearing mice in dose dependant manner. The study revealed that the EVN showed significant antitumour activity in tested animal models. Antioxidant assessment shows significant RSA and XO and extract were able to reduce fe3+.

KEYWORDS: Anticancer, Antioxidant, RSA, Xanthine oxidase, DPPH, Vitex Negundo linn.

INTRODUCTION

Managements of cancer are a global problem and successful treatment is very much essential preventing or at least delaying the onset of long term complication of the disorder. Remedies to treat such chronic state are available in nature in the form of herbal medicine or drug which is minimal adverse effect when compared to available synthetic drugs.[1] Such herbal

drugs as therapeutic agent are a boon when compared to the severe adverse effect of the allopathic medical practice for cancer, though the quest for a complete and permanent cure for the diseases is being pursued relentlessly by eluding physician and researcher.

The benefits from achieving our goal will be to reduce the cancer mortality rate and improve the quality of life of those who develop cancer in the future.

The aim of research thesis is to identify and evaluate new, more effective, natural active principal.

The aim of projects is to identify new cancer drugs. We plan to do this by examining recently discovered biological targets that are predicted to offer the opportunity to develop drugs that are more potent and less toxic that the existing therapies. For instance, the reference with some targets is predicted to kill cancer cell without affecting normal ones and therefore the resulting drug is expected to have few side effects.

MATERIAL AND METHODS

Plant materials and extraction[1,2]

The leaves of Vitex nigundo, were collected from Nimar region of Madhya Pradesh, India in the month September 2008 and identified and authenticated at Govt. PG College, Dept. Of Pharmacognosy, Mandleshwar. A voucher specimen has been kept in Govt. PG College, Dept. of Pharmacognosy. The air-dried and coarsely powdered leaves (600gm) extracted with soxhlet apparatus using petroleum ether within 72 hour. The extracts were found brown and semisolid in nature.

Animals

The experimental protocol was approved by IACUC of Nimar Institute of Pharmacy, Dhamnod and Mature male Swiss albino mice weighing 20-25g were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. They were allowed free access to water, standard laboratory chow (Patanjali Pvt. Ltd Haridwar,) given food and water *ad libitum*. After sufficient period of acclimatization, they were used to evaluate anticancer activity.

Tumour Cell Line

Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of the Cancer Research Centre, Indore, India. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 1 x 106 cells/mouse.8.

Antitumor activity in mice

After acclimatization, mature male Swiss albino mice divided into five groups (n=10) and given food and water *ad libitum.* All the groups (Table 1) except group I were injected with DAL Cells (1×106 cells/mouse.i.p.). This was taken as day 0. Group I served as normal saline control (5 ml/kg, p.o.) and Group II served as DAL control. On day 1, the EVN at a dose of 250, 500 and 100 mg/kg body weight (Gr-III , IV & V) were administered orally and continued for 14 consecutive days.[3,4] The dose of EVN was selected based on previous study on hepatoprotective activity.10 On day 15, five mice of each group were sacrificed 24 h after the last dose and the rest were kept with food and water *ad libitum* to check the increase in the life span of the tumor hosts. The effect of ethanol extract on tumor growth and host's survival time were examined by studying the parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span.[3,4,5]

Determination of tumour volume

The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume determined by centrifuging at 1000 g for 5 min.[5,6]

Determination of tumour cell count

The ascetic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neuberger counting chamber and the number of cells in 64 small squares was counted.[7]

Estimation of viable tumour cell count

The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and non viable cells were counted.[6,7,8]

Cell count = (No. of cells x Dilution) / (Area x Thickness of liquid film).

