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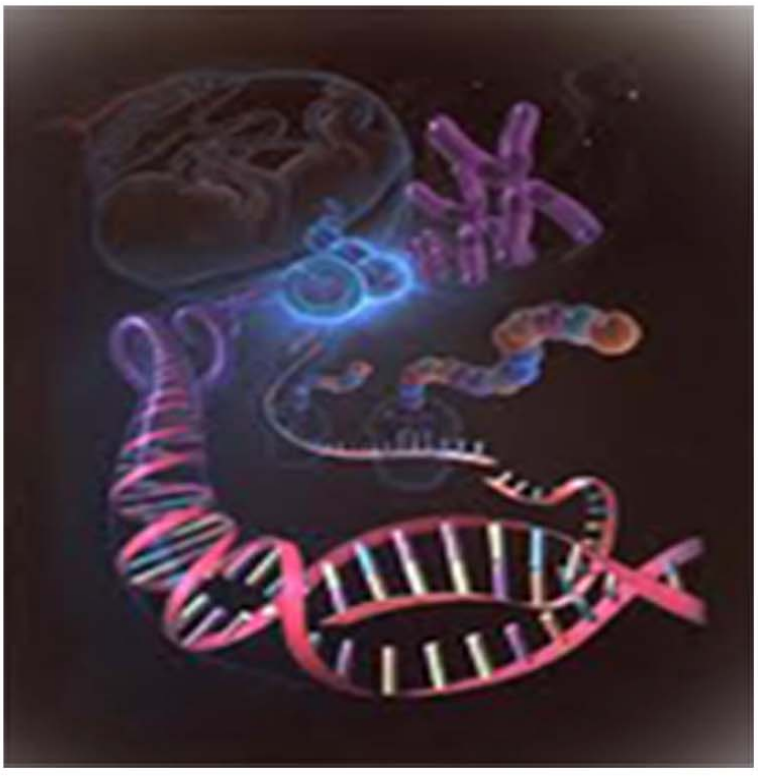

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*Research Paper*

# HAEMATOLOGICAL AND CYTOTOXIC EFFECT OF THE ETHANOLIC EXTRACT OF *VITEX NEGUNDO*

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Cancer is a class of disease in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate from benign tumors, which are self-limited and do not invade or metastasis. Most cancers form a tumor but some, like leukemia, do not. Cancer may affect all ages. Even fetuses but the risk for most varieties increases with ages Cancer is a major public health burden in both developed and developing countries. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively. So the present study was designed to analyze the anti-cancer potential of the *Vitex negundo* extract against Ehrlich's Ascite Carcinoma (EAC) Cell line (In vivo).The ethanolic extract of *Vitex negundo* extract treatment showed decrease in tumor volume, packed cell volume and viable cell count and increases the nonviable cell count and mean survival time (MST), thereby increasing life span of EAC tumor bearing mice. Hematological profile reverted to more (or) less normal levels in extract treated mice. The data presented here clearly encourage the development of *Vitex negundo* for chemopreventive agent.

***Keywords:*** *Vitex negundo, Chemoprevention*

## INTRODUCTION

Cancer is a class of disease in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate from benign tumors, which are self-limited and do not invade or metastasis. Most cancers form a tumor but some, like leukemia, do not. Cancer may affect all ages. Even fetuses but the risk for most verities increases with ages (ACS, 2005).

Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002 (Parkin *et al.,* 2005). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity (Madhumitha *et al*., 2004).

Medicinal plants have not been exploited to their

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potential, as far as treatment of cancer is concerned. The study of anticancer medicinal herbs has been done at animal levels. The need of the hour is to investigate the herbal remedies with open mind so as to find potent remedy for the treatment of cancer (Kantrajian and Talpaz, 2000).

According to World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary healthcare system. The synthetic anticancer remedies are beyond the reach of common man because of cost factor. Medicinal herbs are commonly available and comparatively economical. Medicinal herb is a biosynthetic laboratory, for chemical compounds like glycosides, alkaloids, resins, oleoresins (Kantrajian and Talpaz, 2000).

The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious preparations drugs of natural origin have a share of 60% and 75% respectively (Ranaabu-Dahab and FtmaAfifi, 2007).

