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Research Article

ANTICARCINOGENIC EFFECT OF *ARTEMISIA PALLENS* IN 20-METHYLCHOLANTHRENE INDUCED FIBROSARCOMA IN SWISS ALBINO MICE

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ABSTRACT

Aim: The aim of the present study was to evaluate the anti-carcinogenic effect of Artemisia pallens in 20-methylcholanthrene induced fibrosarcoma in swiss albino mice.

Method: The chloroform extract of Artemisia pallens was screened for its anti-carcinogenic activity. A preliminary phytochemical study of the extract for the presence of different phytoconstituents was carried out. Fibrosarcoma was induced by subcutaneous injection of single dose of 200µg/0.1ml MCA in DMSO into thigh region and the tumors appeared in about six weeks. The fibrosarcoma was isolated and proved by pathological examinations. The antioxidant parameters like Lipid peroxidation, Glutathione, Superoxide dimustase and Catalase and Heamatological parameters like Hb content, RBC count and WBC count were evaluated in this study. The chloroform extract of Artemisia pallens can be given orally in the dose of 200mg/kg and 400mg/kg b.w per day for 30 days.

Result and Conclusion: The chloroform extract of Artemisia pallens (400mg/kg) significantly restored the altered antioxidant and haematological parameters to normal values. It is concluded that CEAP (400mg/kg) possess significant anti-carcinogenic activity.

Keywords: Artemisia pallens, Fibrosarcoma, Haematological, Antioxidant.

INTRODUCTION

Cancer is a leading cause of mortality worldwide and the failure of conventional chemotherapy to effect major reductions in the mortality indicate that new approaches are critically needed. According to a recent report by the WHO, there are now more than 10 million cases of cancer per year worldwide. The goal of basic and clinical research, cancer surveillance, and cancer control is to reduce suffering and death due to cancer. Supporting scientific research to increase knowledge about the molecular and cellular mechanisms that affect the initiation and progression of cancer can help lead to the development of more effective treatment modalities.

The investigation and research on medicinal plants might bring to the scientific world many useful remedies for the alleviation and cure of human sufferings. Despite the phenomenal advances in synthetic chemistry many of the most successful drugs used in western medicine are plant components. Indeed, over 75% of the world's population relies mainly on plants and their extracts for health care. Although the scientific study of traditional medicine is by no means new there has been a renewed interest in obtaining potent anticancer agents from plants. Indeed, **taxol** isolated from the bark of the Pacific yew tree and **artimisin** from the sweet wormwood plant, are very powerful drugs for the treatment of cancer and malaria respectively. Several other plants, which are used in the traditional medicine for cancer, remain unexplored. The incidence of cancer in India is rising steadily. Moreover, many of the plant constituents such as stilbenes, alkaloids, flavanoids, tannins and quinines were found to be potent anticancer agents[1].

Artemisia pallens commonly known as Davana is traditionally used in Indian system of medicine for the treatment of Diabetes[2]. Through the literature survey and folkflore information revealed that the Artemisia pallens was reported in ethanomedical claims possess urinary problem, antibacterial, anticoagulant[1]. This plant is accredited with anthelmintic, antipyretic and tonic properties. It is also considered as a good fodder. The oil possesses antispasmodic, antibacterial, antifungal and stimulant properties[3].The plant

has been screened for the following pharmacological activities, antidiabetic[4], analgesic and anti-inflammatory[5,] antioxidant and antimicrobial[6,7,] antinociceptive, wound healing activity[8] and also possess the tyrosinase inhibitory activity[9], immunomodulatory activity[10].

MATERIALS AND METHODS

Collection of plant material

The whole plant of Artemisia pallens wall was collected from the local area of mandya in Karnataka state. The plants parts were dried under shade, crushed into coarse powder. The coarse powder was subjected to hot continuous extraction with chloroform in a Soxhlet extractor. The extract will be subjected for phytochemical analysis.

Preparation of plant extract

About 1kg of dry coarse powder of the whole plant of Artemisia pallens wall. was extracted with chloroform by hot continuous percolation using Soxhlet apparatus. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated to dry residue, in a desiccators over anhydrous calcium chloride. A dark green residue was obtained. The chloroform extract was then distilled and concentrated to a dry mass. A dark green residue was obtained.

Animals

Swiss albino mice weighing of about 20-25gm were procured from (Bharathi College of Pharmacy) was used for the pharmacological studies. The animals were housed in microloan boxes in a controlled environment (temperature 25±2 ^{o}C, humidity 60-70% and 12 hr dark/lightcycle) with standard laboratory diet and water ad libitum. The study was conducted after obtaining Institutional animal ethical committee's clearance. As per the standard practice, the mice were 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.

