Iranian Journal of Pharmaceutical Research (2007), 6 (2): 141-145
Received: May 2005
Accepted: December 2005

Short Communication

Anticancer Activity of *Indigofera aspalathoides* and *Wedelia calendulaceae* in Swiss Albino Mice

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Abstract

The methanolic extracts of *Indigofera aspalathoides* (MEIA) and *Wedelia calendulaceae* (MEWC) were evaluated for their anticancer activity against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. On day 1, the extract of *Indigofera aspalathoides* at a dose of 250 and 500 mg/kg body weight and the extract of *Wedelia calendulaceae* at a dose of 250 and 500 mg/kg body weight were administered orally and continued for 9 consecutive days. The anticancer activity of MEIA and MEWC were examined by determining the tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span in experimental animal models. Both these extracts increased the life span of EAC treated mice and restored the hematological parameters as compared with the EAC bearing mice. Thus, the present study revealed that the MEIA and MEWC showed anticancer activity in the tested animal models.

Keywords: *Indigofera aspalathoides*; *Wedelia calendulaceae;* Ehrlich acitic carcinoma; Anticancer.

Introduction

The plant *Indigofera aspalathoides* (Leguminosae) is commonly known as 'Shivanarbembu' in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoot are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous affections (1). The leaves are also applied to abscesses. The whole plant is used in odematous tumors and the ashes are used in preparations for dandruff's (2). The methanol extract of *Indigofera aspalathoides* also possess hepatoprotective acvity (3). *Wedelia calendulaceae* (Compositae) is commonly known as 'Bhimraj' in Bengali, which is widely, distributed in Bengal, Assam, Burma and plains districts of Madras. In traditional system of medicine, the leaves are considered as tonic, alterative, and useful in cough, cephalalgia, skin disease and alopecia. An infusion of the plant is given in Indo China for the swelling of abdomen (4). The plant is very specific for viral hepatitis (5). The aim of the present study was to evaluate the anticancer activity of the methanolic extract of *Indigofera aspalathoides* (MEIA) and *Wedelia calendulaceae* (MEWC) against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice.

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Experimental

Preparation of the extract

The plant *Indigofera aspalathoides* was collected in the month of March 2002 from Tamilnadu and the plant *Wedelia calendulaceae* was collected during the month of November 2002 from West Bengal. Both plants were identified by the Botanical Survey of India, Shibpur, Howraw, West Bengal, India. Specimens of both plants (IAP-1 & WCP-1) are kept in laboratory for future reference. After shade dried the plants materials were powdered in mechanical grinder. Then both of the plants materials were extracted with petroleum ether (60-80) and after being defatted they were extracted with methanol in a Soxhlet extraction apparatus. The solvent was then completely removed under reduced pressure and stored in a vacuums dessicator. The yields of the methanol extract of the two plants were 4.5% and 5.8% respectively.

Treatment schedule

Mature male Swiss albino mice weighing 20-25g were kept in identical laboratory condition and divided into six groups (n=10) and given food and water *ad libitum*. All the groups (Table 1) except group I were injected with EAC Cells (2×10^6 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 EAC control. On day 1 the methanol extract of *Indigofera aspalathoides* at a dose of 250 and 500 mg/kg body weight (Gr-III &IV) and methanol extract of *Wedelia calendulaceae* at a dose of 250 and 500 mg/kg body weight (Gr-V & VI) were administered orally and continued for 9 consecutive days. On day 10, 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 (6).

The effect of methanol extract on tumor growth and host's survival time were examined by studying the following parameters-tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span**.**

Table 1. Treatment schedule of various groups.

Group	Treatment	Dose
Group I	Control	[5 ml/kg saline/normal p.o]
Group II	EAC	[2×10^6 cells/mouse i.p.]
Group III	EAC+MEIA	[250 mg/kg body weight p.o]
Group IV	EAC+MEIA	[500mg/kg body weight p.o]
Group V	EAC+MEWC	[250mg/kg body weight p.o]
Group VI	EAC+MEWC	[500mg/kg body weight p.o]

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 and packed cell volume determined by centrifuging at 1000 g for 5 min.

Determination of tumor cell count

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

Estimation of viable tumor 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 nonviable cells were counted.

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

Percentage increase life span

Recording the mortality monitored the effect of the MEIA and MEWC on tumor growth and percentage increase in life span (ILS%) were calculated (7).

ILS (%)=[(Mean survival of treated group/ Mean survival of control group)-1]×100
Mean survival time=[1st Death+Last Death]/2

Hematological studies

The effect of the MEIA and MEWC on peripheral blood was investigated. RBC, WBC counts and estimation of hemoglobin were done by standard procedures from freely flowing tail

Table 2. Effect of *Indigofera aspalathoides* and *Wedelia calendulaceae* extracts on survival time, life span, tumor volume, viable and non-viable cell count in EAC bearing mice.

