EVALUATION OF FAGONIA INDICA ANTI-TUMOR ACTIVITY AGAINST EHRLICH'S ASCITES (EAC) CARCINOMA IN SWISS ALBINO MICE

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ABSTRACT

To evaluate the Effect of Fagonia indica extract (FIE) on tumor growth response, hematological studies and measurement of normal peritoneal cells in Ehrlich's ascites carcinoma (EAC) in Swiss albino mice. The intraperitoneal administration of the extract effectively reduced the development of solid tumor mass induced by EAC cells. Notably, significant anti-tumor activity was observed in mice treated with different doses over a 14-day period. The administration of the extracted substance led to a noticeable decrease in tumor volume and successfully reversed the alterations observed in hematological parameters caused by tumor inoculation. These findings strongly support the anti-tumor potential of the extracted substance against EAC-induced tumors, with the effectiveness varying depending on the dosage employed.

Keyword *: Fagonia indica, Ehrlich's ascites carcinoma, 5 FluoroUracil*

1. INTRODUCTION

Cancer is a cellular tumour that, unlike benign tumour cells, can metastasize and invade the surrounding and distant tissues [1]. Cancer is the abnormal growth of cells in our bodies that can lead to death. These cells are born due to imbalance in the body and by correcting this imbalance the cancer may be treated. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation. The important preventive methods for most of the cancers include dietary changes, stopping the use of tobacco products, treating inflammatory diseases effectively, and taking nutritional supplements that aid immune functions [2]. The eluted flavanoid has broad antibiotic properties – including nematocidal, antimicrobial, antiprotozoal and insecticidal activities. A thorough and complete literature search on *Fagonia indica*.was performed from the chemical abstracts, national and international journals, E-library, internet and other research materials. *Fagonia indica* is a medicinal plant of the Bignoniaceae family. It is widely distributed in Eastern Ghats and Deccan plateau in India. The leaves of *Fagonia indica* are used in traditional medicine for relieving headache, anti-inflammatory activity, preservative, anti-bacterial activity[3]. General pharmacological studies revealed analgesic, inflammatory, anti- microbial activity, anti-malarial activity, anti-fungal activity, anti-ulcer activity of aqueous and methanolic extracts of the leaves *Fagonia indica*[4]. The anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves of *Fagonia indica* in acute inflammation model has been reported. The phytoconstituents present in this plant are saponins, alkaloids, glycosides, tannins, flavanoids, sterols and triterpenoids. Three sapogenins named nahagenin, hederagenin and urosolic acid have been isolated from aerial part of Fagonia indica[5]. Based on this information the present study was carried out to evaluate the antitumor activity, status of ethanolic extracts of *Fagonia indica* against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Plant material and preparation of extract

The fresh leaves of *Fagonia indica* were collected from local area of Gajwel, Medak distrct,Telangana state, India. The plant was identified and authenticated in the Department of studies in Botany Osmania University, Telangana state, India. The leaves of the plant were shade dried on a laboratory table for 6 days and reduced to powder by using dry grinder. This powder (100g) was then packed into soxhlet apparatus and extracted using 95% ethanol (40-50°C) (Voucher No: SDRCP/HS/1/2022) was preserved in our laboratory herbarium.The extraction was carried out for 40h. The extract obtained was dried at 45 °C in hot air oven till green colored semisolid mass was obtained. The yield obtained was 4.5% and the semisolid extract was stored in a refrigerator at 4 °C until further use.

2.2 Experimental animals

The complete course of experiment was carried out using healthy Swiss albino mice weighing between 25-30 gm. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle. The animals were provided with standard pelleted diet obtained commercially from the manufacturer (Vivo Biotech, Turkapally) and water ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee Surabi Dayakar Rao College of Pharmacy before conducting the experiment

2.3 Drugs and Chemicals:

5-fluorouracil (5-FU) was purchased from Biochem Pharmaceuticals Ltd (Biochem, Mumai). Trypan blue was purchased from Sigma Chemicals (Sigma, U.S.A.). All other chemicals used were of analytical grade