*Vitex negundo* grows all over India, in wastelands, upto 1500 meters elevation. Commonly it is cultivated as a hedge plant. A large shrub or rather small tree grows 2-4 meters in height, with quadrangular branches and thin grey bark. The leaves petiolate, smooth, exstipulate, have a typical pungent odor. The flowers are bluish purple in color, lanceolate, in panicles upto 30 cm long. The fruits are ovoid or obovoid, four-seeded drupes, black when ripe. (Chopra R.N *et.al.,*(1956)The leaves are astringent, febrifuge, sedative, tonic and vermifuge. They are useful in dispersing swellings of the joints from acute rheumatism and of the testes from suppressed gonorrhea. The juice of the leaves is used for removing foetid discharges and worms from ulcers, whilst oil prepared with the leaf juice is applied to sinuses and scrofulous sores. A decoction of the stems is used in the treatment of burns and scalds (Chaturvedi *et al.,* 1965).

The plant is said to be a malarial preventative and is also used in the treatment of bacterial dysentery – extracts of the leaves have shown bactericidal and antitumor activity. The leaves are used to repel insects in grain stores. Extracts of the leaves have insecticidal activity. The fresh leaves are burnt with grass as a fumigant against mosquitoes (Hansal *et al.,* 1965)

The present study was designed to analyze the anticancer potential of the *vitex negundo* extract against Ehrlich's Ascite Carcinoma (EAC) Cell line (*In vivo*).

## MATERIALS AND METHODS

### Collection and Preparation of *Vitex negundo* Sample

The Leaf of *vitex negundo* were collected from the coimbatore district and authenticated in Botanical Survey of India, Coimbatore. Tamil Nadu, India. The leaves were washed under running tap water, dried and cut into small pieces. The leaves were shade dried for 30 days. Then homogenized to get a coarse powder. This powder was stored in an air tight container and used for further successive extraction.

### EXTRACTION OF *vitex negundo*

The method used in the extraction of active constituents from *vitex negundo* was the continuous hot percolation method. Extraction can be defined as the removal of soluble material from an insoluble residue either by solid or liquid

treatment with a liquid solvent. The raw material was placed in a thimble and inserted into the wide extractor. Solvent was placed in the flask and brought to its boiling point. Then the extract was collected from the flask. The extract was filtered while hot and the resultant was distilled in vacuums in order to remove the solvent completely.

## Antitumor Effect of the Ethanolic Extract of *vitex negundo*

The Swiss albino mice were obtained from KMCH Pharmacy College, Coimbatore. The animals were kept in standard cages and were maintained at (27 ± 2°c) under 12 h light/12 h dark cycle. They were fed with standard laboratory pellets, Hindustan Lever Ltd. India) double deionised water and were given *adlibitum.* They were given a week's time to get acclimatized to the laboratory conditions.

## Collection of Cell Line

The Ehrlich's Ascites Carcinoma cells were collected from Amala Cancer Institute, Kerala, India.

## Antitumor Activity

After acclimatization, mature male swiss albino mice divided into four groups (n=6) and given food and water *ad libitum.* All the groups (Table 1) except group I were injected with EAC cells ($1 \times 10^6$ cells/mouse.i.p.). This was taken as day 0. Group I served as normal saline control (5ml/kg, p.o.) and group II served as EAC control. On day1, the EVN (Ethanolic extract of *Vitex Negunda*) at a dose of 250 and 500 mg/kg body weight (Gr- III & IV) were administered orally and continued for 14 consecutive days on day 5, four mice of each group were sacrificed 24 h after the last dose and the rest were kept with food and ad libitum to check the ILS 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, ILS (Increase of Life Span), MST (Mean Survival Time), viable & non-viable cell count, PCV (Packed Cell Volume).

**Group I**: Served as control group, receiving normal saline (5ml/kg.p.o.) for a period of 15 consecutive days.

**Group II** : Served as EAC control

**Table 1: Level of Ethanol Extract of The *Vitex negundo* on Tumor Volume, Packed Cell Volume, Survival Time, Increase of Life Span, Viable and Non-viable Cell of Eac Bearing Mice**

| Treatement Group | Survival Time (Days) | Increase of Life Span (%) | Tumor Volume (Ml) | Viable Cell Count X $10^6$ Cells/Ml | Non-viable Cell Count X $10^6$ Cells/Ml |
|---|---|---|---|---|---|
| GROUP-I Normal saline (5ml/kg) | - | - | - | - | - |
| GROUP-II EAC control ($1x10^6$cell) | 21.40 ± 1.41a* | 0 | 3.68 ± 0.11a* | 9.42 ± 0.14a* | 3.41 ± 0.21a* |
| GROUP-III EAC ($1x10^6$cell) +EVN(250mg/kg p.o) | 30.81 ± 1.02b* | 43.50b* | 2.40 ± 0.11b* | 3.89 ± 0.04b* | 1.91 ± 0.09b* |
| GROUP-IV EAC ($1x10^6$cell) +EVN (500mg/kg p.o) | 35.64 ± 1.05c* | 66.35 | 1.01 ± 0.04c* | 2.62 ± 0.09c* | 2.14 ± 0.19c* |