Chemicals

All the chemicals and reagents used were purchased from M/s Sigma Chemicals, USA.

Induction of fibrosarcoma

Fibrosarcoma was induced by subcutaneous injection of single dose of 200 µg/0.1 ml MCA in DMSO into the thigh region and the tumors appeared in about six weeks. The entire procedure was carried out as per stated guidelines of Institutional Animal Ethical Committee (Ref No: BCP/IAEC/PCOL02/2015).

Experimental design

The experiments were conducted on animal groups to see the effect of CEAP on fibrosarcoma induced by 20-methylcholanthrene. Ten mice were used in each of the four groups which were as follows:

Group I: Served as the vehicle control, which received normal saline (5ml/kg b.w).

Group II: Were administered with a single dose of 200 µg/ 0.1 ml MCA in DMSO into the thigh region subcutaneously. These were fibrosarcoma bearing animals.

Group III: Were administered with a single dose of 200 µg/ 0.1 ml MCA in DMSO into the thigh region subcutaneously. After six weeks of tumor inoculation CEAP (200 mg/kg b.w/day) administrated for 45 subsequent days.

Group IV: Were administered with a single dose of 200 µg/ 0.1 ml MCA in DMSO into the thigh region subcutaneously. After six weeks of tumor inoculation CEAP (400 mg/kg b.w/day) administrated for 45 subsequent days.

Estimation of Antioxidant parameters

All the experimental animals were killed by cervical decapitation after the experimental period. For the estimation of antioxidants liver was rinsed and homogenized (10% w/v) in 0.1 M phosphate buffer (PH 7.0) and centrifuged for 10 min and the resulting supernatant was used for estimation of lipid peroxide (Thiobarbituric acid reactive substances (TBARS))[11],catalase activity[12] and Superoxide dismutase (SOD)[13].

The remaining supernatant was centifused at 3200rpm for 20min and used for the estimation of reduced glutathione (GSH) of activity[14].

Estimation of Heamatological parameters

Blood was withdrawn from animals by retro orbital plexus method for the estimation of Haemoglobin content, Red Blood cell count, White blood cell count and differential leucocyte count of WBC from the blood smears of normal, Tumor control and treated groups[15].

Statistical analysis:

The data were expressed as Mean ± S.E.M. The data were statistically analyzed by One way analysis of variance (ANOVA) followed by Dunnet's test. $^{*}P < 0.05$ were considered as significant, $^{**}P < 0.01$were considered as highly significant and $^{***}P < 0.001$ were considered as very highly significant (compared with respective control).

RESULTS

a) Effect on Antioxidant parameters

Lipid peroxidation levels indicated by TBARS were considerably higher in animals treated with 20-methylcholanthrene as compared to the normal animals. A significant decrease in the Glutathione and Enzymatic antioxidants (SOD & CAT) were noted after the administration of 20-methylcholanthrene.Treatment with CEAP (400mg/kg) resulted in a significant decrease in levels of TBARS to near normal values. The activities of glutathione and enzymatic antioxidants were also significantly reversed to near normal values. In the same way CEAP (200mg/kg) was found to be less effective in normalizing the altered parameters than chloroform extract of Artemisia pallens (400mg/kg) in table:1.

b) Effect on Haematological parameters

Haematological parameters in 20-methylcholanthrene induced fibrosarcoma group animals were found to be significantly altered as compared to that of normal group. Hb content and total RBC count in the tumor control group was significantly decreased whereas total WBC count was significantly increased in 20-methylcholanthrene control group compared with the normal group. In a differential count of WBC, the presence of Neutrophils increased, while the Lymphocyte count and Monocyte decreased in the 20-methylcholanthrene

control group. Treatment with Artemisia pallens (200mg/kg) restored these altered parameters approximately to the normal values. In the same way Artemisia pallens (400mg/kg) restored these altered parameters significantly to the normal values compared to chloroform extract of Artemisia pallens (200mg/kg) in table 2.

Table 1: Effect of Artemisia pallens on liver LPO, GSH, SOD and CAT levels of fibrosarcoma bearing animals

Parameters	Group I Normal control	Group II Fibrosarcoma bearing	Group III Fibrosarcoma+ CEAP 200mg/kg	Group IV Fibrosarcoma+ CEAP 400mg/kg
LPO Abs at 532nm	0.053±0.002***	0.368±0.029	0.219±0.019***	0.16±0.011***
GSH Abs at 412nm	0.338±0.014***	0.177±0.005	0.261±0.015***	0.309±0.012***
SOD Abs at 480nm	1.58±0.042***	0.196±0.035	0.311±0.037	0.587±0.019***
CAT Abs at 620nm	0.53±0.012***	0.234±0.010	0.348±0.018***	0.418±0.007***

Values are expressed as Mean±SEM (n=10). The data were statistically analyzed by One way ANOVA followed by Dunnet's test. *P < 0.05 were considered as significant, **P< 0.01were considered as highly significant and ***P< 0.001 were considered as very highly significant (compared with respective control.