2.4 Cell lines

Ehrlich's ascites carcinoma (EAQ cells were obtained from the courtesy of Amala Cancer Research Centre, Thrissur. EAC cells maintained by weekly intraperitoneal (i.p.) inoculation of 106 cells/mouse

2.5 Treatment schedule

Effect of EEFI on tumor growth and host survival were determined by evaluation tumor volume, tumor cell count and percentage increase in lifespan (% ILS) of the tumor hosts, respectively. For calculating the survival time four groups of mice were used and given food and water ad libitum. Tumor was induced to all the groups by injecting 0.2 ml of 2.3 x 105/ ml of EAC cells in to the peritoneal cavity of mice, except normal group. This was taken as day 'O'. On the first day the normal saline (0.9% w/v, NaCl 5 ml/kg/mouse/day) administered in to normal group. Two different doses of EEFI (50 mg/kg and 100 mg/kg i.p.) and standard drug 5-flurouracil (5-FU, 20 mg/kg i.p.) were subsequently administered for 9 days. On the 9 th day, after the last dose and 18 h fasting six from each group were sacrificed for the anti-tumor activity and the rest of the animals were kept for check the mean survival time (MST) and increase in the lifespan of the tumor bearing mice.

2.6 Dose selection

i) 5-fluorouracil (5-FU) dose of 20mg/kg dissolved in distilled water and administered orally for 9 days.
ii) Swiss albino mice were given at two different doses of ethanolic leaf extract of *Fagonia indica* (100 mg/kg and 200mg/kg body weight) 1ml with vehicle by oral administration daily, for 9 days..
iii) Tumor was induced to all the groups by injecting 0.2 ml of 2.3 x 105/ ml of EAC cells in to the peritoneal cavity of mice, except normal group.

2.7 Tumor growth response:

Antitumor growth effect of EEFI was assessed by observation of changes with respect to ascitics tumor volume, packed cell volume, viable and nonviable tumor cell count, MST and percentage increase in the lifespan (% ILS). Transplantable murine tumor was carefully collected with the help of s sterile 3 ml syringe and measured the tumor volume and the ascetic fluid with was withdraw in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1,000 rpm for 5 min. viable and nonviable cell count of asciatic cell were stained by the tryban blue dye (0.4% in normal saline) exclusion test and count was determined in a neubaur counting chamber. The effect of EEFI on tumor growth was monitored daily by recoding the mortality and percentage increase in life span (5 ILS) was calculated using the following formula ILS (%) = (Mean survival of treated group/Mean survival of control group) -1 x 100).

2.8 Hematological studies

On the 14 day after tumor inoculation blood was obtained through tail vein from normal mice, tumor bearing mice and tumor bearing mice treated with EEFI (100 mg/kg/mouse/day i.p. for the 9 days. For the total count blood was drawn into RBC or WBC pipettes, diluted and counted in a neubauer counting chambers. Sahil's Hemoglobinometer was used for the determination of hemoglobin concentration. Differential count of leukocytes was done on a freshly

drawn blood film using Leishman's stain. Hemoglobin content, RBC and WBC count and differential leukocyte count was estimated from the peripheral blood of normal, EAC control and treated groups.

2.9 Measurement of normal peritoneal cells:

Three groups of normal mice (n = 6) were used for his study. One group was treated with 100 mg/kg, i.p. of EEFI only once for a single day, while the second group received the same treatment for two consecutive days. The untreated third group was used as control and treated with equivalent volume of normal saline. After 24 h intraperitoneal fluid was collected by repeated intraperitoneal wash with cold normal saline and intraperitoneal cells were counted using hemocytometer in each of the treated groups and compared with untreated control group.

2.10 Statistical analysis

All the values are expressed in mean ± SEM. The data were statistically analyzed using one way analysis of ANOVA followed by Dunnett's multiple comparison test using Graphat Instat Software (San Diego, U.S.A.) and student's f-test Difference in means were considered to be significant when $P < 0.01$.