**Note:** Values are mean ± SD of six observations; Group Comparison: a – Group II vs Group III; b – Group III vs Group IV; c – Group IV vs Group II; Statistical significance: * – Significant at 1% ($p<0.01$); ns – Not significant.

**Group III**: EAC treated mice receiving the extracts of *vitex negundo* (250 mg/kg,p.o.) for a period of 15 consecutive days.

**Group IV**: EAC treated mice receiving the extracts of *vitex negundo* (500 mg/kg, p.o.)for a period of 15 consecutive days.

All the animals were sacrificed at the end of 15th day after EAC administration. Blood was drawn from tail. The blood was used for the hematological parameters.

## Effect of Ethanolic Extract of the *Vitex negundo* on Tumor Volume

The determination of tumor volume in the experimental micewas carried out by the method of (Ghai, 1995).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.

## Estimation of Viable and Non-viable Tumor Cell Count

The Estimation of viable and non-viable tumor cell count in the experimental mice was carried out by the method of (Method of Green, 1990).

The cells were then stained with Tryphan 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 nonviable cells were counted.

## Effect of Ethanolic Extract of the *Vitex negundo* on Hematological Parameters of EAC Treated Mice

Hemoglobin content, red blood cell (RBC) and white blood cell (WBC) counts were measured from freely flowing retro orbital plexus blood. Differential leukocyte count of WBC was carried out from Leishman stained blood smears of normal and EAC control.

## Estimation of Haemoglobin

The Estimation of Haemoglobin (Hb) in the experimental mice was carried out by the method of **(**Sahli's Method of Ramnik Sood,1987) **.**

0.1 N HC1 was filled up to the mark 20 in the Hb calibrated tube. Blood was gently sucked up to 20 cu mm mark of Hb pipette. Emptied the Hb pipette in to acid in the tube by keeping the point of the pipette to the bottom of the tube and gently blowed off the blood without causing bubbles. The acid haematin solution was mixed with glass rod and allows the tube to stand for 10 min. The acid haematin solution was diluted by adding distilled water drop by drop with frequent stirring, until the colour matches with the standard.

## Determination of Total RBC Count

The Determination of Total RBC Count in the experimental mice was carried out by the method of (Method of Ramnik Sood, 1987).

The RBC pipette was filled with blood up to 0.5 marks. RBC diluting fluid was immediately drawn up to the mark 101 (dilution 1 in 200). The content of the pipette was gently rotated for 3 min to obtain good mixing. The cover slip was placed over the Neubauer's chamber so as to cover both the ruled platforms evenly. Few drops of the pipette content were expelled, and an appropriate drop of the mixture was allowed to run between the covering slip and chamber. Allowed 2 min for setting of the cells and count was done on 80 small squares under high power lens. The total RBC count was calculated by using the following equation,

RBC count = Dilution x 50 x N Cells/cumm

### Determination of Total WBC Count

The Determination of Total WBC Count in the experimental mice was carried out by the method of (Method of Ramnik Sood, 1987).

The WBC Pipette was filled with blood up to 0.5 marks. WBC diluting fluid was immediately drawn up to the mark 11 (dilution 1 in 20). The content of the pipette was gently rotated for 3 min to obtain good mixing. The cover slip was placed over the Neubauer's chamber so as to cover both the ruled platforms evenly. Few drops of the pipette content were expelled, and an appropriate drop of the mixture was allowed to run between the covering slip and chamber. Allowed 2 min for setting of the cells and count was done on 64 small squares under low power lens. The total WBC count was calculated by using the following equation, WBC count = *50* x N Cells/cumm

### Effect of Ethanolic Extract of the *Vitex negundo* on Differential Counts of White Blood Cells in EAC Bearing Mice

The differential counts of white blood cells, Lymphocyte, Neutrophil and Monocyte**,** in EAC bearing mice was carried out by the method of **(**Method of Ramnik Sood, 1987)**.**