Table 2: Effect of Artemisia pallens on Haematological Parameters of fibrosarcoma bearing animals

Parameters	Group I Normal control	Group II Fibrosarcoma bearing	Group III Fibrosarcoma+ CEAP 200mg/kg	Group IV Fibrosarcoma+ CEAP 400mg/kg
Hb content(g/dl)	12.04±0.24***	7.07±0.390	9.76±0.401***	11.35±0.291***
Total RBC count 10^6cells/mm^3	8.30±0.104***	3.95±0.22	5.61±0.197***	7.14±0.173***
Total WBC count 10^3cells/mm^3	6.35±0.166***	17.71±0.199	10.97±0.909***	7.58±0.316***
Monocytes (%)	2.07±0.022***	1.09±0.023	1.55±0.086***	1.91±0.038***
Neutrophils (%)	16.06±0.53***	69.31±0.486	42.4±0.594***	22.52±0.931***
Lymphocytes (%)	85.37±0.44***	34.19±0.442	65.94±4.082***	73.63±2.395***

Values are expressed as Mean±SEM (n=10). The data were statistically analyzed by One way ANOVA followed by Dunnet's test. *P < 0.05 were considered as significant, **P< 0.01were considered as highly significant and ***P< 0.001 were considered as very highly significant (compared with respective control).

DISCUSSION

Cancer is actually a family of diseases having the common characteristic of uncontrolled cell growth. In normal tissue, there are a myriad of regulatory signals that instruct cells when to replicate, when to enter a resting state, and even when to die. In a cancer cell these regulatory mechanisms become disabled and the cell is allowed to grow and replicate unchecked. Cancer is largely a disease of aging. Carcinogenesis, or the sequence of events leading to cancer, is a multistep process involving both intrinsic and extrinsic factors[16].

Free radicals and reactive oxygen species (ROS) are continuously produced in the human body. These oxygen species are the cause of cell damage and the progression of tumor cells to cancer cells. The increased levels of lipid peroxides were observed in fibrosarcoma bearing animals. The formation of Malondialdehyde (MDA) is considered as an index of lipid peroxidation that causes cell injury. Elevation in lipid peroxidation as indication by increased MDA was observed in fibrosarcoma bearing animals. The naturally occurring antioxidant properties of Artemisia pallens lowered the MDA levels suggesting reduced lipid peroxidation due to its antioxidant activity. Artemisia pallens helps to improve the antioxidant defense system and prevent the damage induced by free radicals. The significant increases in lipid peroxidation in carcinogenic process may be due to abnormal levels of Reactive oxygen species (ROS). The antioxidant status has improved during the chemotherapy of chloroform extract of Artemisia pallens. The animals treated with CEAP (400mg/kg) shows significant decrease in LPO level compared to animals treated with CEAP (200mg/kg).

Glutathione is an important non-enzymatic antioxidant preventing damage to important cellular components caused by ROS. It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds. The level of GSH is decreased in fibrosarcoma bearing animals due to elevated level of free radicals being formed by reaction of superoxide radicals with hydrogen peroxide. The GSH level is increased after treatment with Artemisia pallens. The CEAP (400mg/kg) significantly increase the Glutathione level compared to CEAP (200mg/kg).

Superoxide dimustase is one of the body's primary internal antioxidant defenses and play a critical role in reducing the oxidative stress. In the fibrosarcoma bearing animals the reduced level of SOD is observed due to vast metabolic consequences because of production of chemical species from superoxide. The superoxide dimustase level is improved after treatment with Artemisia pallens. Treatment with CEAP (400mg/kg) resulted in a significant increase in levels of SOD to near normal values compared CEAP (200mg/kg).

Catalase is an enzymatic antioxidant. It is very important in protecting the cell from oxidative damage by reactive oxygen species. Catalase has one of the highest turnover numbers of all enzymes, one catalase molecule can convert approximately 5 million molecules of hydrogen peroxide to water and oxygen each minute. Catalase level is decreased in fibrosarcoma bearing animals due to increased levels of free radicals. The level of catalase is significantly increased after treatment with CEAP (400mg/kg) compared to CEAP (200mg/kg).