3. RESULTS AND DISCUSSION

Effect of EEFI on survival time of tumor bearing mice The effect EEFI on the survival of tumor bearing mice is shown in Table 1 and Fig. 1. Median survival time for the control group was 19.7 ±1.0 days, while it was 27.0 ± 0.9,31.5 ± 1.1, and 36.1 ± 0.8, days for the groups treated with Euphorbia hirta 50 mg/kg, 100 mg/kg and 5 FU 20 mg/kg, respectively. Significant ($P < 0.001$) increases in the lifespan of EAC bearing mice

Table 1. Effect of EEFI treatment on the survival of EAC bearing mice

S.NO	Experiment	Median survival time (days)	Increase in lifespan (%)
1	EAC Control	19.7 ±1.0	
2	EEFI (100	27.09 ± 0.9*	41.6%
3	mg/kg)	31.5 ±1.1*	59.9%
4	EEFI (200 mg/kg	36.1 ± 0.8*	83.2%
	5 FU (30 mg/kg)		

Values are expressed as mean ± SEM (n -10). Days of drug treatment = 9; $P< 0.001$, significantly differ from control group.

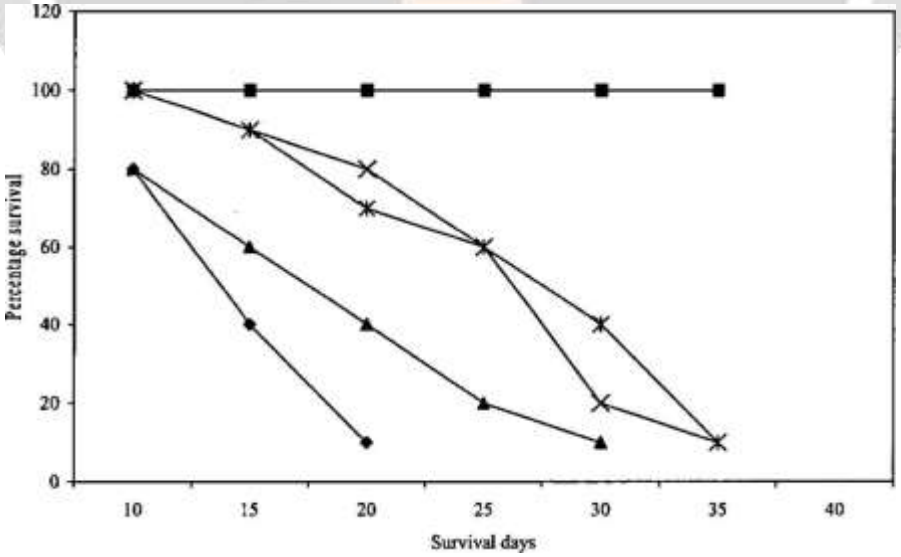

Fig. 1. Effect of EEFI and 5-FU on the percentage survival days of mice treated with EAC cells. Tumor bearing mice treated with EEFI 100,200 mg/kg and 5 FU- 30 mg/kg was found to be 41.6%, 59.6% and 83.2%, respectively, as compared to the control group.

3.1 Effect of EEFI on normal peritoneal cells

The average number of peritoneal exudates cells per normal mouse was found to be 5.98 ± 0.1. EEFI (100 mg/kg/day i.p.) treatment increased the number of peritoneal cells as shown in Table 2. Single treatment with EEFI

(200 mg/kg) enhances the number to 8.2 ± 0.1, while two consecutive treatments enhanced the number to 8.9 ± 0.1 (P < 0.001).

Table 2. Effect of EEFI (100 mg/kg/day i.p.) on peritoneal cells

Experiment	**Number of peritoneal cells (xlO6) per mouse**
Control	5.98 ± 0.1
Once treated	8.2 ± 0.1
Treated two consecutive days	8.9 ± 0.1*

Values are expressed as mean ± SEM (Number of animals used = 6 in each groups).
*P < 0.001 as compared to control group

3.2 Effect of EEFI on tumor growth

Intraperitoneal administration of EEFI (100 mg/kg) to tumor bearing mice was found to be effective in controlling the tumor volume development was shown in Table 3. The untreated control mice showed a tumor volume of 4.0 + 0.09 ml. A tumor volume of 1.8 ± 0.14 ml, 1.6 ± 0.1 ml and 1.4 ± 1.12 ml (induced by EAC cells) were recorded for the animals treated with the dose of EEFI 50 mg/kg, 100 mg/kg, and 5 FU 20 mg/kg, respectively. Tumor volumes in mice for the treated groups were lesser as compared to controls and statistically it was significant (P < 0.001).