Percentage increase life span

Recording the mortality monitored the effect of the EVN on tumor growth and percentage increase in life span (ILS %) were calculated.[9,10]

ILS (%) = [(Mean survival of treated group/ Mean survival of control group)-1] x100
Mean survival time = [1st Death + Last Death] / 2

Hematological studies

The effect of EVN on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin were done by standard procedures from freely flowing tail vein blood. Serum protein conc. was estimated by Lowry's method and packed cell volume (PCV) was determined by the method described by Docie et al.[11,12]

Antioxidant activity

For measuring radical scavenging activity[13,14] (RSA) against the stable radical N,Ndiphenyl-N'-picrylhydrazyl (DPPH), 0.1 and 0.2 ml of plant extract was added to 2.9 ml of DPPH 10-4 M solution in ethanol and the absorbance (A) was measured at 517 nm after 30 min incubation at 30°C (Brand-Williams et al., 1995). RSA was calculated in percent by the following formulae:

RSA = (Acontr. - Asample / Acontr. - Ablank) × 100.

Inhibition of xanthine oxidase was expressed as decreasing of uric acid generation (Noro et al., 1983). The mixture of 2.6 ml of 0.225 M xanthine solution in 0.65 M PBS (pH=7.4) with 0.1 ml and 0.2 ml of plant extract (30 mg ml-1) in ethanol (control ñ 0.1 ml ethanol) was incubated 5 min at 37°C. Afterwards 0.2 ml of XO (0.15 U ml-1) in 0.65 M PBS (pH=7.4) was added and absorbance (A) at 290 nm was measured after 5 min. The inhibition was calculated in percent by the formulae.[14,15,16]

IE = 100 × (Asample - Acontrol)/Asample.

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of Oneway ANOVA test. P value of <0.05 was considered as statistically significant.

RESULTS

On the previous study and the current study shows the significant anticancer and antioxidant activity of vitex negundo linn leaf extracts. The Phytochemical findings revealed the presence of flavanoids, glycosides, alkaloids, saponins.[2,3] The anticancer activity revealed decrease in mortality rate in EVN treated group as compared to non treated group. The increase in plant extract concentration does not shown any significant increase in activity. The effect of EVN on tumour volume was significant. Viable cell count in controlled (group II) was significantly decreased while non viable cell count was significantly increased in EVN treated group. Haematological parameter was of group II was altered from normal group on day15. WBC, protein and PCV parameter were decrease and RBC and haemoglobin parameter was increase in EVN treated group. EVN extracts reflects the activity towards week free radicals and ability to decrease ferric ions into ferrous along with inhibition of XO in both concentration.

Table 1. Effect of Ethanolic extracts of Vitex negundo on survival time, life span, tumour volume, viable and non-viable cell count in DAL bearing mice.

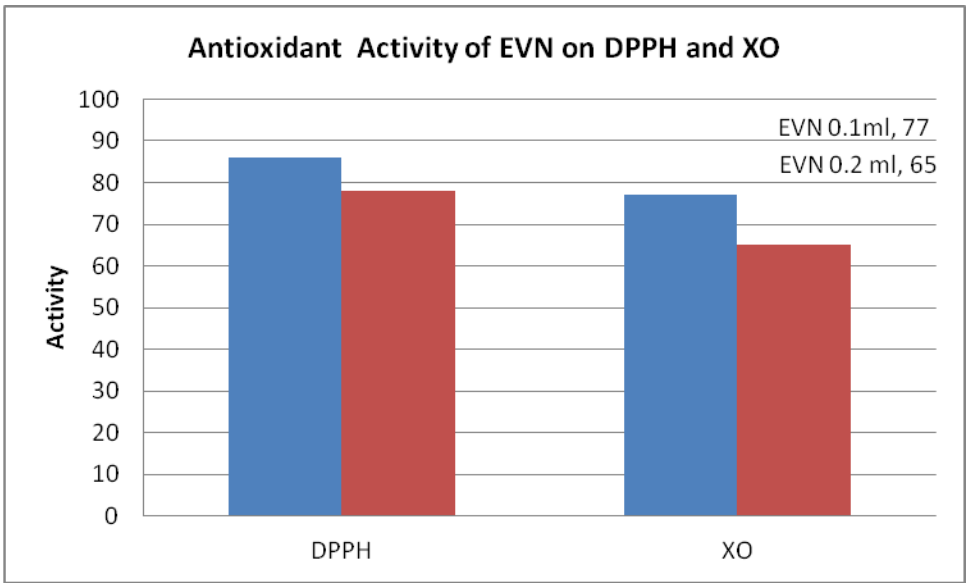
Treatment group	Survival time	Increase of life span	Tumour volume	Viable cell count x 10^6 cells/ml	Non-Viable cell count x 10^6 cells/ml
Normal Saline (5 ml/kg P.O)	-	-	-	-	-
DAL control (1 x 10^6 cells)	22.40±0.20	-	3.80±0.80	10.46±0.20	3.89±0.20
DAL control (1 x 10^6 cells) + EVN (250 mg/kg p.o)	33.61±1.02	46.90±1.56	3.00±0.01	7.50±1.05	2.89±1.20
DAL control (1 x 10^6 cells) + EVN (500 mg/kg p.o)	39.40±1.02	71.80±0.50	1.89±2.05	4.50±1.02	1.89±0.105
DAL control (1 x 10^6 cells) + EVN (1000 mg/kg p.o)	41.89±0.80	74.50±0.90	1.60±0.20	2.5±0.10	2.46±0.20

