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Screening of Hydro-alcoholic Extract of *Eclipta alba* for its Anticancerous Efficacy

Anjali Singh[1], Ayodhya Singh[2], Vandana Dwivedi[3]

Department of Chemistry (P.G. Studies and Research) M.M.H. College, Ghaziabad (UP), 201002, India

Abstract: ***Cancer is one of the most dreaded diseases of the 20th century and is spreading further with continuance and increasing incidence in 21st century. Cancer is an abnormal growth of cells. There are more than 100 types of cancer, including breast cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Plants have been extensively used as natural sources to develop anticancerous activity due to their active chemical constituents. In our study anticancerous activity of Eclipta alba was examined in-vivo against Ehrlich Ascites Carcinoma on Swiss albino mice. The methanolic extract of E. alba restore the mean survival time and decrease tumour volume count in treated mice. Consequently, our study revealed that the methanolic extract of E. alba showed anticancer activity.***

Keywords: MEEA (Methanol Extract of E*clipta alba*), EAC (Ehrlich Ascites Carcinoma), EAE (Hydroalcoholic Extract of E*clipta alba*), RCT (Randomised Controlled Clinical Trial)

1. Introduction

Abuse of nature's law upsets the human system and ends up in diseases like cancer. It is again the nature, the foremost physician who brings the cure. The Ayurvedic system of medicine was well founded on the basic principles of nature and its elements after a careful and thorough study of human physiology. This is the first system to emphasize health as the perfect state of physical, psychological, social and spiritual component of a human being.

Learning from the past, examining the present and advancing to the future and because a large population uses Ayurvedic medicine worldwide **[1, 2]**, there is an urgent need for additional, carefully conducted high-quality intensive research to evaluate its efficacy and to develop this discipline to meet ever-new challenges of modern medicine in the field of oncology.
The most stringent evaluation should take place with gold standards for clinical research the randomised controlled clinical trial (RCT). Priority for research funding should be given to clinical investigations in Ayurveda involving well-designed studies with encouraging results especially for diseases like cancer to which conventional medicine has been shown to be less effective. Attention should be given not only to the evaluation of safety and examination of effectiveness in treatment strategy, but also to the consideration of community practice settings, patient expectations, compliance and cost effectiveness. Standardization and quality production of herbal products may allow us to develop low cost therapies with reduced risk over pharmaceuticals. In any case, studies on anticancer Ayurvedic drugs will be popular from the economic point of view because cancer is becoming the major cause of death.

In Ayurveda, *Eclipta alba* has been described for the treatment of *Kapha* and *Vata* imbalances. *Eclipta alba* is considered a primary liver herb in Ayurveda, where it is called *Bhringaraja*, and in Arabian medicine, it is known as *Kadim-el-bint* **[3]**. The *Eclipta alba* has been extensively studied for its hepatoprotective activity and a number of herbal preparations comprising of *Eclipta alba* are available for treatment of jaundice and other ailments of the liver and gallbladder and viral hepatitis **[4-8]**.The coumeston constituents of plant wedelolactone and dimethyl wedelolactone are responsible for potent anti-hepatotoxic activities in carbon tetrachloride, galactosomine and phalloidin induced liver cancer damage in rats **[9]** and are used in phytopharmaceutical medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis **[10]**. Although there have been various studies for antioxidant, hepatoprotective, antibacterial activities of *Eclipta alba* but there has been less focus on its anti-cancerous properties. Hence, the present study was carried out to assess and establish the role of *Eclipta alba* extract as anti-cancer agent using different tumour cell lines with emphasis on liver cancer.

Our present study was to evaluate the anticancer activity of the methanolic extract of *Eclipta alba* (MEEA) against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice.