Tested group	Survival time (days)	Increase of life span (%)	Tumor volume (ml)	Viable cell count $\times 10^6$ cells/ml	Non-Viable cell count $\times 10^6$ cells/ml
Normal saline (5 ml/kg)	-	-	-	-	-
EAC control (2×10^6 cells/mouse)	21.40±1.41	-	3.69±0.11	9.42±0.14	3.41±0.21
EAC (2×10^6 cells)+MEIA (250 mg/kg)	33.41±1.60*	56.07	2.89±0.25	3.89±0.04*	1.64±0.12
EAC (2×10^6 cells)+MEIA (500 mg/kg)	36.83±1.32*	71.96	1.69±0.18*	2.61±0.05*	2.03±0.11*
EAC (2×10^6 cells)+MEWC (250 mg/kg)	30.81±1.02	43.93	2.48±0.11*	4.09±0.03	1.91±0.09*
EAC (2×10^6 cells)+MEWC (500 mg/kg)	35.64±2.05*	66.51	1.91±0.04*	3.13±0.07*	2.34±0.19*

Statistical significance (p) calculated by one-way ANOVA between the treated groups and the EAC control followed by Dunnett's post hoc test of significance.
*$p<0.05$

vein blood (8, 9).

Results

Oral administration of the methanol extract of *Indigofera aspalathoides* (MEIA) and *Wedelia calendulaceae* (MEWC) at the dose of 250 and 500 mg\kg body weight increased the life span and non-viable cell count, decreased tumor volume and viable cell count of the tumor bearing mice, when compared to that of EAC control mice (Table 2). Both MEIA and MEWC also restored the hematological parameters. The number of RBC count and Hemoglobin content also increased as compared to that of EAC control. In the differential count the percentage of Lymphocytes was increased with decreased level of Neutrophils (Table 3).

Discussion

The present study shows that MEIA and MEWC were significantly increased the life span than that of EAC bearing mice. The reliable criteria for judging the value of any anti cancer drug are prolongation of life span and decrease of WBC from blood (10, 11).

Further more the reduced volume of EAC and increased survival time of mice suggest the delaying impact of MEIA and MEWC on cell division. Usually in cancer chemotherapy, the major problem is anemia, due to reduction in RBC or hemoglobin concentration and leucocytes.

Our results says that MEIA and MEWC have significantly enhanced the erythrocyte count and hemoglobin level when compared to that of EAC bearing mice. The WBC level is reduced when compared to that of EAC bearing mice. These indicating parameters reveal that MEIA and MEWC possess less toxic effect on hematological system.

Viable cell count decreased with increased level of non-viable cell count. These suggested that MEIA and MEWC have direct relationship

Table 3. Effect of *indigofera aspalathoides* and *wedelia calendulaceae* extract on hematological parameters in EAC bearing mice.

Parameter	Normal Saline (0.9%NS/W)	EAC Control (2×10^6 cells/ mouse)	5-Fluorouracil (2 mg/kg)	MEIA (250 mg/kg)	MEIA (500 mg/kg)	MEWC (250 mg/kg)	MEWC (500 mg/kg)
Hb (g %)	13.9±0.1	8.9±0.6	12.4±0.4	10.2±0.3	11.3±0.3*	10.2±0.3	11.0±0.6*
RBC (Cells$\times10^6$/mm^3)	6.3±0.1	4.3±0.1	5.8±0.3	5.3±0.5*	5.6±0.4	4.8±0.5*	5.4±0.7
WBC(Cells$\times10^3$/mm^3)	7.3±0.2	17.6±1.2	8.6±0.7	11.2±1.6*	9.8±0.6*	11.9±1.3*	11.3±0.5*
Lymphocytes (%)	71.2±1.4	25.1±0.4	66.3±2.1	55.8±1.1*	61.5±3.1	54.8±2.1*	58.3±2.3
Neutrophil (%)	26.8±1.1	62.6±1.8	30.1±2.2	42.1±1.7*	37.3±1.7*	43.5±1.5*	39.8±2.7*
Monocyte (%)	2.2±0.4	4.2±0.5	2.9±0.4	3.5±0.6	3.8±0.3*	3.6±0.6*	3.2±0.3*

Statistical significance (p) calculated by one-way ANOVA between the treated groups and the EAC Control followed by dunnett's post hoc test of significance.
*$p<0.05$

with tumor cells. Because these tumor cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and these anticancer agents lysis the cells by direct cytotoxic mechanism.