The viable tumor cell counts were shown in Table 3. The viable tumor cell counts for the control group 8.4 ± 0.13, while it was 5.77 ± 0.13,3.51 ± 0.22 and 2.5 ± 0.28 for the groups treated with EEHE 50 mg/kg, 100 mg/kg and 5 FU 20 mg/kg, respectively. The reduction of viable tumor cell count was found to be effective (P < 0.001) as compared to the control group.

Table 3. Effect of EEFI on tumour growth and tumor cell count induced by EAC

S.NO	**Treatment**	**Tumor volume (ml)**	**Viable tumor cell count (x 107cells/ml)**
1	EAC control	4.0 ± 0.09	8.4 ± 0.13
2	EEFI(100mg/kg/dayi.p)	1.8 ± 0.14*	5.7 ±0.13*
3	EEFI(200mg/kg/dayi.p)	1.6 ±0.1*	3.5 ± 0.22*
4	5FU (30 mg/kg/day i.p.)	1.4 ± 1.12	2.5 ± 0.28*

mean ± SEM. Days of treatment = 7; P < 0.001 as compared to control group.

3.3 Effect of EEFI on hematological parameters

Hematological parameters of tumor bearing mice on 14th day were found to be significantly altered from group was shown in Table 4. The total WBC count, protein and PCV were found to be increased along with the hemoglobin content of RBC.

Table 4. Effect of EEFI (100 mg/kg/day, i.p.) on hematological parameters

S.NO	**Hematological Parameters**	**Normal**	**Tumor bearing mice (14 days)**	**EEFI Treated Tumor bearing mice**
1	Hb (%)	14.6 ± 0.3	8.9 ± 0.1’	12.8 ± 0.3*
2	RBC Cells/ml *10^{w}	3.9 ± 0.3	7.7 ±0.2*	2.8 ± 0.3
3	WBCCells/mlx10^{10}	7.5 ± 0.2	16.7 ± 0.2	10.8 ± 0.2
4	Protein (g%)	8.4 ±0.3	13.5 ± 0.3	0.8 ± 0.2
5	PCV(mm)	16.9 + 0.3	25.8 ± 0.3	18.9 ± 0.2
6	Neutrophil	69.7 ±0.3	25.8 ± 0.2*	56.2 ± 0.2
7	Lympocytes	30.1 ± 0.3	72.2 ± 0.2	42.7 ± 0.3
8	Monocytes	2.33 + 0.42	1.33 ± 0.33a	1.17±0.31b

Days of drug treatment = 14, n = 6 animals in each group. *P < 0.001 as compared to normal mice; *P < 0.01, P < 0.001 as compared to tumor bearing mice. A Non significant as compared to normal group; on significant as compared to control group.

Differential counts were significantly reduced by EEFI treatment and monocytes counts were not significant when compared with the control group (tumor treated groups). At the same time interval, EEFI (100 mg/kg/day i.p) treatment could change altered hematological parameters to near normal.

4. CONCLUSION

The cytotoxicity and anti-tumor properties of the extract of Fagonia indica may be due do its quinovic acid. Direct inhibition of lipid peroxidation is another protective measure. Quinovic acid and Quercetin inhibited melanoma growth and influenced the invasive and meatastatic potential in mice. A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequent incidence of lung cancer. This effect was mainly ascribed to quinovic acid, which provided > 95% of the total flavonoid intake in that particular study. quinovic acid inhibited melanoma growth and influenced the invasive and metastatic potential in mice. Quercetin, in particular, inhibits both cyclo-oxgenase and lipo-oxygenase activities, thus diminishing the formation of these inflammatory metabolites. The increase of lifespan and reduced tumor volume, viable tumor cell count by EEFI treatments seems to be playing a significant role in mediating cytotoxic and anti- tumor activity against Ehrlich's ascites carcinoma.

5. REFERENCES

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