2. Material and Method

2.1 Preparation of *Eclipta alba* hydroalcoholic extracts (EAE)

After shade dried the plant material was powdered in mechanical grinder. Then the plant material was extracted with petroleum ether (60-80° C) and after being defatted it was extracted with methanol in a Soxhlet extraction apparatus. The solvent was then completely removed under reduced pressure and stored in a vacuum desiccator. The yield of the methanol extract of the plant was 5.5%.

2.2 Treatment

Mature male Swiss albino mice weighing 22-26g were kept in identical laboratory condition and divided into six groups (n=10) and given food and water *ad libitum*. In Table-1, all the groups except Group 1 were injected with EAC Cells (2×10^6 cells/mouse.i.p.).This was taken as day 0. Group 1 served as normal saline control (5 ml/kg, p. o.) and Group 2 served as EAC control. On day 1 the methanol extract of *Eclipta alba* at a dose of 250 and 500 mg/kg body weight (Group -3 & 4) was 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 tumour hosts **[11]**.The effect of methanol extract on tumour growth and host's survival time were examined by studying the following parameters- tumour cell count, viable tumour cell count, nonviable tumour cell count, tumour volume, mean survival time and increase in life span.

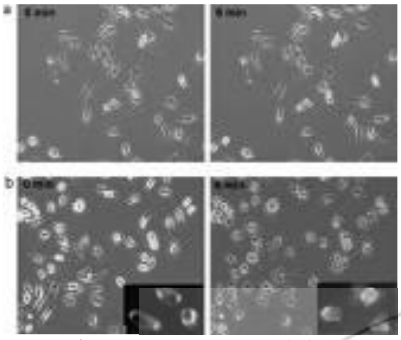

Figure 1: Cancer activity

Table 1: Treatment of various groups

Group	*Parameter*	*Dose*
Group 1	Normal saline Control	5 ml/kg, p. o.
Group 2	EAC	2×10^6 cells/mouse.i.p
Group 3	EAC+MEEA	250 mg\kg body weight
Group 4	EAC+MEEA	500 mg\kg body weight

2.3 Determination of tumour 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 64small squares was counted.

2.4 Estimation of viable and nonviable 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 nonviable cells were counted.
Cell count = (No. of cells × Dilution) / (Area × Thickness of liquid film)

2.5 Determination of tumour 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.

2.6 Mean survival time

Mean survival time = [1st Death + LastDeath]/2

2.7 Increase life span percentage (ILS%)

Recording the mortality monitored the effect of the MEEA on tumour growth and percentage increase in life span were calculated **[12]**.
ILS (%) = [(Mean survival of treated group/Mean survival of control group)-1] × 100

3. Haematological Studies

The effect of the MEEA on peripheral blood was investigated. RBC, WBC counts and estimation of haemoglobin were done by standard procedures from freely flowing tail vein blood **[13, 14]**.

Table 2: Effect of *Eclipta alba* extract on viable, non-viable cell count, tumour volume, survival time and life span in EAC bearing mice

Control Group	Viable cell count (10^4 cells/ml)	Non-viable cell count (10^4 cells/ml)	Tumour Volume(ml)	Survival time(days)	Increase in Life span (%)
Normal saline (5 ml/kg, p. o.)	-	-	-	-	-
EAC Control(2×10^6 cells/mouse)	9.82±0.24	3.61±0.11	3.39±0.20	21.80±1.61	-
EAC(2×10^6 cells/mouse)+MEEA(250 mg\kg body weight)	3.95±0.08	1.54±0.22	2.29±1.06	34.41±1.04	38.05
EAC(2×10^6 cells/mouse)+MEEA(500 mg\kg body weight)	2.41±0.18	2.04±0.21	1.79±1.08	37.75±2.62	73.54

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

4. Results and Discussions

In Table-2, oral administration of the methanol extract of *Eclipta alba* (MEEA) at the dose of 250 and 500 mg\kg body weight increased the life span and non-viable cell count, decreased tumour volume and viable cell count of the tumour-bearing mice, when compared to that of EAC control mice. MEEA restored the haematological parameters. The number of RBC count and Haemoglobin content also increased as compared to that of EAC control. Table-3 shows that in the differential count the percentage of Lymphocytes was increased with decreased level of Neutrophils.