Preliminary phytochemical screening indicated the presence of flavonoids, alkaloids and tannins in MEIA. Flavonoids have been shown to possess antimutagenic and antimalignant effects (12, 13). Further more, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation (14) and angiogenesis (15). The cytotoxicity and anticancer properties are due to the presence of flavonoids.

According to the previous reports wedelolactones present in *Wedelia calendulaceae* were found to have 5-lipooxygenase and caspase inhibiting activities (16, 17). The current studies indicate that both 5-LOX & 12-LOX expression such as human pancreatic cancer cell. LOX plays a critical role in human pancreatic cancer cell proliferation. LOX inhibitor may play a very important role in the treatement of cancer. Thus the anticancer activity of *Wedelia calendulaceae* may be due to the LOX inhibition or due to induction of detoxifying system.

The methanolic extract of *wedelia calendulaceae* restore the mean survival time, decrease tumor volume count in treated mice.

Thus our present study suggests that both MEIA and MEWC possess potent anticancer activity and increase life span.

Acknowledgement

The financial assistance of Jadavpur University, Department of Pharmaceutical Technology, Kolkata, India as well as Gupta College of Technological Sciences, Ashram More, Asansol, India is gratefully acknowledged.

References

(1) Kirtikar KR and Basu BD. G*lossary of Indian Medicinal plants*. Vol I, m/s Periodical Experts, New Delhi (1975) 338

(2) *The Wealth of India. A Dictionary of Indian Raw Materials and industrial Products, Raw Materials.* Vol V, H-K, Publication and Information Direction, Council of Scientifit and Industrial Research New Delhi, India (1959) 176

(3) Gupta M, Mazumder UK, Haldar PK, Kandar CK, Manikandan L and Senthil GP. Hepatoprotective activity of *Indigofera aspalathoides* against carbon tetrachloride induced liver damage in rats. *OPEM.* (2004) 100-103

(4) Kirtikar KR and Basu BD. *Indian Medicinal Plants*. Vol 3, 2nd ed. Oriental Enterprises, Dehra Dun (2001) 997-999

(5) *Indian Medicinal Plants, a Compendium of 500 Species*. Vol 5, Orient Long Man, Chennai (1997) 404-406

(6) Mazumder U K, Gupta M, Maiti S and Mukherjee M. Antitumor activity of *Gygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian J. Expt. Biol.* (1997) 35: 473-477

(7) Sur P and Ganguly DK. Tea plant root extract (TRE) as an antineoplastic agent. *Planta Med.* (1994) 60: 106-109

(8) Dacie JV and Lewis SM. *Practical Hematology*. 2nd ed. J and A Churchill, London (1958) 38-48

(9) D'Armour FE, Blood FR and Belden DA. (*The Manual for Laboratory Work in Mammalian Physiology*. 3rd ed. The University of Chicago Press, Illinois (1965) 4-6

(10) Clarkson BD and Burchenal JH. Preliminary screening of antineoplastic drugs. *Prog. Clin. Cancer* (1965) 1: 625- 629

(11) Oberling C and Guerin M. The role of viruses in the production of cancer. *Adv. Cancer Res.* (1954) 2: 353-423

(12) Brown JP. A review of the genetic effect of naturally occurring flavonoids, anthraquinones and related compounds. *Mutat. Res.* (1980) 75: 243-247

(13) Hirano T, Oka K and Akiba M. Antiproliferative effect of synthetic and naturally occurring flavonoids on tumor cells of human breast carcinoma cell lines, ZR-75-1. *Res. Commun. Pathol. Pharmacol.* (1989) 64: 69-78

(14) Weber G, Shen F, Prajda N, Yeh YA and Yang H. Increased signal transduction activity and down regulation in human cancer cells. *Anticancer Res.* (1996) 16: 3271-3282

(15) Fotsis T, Pepper M S, Aktas E, Breit S, Rasku S and Adlercreutz H. Flavonoids, dietary-derived inhibitors of cell proliferation and i*n vitro* angiogenesis. *Cancer Res.* (1997) 57: 2916-2921

(16) Wagner H, Geyer B, Yoshinobu K and Govind SR. Coumestan as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulaceae*. *Planta Med.* (1986) 5: 370-372

(17) Kobori M, Yang Z, Gong D, Heissmeyer V, Zhu H, Jung YK, Gakidis MA, Rao A, Sekine T, Ikegami F, Yuan C and Yuan J. Wedelolactone suppresses LPS-induced caspase-11 expression by directly inhibiting the IKK complex. *Cell death Differ.* (2004) 11: 123-130

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