## RESULTS AND DISCUSSION

In the EAC control group the mean survival time was 21.40 ± 1.41days, while it increased to 30.81 ± 1.02 days (43.50%) and 35.64 ± 1.05 days (66.35) for the group treated with Extract of *vitex negundo* (EVN) at the dose of 250 & 500 mg/kg respectively. The average number of tumor volume (Table 1) in EAC treated animals was found to be 3.68 ±0.11. EVN treatments at both dose level significantly ($p<0.05$) reduced tumor volume which was found to be 2.40 ± 0.11 and 1.01 ± 0.04 respectively. Viable cell count of the tumor bearing mice was significantly decreased while non- viable cell count were increased in EVN treated groups in dose dependent fashion when compared with EAC treated group.

Reliable criteria for judging the value of any anticancer agent is the prolongation of life span of animals (Hogland, 1982). A decrease in tumor volume and viable tumor cell count as mentioned above finally reduced the tumor burden and enhanced the life span of EAC bearing mice.

The effect of Extract of *Indigofera aspalathoides* (EIA) was experimented on the survival of tumor-bearing mice. The MST for the control group was 21+1.20 days, whereas it was 33+1.20 days and 40+2.10 days for the groups treated with EIA (250 mg/kg/day, p.o.) and 5-Flurouracil (20mg/kg/day,i.p.) respectively. The increase in the lifespan of tumor-bearing mice treated with EIA and 5-Flurouracil was found to be 57.14% and 90.47% respectively ($P< 0.01$) as compared to the control group (Rajkapoor *et al.,* 2003).

### Effect of Ethanolic Extract of the *Vitex negundo* on Hematological Parameters of EAC Bearing Mice

Moreover, hematological parameters of (Table 2) tumor bearing mice on day 15 were found to be significantly altered from normal group. The total WBC count, protein and PCV were found to be increased with a reduction of the haemoglobin and RBC. In a differential count of WBC, the percent of neutrphils increased while the lymphocyte count decreased. At the same time interval, EVN treatment could change those altered parameters to near normal.

Haemoglobin content and RBC count in the EAC control group was decreased when compared to normal group. Treatment with *Vitex*

**Table 2: Effect of Ethanolic Extract of the *Vitex negundo* on Hematological Parameters of EAC Bearing Mice**

| Groups | Hb (g%) | RBC (million mm$^3$) | WBC ($10^3$ cells/mm$^3$) | PCV (mm) | Different Count % | | |
|---|---|---|---|---|---|---|---|
| | | | | | Lymphocytes | Neutrophils | Monocytes |
| GROUP-I Normal saline (5ml/kg) | 14.5 ± 0.2a* | 6.5 ± 0.5a* | 7.2 ± 1.2a* | 17.8 ± 1.7a* | 70.2 ± 2.31a* | 29.8 ± 2.1a* | 2.2 ± 0.4a* |
| GROUP-II EAC control ($1x10^6$cell) | 7.8 ± 0.6b* | 3.8 ± 1.03 b* | 15.2 ± 1.3b* | 27.5 ± 1.4b* | 30.3 ± 2.4b* | 68.6 ± 3.6b* | 3.8 ± 0.5b* |
| GROUP-III EAC ($1x10^6$cell) +EVN (250mg/kg p.o) | 10.2 ± 0.6c* | 5.1 ± 0.1.09c* | 11.2 ± 1.7c * | 21.4 ± 1.4c* | 55.8 ± 3.1c* | 42.1 ± 3.42c* | 2.9 ± 0.4c* |
| GROUP-IV EAC ($1x10^6$cell) + EVN (500mg/kg p.o) | 12. ± 0.4d*e* | 5.8 ± 1.3d*e$^{ns}$ | 8.6 ± 1.7d*e* | 18.4 ± 1.1d*e$^{ns}$ | 67.3 ± 3.1d*e* | 30.1 ± 2.2d*e$^{ns}$ | 2.8 ± 0.3d*e* |

**Note:** Values are mean ± SD of six observations; Group Comparison: a – Group I vs Group II; b – Group II vs Group III; c – Group III vs Group I d – Group IV vs Group II; e – Group IV vs Group I ; Statistical significance: * – Significant at 1% ($p<0.01$); ns – Not significant.