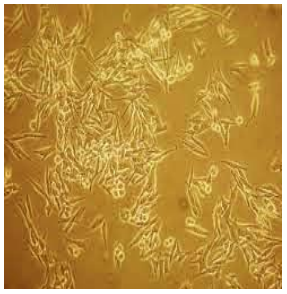

Figure 2: Before treatment

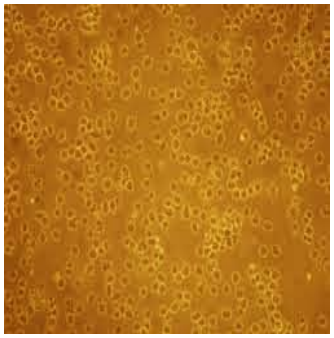

Figure 3: After treatment

The present study shows that MEEA was significantly increased the lifespan than that of EAC bearing mice. The reliable criteria for judging the value of any anticancer drug are prolongation of life span and decrease of WBC from blood **[15, 16]**. In addition to this, the reduced volume of EAC and increased survival time of mice suggest the delaying impact of MEEA on cell division. In cancer chemotherapy, the major problem is anaemia, due to reduction in RBC or haemoglobin concentration and leucocytes. Our findings say that MEEA have significantly enhanced the erythrocyte count and haemoglobin 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 MEEA possess less toxic effect on haematological system.

It was observed that viable cell count decreased with increased level of non-viable cell count. These suggested that *Eclipta alba* is directly related with tumour cells. Because these tumour cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and these anticancer agents lysis the cells by direct cytotoxic mechanism.

Table 3: Effect of *Eclipta alba* extract on haematological parameters in EAC bearing mice

Parameter	Normal saline (5 ml/kg, p. o.)	EAC Control(2×10^6 cells/mouse)	5-Furouracil	MEEA (250 mg\kg body weight)	MEEA (500 mg\kg body weight)
Haemoglobin (gm%)	13.8±0.3	5.9±0.8	12.8±0.1	10.2±0.5	11.5±0.3
RBC(10^6/mm^3)	6.4±0.2	4.5±0.3	1.6±0.5	5.0±1.0	5.8±0.2
WBC(10^3/mm^3)	7.5±0.3	17.7±1.4	8.4±0.9	11.4±1.4	9.7±0.2
Lymphocytes (%)	71.8±1.2	25.5±0.6	65.3±3.1	54.2±2.2	60.5±2.1
Neutrophils (%)	25.4±1.4	51.5±2.5	28.1±4.2	40.2±3.9	36.3±2.9
Monocytes (%)	2.3±0.5	4.3±0.4	2.9±0.6	3.7±0.2	3.1±0.4

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

Preliminary phytochemical screening indicated the presence of flavonoids, alkaloids and tannins in MEEA. Flavonoids have been shown to possess antimutagenic and antimalignant effects **[17, 18]**. In addition to this, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation **[19]** and angiogenesis **[20]**.

The anticancer properties are due to the presence of flavonoids. Reported results shows wedelolactone present in *Eclipa alba* were found to have 5-lipooxygenase and caspase inhibiting activities **[6, 21]**. The current studies indicate that both 5-LOX & 12-LOX expression such as human pancreatic cancer cell. LOX (Lipoxygenase) plays a critical role in human pancreatic cancer cell proliferation. LOX inhibitor may play a very important role in the treatment of cancer. Thus the anticancer activity of *Eclipa alba* may be due to the LOX inhibition or due to induction of detoxifying system.