Administration of Artemisia pallens(400mg/kg) reveals its effectiveness in affording protection to cell membrane by lowering of lipid peroxidation and increases in levels of Glutathione, Superoxide dimustase and Catalase. The observation made in this study highlights the antioxidant property of Artemisia pallens in fibrosarcoma bearing animals.

The reliable criteria for judging the value of any anticancer drugs are prolongation of lifespan and disappearance of leukemic cells from blood. WBCs are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. Increasing numbers of abnormal white blood cells cause a decrease in the number of normal cells. In fibrosarcoma bearing animals the WBCs are increased compared to normal control group. Treatment with CEAP (400mg/kg) demonstrated very significant reduction in abnormal WBCs from blood of fibrosarcoma bearing mice compared to CEAP (200mg/kg).

Anaemia is also another problem in cancer chemotherapy. The anemia encountered in fibrosarcoma bearing animals 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. Present study indicate that treatment of CEAP (400mg/kg) brought back the haemoglobin content and RBC count more or less to normal levels which is comparable to the effect of CEAP (200mg/kg). This indicates that the test compounds possess protective action on haemopoietic system.

In a differential count of WBC, the presence of Neutrophils increased, while the Lymphocyte count and Monocyte decreased in the 20-methylcholanthrene control group. Treatment with Artemisia pallens (200mg/kg) restored these altered parameters approximately to the normal values. In the same way Artemisia pallens (400mg/kg) restored these altered parameters significantly to the normal values.

CONCLUSION

It is concluded that based on improvement in Antioxidant parameters and Haemotological parameters it is concluded that the chloroform extract of Artemisia pallens (400mg/kg) possess significant anti-carcinogenic activity. Thus it proves the traditional information of the plant Artemisia pallens on Anti-carcinogenic activity.

REFERENCES

1. Wealth of India, NISCOM, CSIRpublications, I, 1985; 439-440.
2. Swanston SK, Flatt P R, Bailey D C. Traditional dietary adjuncts for the treatment of diabetes mellitus. Proceedings of nutritional society. 1991;50(3):641–651.
3. Mehrota S, Rawat AKS, Shome. Antimicrobial activity of the essential oil of some Indian Artemisia species. Fitoterapia. 1993;64(1):65-68.
4. Subramoniam A, Pushpangadan P, Rajasekharan S, Evans D A, Latha P G, Valsaraj R. Effects of Artemisia pallens Wall on blood glucose levels in normal and alloxan-induced diabetic rats. J Ethnopharmacol. 1996;50:13-17.
5. Praveen Kumar Ashok, Kumud Upadhyaya. Analgesic and anti-inflammatory properties of Artemisia pallens wall ex.dc. T Ph Res.2010;3:249-256.
6. Suresh J, Sri Vasavi Reddy A, Ahuja J, Mary Sebastian, Shimna K. Rajan. antioxidant and antimicrobial activity of Artemisia pallens. J PharmacognHerbal Formulations. 2011;1(2):13-18.
7. Tekade A R, Bagali R S, Kale R H. Antibacterial activity of Artemisia pallens Wall Extract. Indian Drugs. 2005;42(12):829-831.
8. Rasal VP, Ajay Kshirsagar, Raj Bagali, Santosh K Rai. Influence of Artemisia pallens Wall plant on experimental wounds in albino Rats. In Rescent Adv Med Plant Res. 2005;393-401.
9. Kanimozhi P, K Ilango, Anupam Chowdary, Arun B, Ammar Mohammed musa adam, Nethaji A. Microwave assisted extraction of Artemisia pallens for tyrosinase inhibitory activity. Int J Pharm and Bio Sci. 2010;1(1):1-7.
10. Joghee suresh, Nagamani. Immunomodulatory activity of ethanolic extract of aerial parts of Artemisia pallens. Int J Pharm Res Bio-Sci. 2014;3(2):1-11.
11. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products:Malondialdehyde and 4-hydrpxynonenal. Meth. Enzymol. 1990;186:407-421.
12. Sinha BB, Peterson GA, Whitny RR. Nuclear change distribution of isotone. Pairs phys. 1972;6:1657-1663.
13. Kakkar P, Awasthi S, Viswanathan PN. Effects of anesthetic ether on lipid peroxidation and superoxide dimulatase isoenzymes of young and adult rat brain. Ind J Exp Biol. 1984;27:647-649.
14. Ellman GL, Fiches FT. Quantitative determination of peptides by sulfhydryl groups Arch. Biochem Biophys.1959;82:70-72.
15. Mohan H. Pathology practical book. New Delhi: Vora Jaypee Brother's medical publishers. 2005.
16. James RC, Roberts SM, Williams PL. General principles of toxicology. 2nd ed.USA (NJ): A John Wiley & Sons, Inc., Publication. 2000.

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