*negundo* (group-IV) at the dose of 500 mg/kg increased the hemoglobin content and RBC count to more or less normal levels. The total WBC counts found to be increased in EAC control group when compared with normal group. Administration of *Vitex negundo* at the dose of 250 mg/kg in EAC bearing mice reduced WBCcount compared with EAC control. In the differential count of WBC, increase of neutrophils and the lymphocyte count decreased in EAC control group. Treatment with *Vitex negundo* dose was changed these altered parameters more or less normal (Table 2). In cancer chemotherapy the major problem are of myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with *Vitex negundo* brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that *Vitex negundo* possess protective action on the heamoto-poietic system.

Treatment with MEMP brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that MEMP possess protective action on the hemopoietic system (Yerra Rajeshwar *et al.,* 2005)

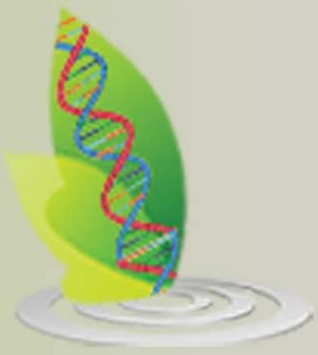
## CONCLUSION

Cancer is perhaps the most progressive disease posing a threat of mortality to the entire world despite significant advances in medical technology for its diagnosis and treatment.

The ethanolic extract of *Vitex negundo* extract treatment showed decrease in tumor volume, packed cell volume and viable cell count and increases the nonviable cell count and mean survival time (MST), thereby increasing life span of EAC tumor bearing mice. Hematological profile reverted to more (or) less normal levels in extract treated mice. The data presented here clearly encourage the development of *Vitex negundo* for chemopreventive agent.

## REFERENCES


1. ACS (American Cancer Society) (2005), "A History of Cancer", American Cancer Society, inc www. Cancer.org
2. Chaturvedi G N and Singh R H (1965), "Experimental Studies on Anti Arthritic Effect of an Indigenous Drugs", *Indian Journal of Medicinal Research*, Vol. 53, No. 1, pp. 71-80.
3. Chopra R N, Nayar S L and Chopra I C (1956), *Glossary of Indian Medicinal Plants*, New Delhi, CISR, p. 256.
4. Ghai C L (1995), A Text Book of Practical Physiology, Jaypee Brothers, India, pp. 119-202.
5. Green F (1990), "Dyes & Indicators", The sigma-Aldrich Handbook of Stains, pp. 721-722, Aldrich Chemical Co. Milwaukee.WI.
6. Hansal R, Leckert C, KimplerH and Schaaf K D (1965), *Phytochemistry*, Vol. 4, p. 19.
7. Eric X Chen and Lillian L Siu (2005*),* "Development of Molecular Targeted Anticancer Agents: Successes, Failures and Future Directions", *Current Pharmaceutical Design,* Vol. 11, pp. 265-272.
8. Parkin D M, Bray F, Ferlay J and Pisani P (2003), "Global Cancer Statistics", CA *Cancer J Clin*., Vol. 55, pp. 74-108.
9. RamnikSood (1987), *Textbook of Medical Laboratory Technology*, 2nd Edition, Jaypee Publishers, pp. 200-208.
10. Madhumitha R Y, Chakaraborty S, Roy M, Taraphdar A K and Bhattacharya R K (2004), "Cytotoxic Effect of Root Extract of *Tiliacoraracemosa* and Oil of *Semecarpus-anacardium* nutin Human Tumour Cells", *Asian Pacific J. Cancer Prev*., Vol. 3, pp. 61-67.
11. Rana Abu-Dahab and FatmaAfifi, (2007), "Antiproliferative Activity of Selected Medicinal Plants of Jordan Against A Breast Adenocarcinoma Cell Line", *Scientia pharmaceutica* (Sci. Pharm.)*,* Vol. 75, pp. 121-136.
12. Rajkapoor B, Jayakumar N and Murukesh, (2003), "Antitumor Activity *of Indigofera Aspalathodes* on Ehrlich Ascites Carcinoma In Mice", *Indian J Pharmacol.,* Vol. 36, 1; pp. 38-40.
13. Yerrarajeswhar, Malaya Gupta and UpalKantiMazunder (2005), "Antioxidant Activity and *in Vivo* Antioxidant Status *of Mucunapruriens* (Fabaceae) Seeds Against Ehrlich Ascites Carcinoma In Swiss Albino Mice", *Iranian Journal of Pharmacology & Therapeutics*, 1735-2657/05/41-53.



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