Table 2. Effect of EVN extracts on haematological parameter in DAL bearing mice.

Treatment	Hb(g%)	RBC (Million/mm^3)	WBC (10^3cells/mm^3)	Proteins (g%)	PCV (mm)	Differential count %		
						Lymphocytes	Neutrophils	Monocytes
Normal Saline (5 ml/kg P.O)	14.2±0.2	6.40±0.2	7.8±0.2	9.40±0.2	20.20± 0.2	70.2±1.20	29.80±1.20	2.10±0.2
DAL control (1 x 10^6 cells)	6.80±0.2	3.80±0.5	14.50±0.5	16.80±0.2	31.50± 1.50	40.106±1.06	68.6±1.6	4.80±0.2
DAL control (1 x 10^6 cells) + EVN (250 mg/kg p.o)	11.20±0.5	5.90±0.2	10.50±1.02	13.50±1.0 6	24.50± 1.00	46.20±0.2	41.1±0.6	3.1±0.6
DAL control (1 x 10^6 cells) + EVN (500 mg/kg p.o)	13.50±0.20	6.10±0.2	9.20±0.2	11.20±0.9	19.89± 1.06	51.60±1.6	38.20±1.80	2.4±0.2
DAL control (1 x 10^6 cells) + EVN (1000 mg/kg p.o)	13.80±0.90	6.50±0.5	8.22±1.02	10.80±0.2	17.50± 0.2	55.20±1.8	38.00±0.2	2.0±0.5

Statistical significance calculated by one way ANOVA followed by Dunnett's test.

Table 3. Effect of EVN extract on XO and DPPH



DISCUSSION

The Phytochemical study indicated the presence of flavonoids, Alkaloids and terpenoids in EVN. Flavonoids have been shown to possess antimutagenic and antimalignant effects. The above results demonstrated the antitumour effect of EVN against DAL is Swiss albino mice. A significant ($P<0.05$) enhancement of MST and non-viable cell count in peritoneal exudates ($P<0.05$) was observed due to EVN treatment. To evaluate whether EVN treatment indirectly inhibited tumour cell growth, the effect of EVN treatment was examined on the viable & non-viable cell counts against tumour bearing mice. Normally, each mouse contains about 5 x 106 intraperitoneal cells, 50% of which are macrophage. EVN treatment was found to enhance nonviable cell counts in peritoneal exudates and decrease the viable cell count. EVN extracts increase the level of haemoglobin and RBC to normal level while decrease the WBC, protein, and PCV as compared to control group although there is anaemia which usually occurred in cancer chemotherapy. The presence of flavanoids and antioxidant activity of Vitex negundo linn shows the possible potant therapy for cancer treatment. Further need to characterize the active principal and elucidation of mechanism of action behind the ethnolic extracts.

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