5. Conclusion

The anticancer activity of *Eclipta alba* was examined. The methanolic extract of *Eclipa alba* restore the mean survival time, decrease tumour volume count in treated mice. *Eclipta alba* extract increased the life span of EAC treated mice and restored the hematological parameters as compared with the EAC bearing mice. Consequently, our study revealed that the methanolic extract of *Eclipta alba* showed anticancer activity.

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References

[1] Ayodhya Singh, Anjali Singh, Vandana Dwivedi; Antidiabetic effect of *Eclipta alba; International Journal Of Scientific & Engineering Research;* Vol. 5, Issue 2, Issn 2229-5518, pp 1462-1466, February-2014.
[2] Archana Tomar, Ayodhya Singh and Anjali Singh; Management of Diabetes Mellitus Utilizing Folk Medicinal Herbs; *Flora and Fauna*; Vol. 15, No. 1, pp 03-11, 2009.
[3] Puri, H.S.; Rasayana: Ayurvedic Herbs for Longevity and Rejuvenation; Taylor& Francis, London, pp. 80–85, 2003.
[4] Kim, D.I., Leeb, S.H., Choia, J.H., Lillehojb, H.S., Yud, M.H., Lee, G.S. The butanol fraction of Eclipta prostrata (Linn) effectively reduces serum lipid levels and improves antioxidant activities in CD rats. *Nutrition Research* **28**, pp 550–554, 2008.
[5] Orning, L., Hammarstrom, S., Samuelsson, B., Leukotriene, D., 1980. A slow reacting substance from

rat basophilic leukemia cells. Proceedings of the National Academy of Sciences of the United States of America, 2014–2017.

[6] Wagner, H., Geyer, B., Kiso, Y., Rao, G.S. Coumestans as the main active principles of the liver drugs *Eclipta alba* and Wedelia calendulacea. *Planta Medica* **52**, 370–37, 1986.

[7] Saxena, A.K., Singh, B., Anand, K.K. Hepatoprotective effects of *Eclipta alba* onsubcellular levels in rats. *Journal of Ethnopharmacology* **40**, 155–161,1993.

[8] Singh, B., Saxena, A.K., Chandan, B.K., Agarwal, S.G., Bhatia, M.S., Anand, K.K.; Hepatoprotective effect of ethanolic extract of *Eclipta alba* on experimental liverdamage in rats and mice. *Phytotherapy Research***7**, 154–158,1993.

[9] Handa, S.S., Prakash, P., Roy, B.; Bioactivity directed extraction and fraction of *Eclipta alba*—an antihepatotoxic drug of Indian origin; *Indian Journal of Pharmaceutical Science*, **50,** 1984.

[10] Murphy, R.C., Hammarstrom, S., Samuelsson, B., Leukotriene, C.; A slow reacting substance from marine mastocytoma cells. Proceedings of the NationalAcademy of Sciences of the United States of America, 4275–4279, 1979.

[11] 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*. **35,** pp 473-477, 1997.

[12] Sur P and Ganguly DK.; Tea plant root extract (TRE) as an antineoplastic agent. *Planta Med.* **60**, pp106-109, 1994.

[13] Dacie JV and Lewis SM. Practical Hematology. 2nd ed. *J and A Churchill*, London, pp 38-48, 1958.

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

[15] Clarkson BD and Burchenal JH. Preliminary screening of antineoplastic drugs. *Prog. Clin. Cancer* **1,** pp 625-629, 1965.

[16] Oberling C and Guerin M. The role of viruses in the production of cancer. *Adv. Cancer Res.* **2,** pp 353-423, 1954.

[17] Brown JP. A review of the genetic effect of naturally occurring flavonoids, anthraquinones and relatedcompounds. *Mutat. Res*. **75,** 243-247, 1980.

[18] 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.* **64,** pp 69-78, 1989.

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

[20] Fotsis T, Pepper M S, Aktas E, Breit S, Rasku S and Adlercreutz H. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. *Cancer Res.* **57**: 2916-2921, 1997.

[21] 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*, **11**: pp123-130, 2004.