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RESEARCH ARTICLE

PHARMACOLOGY

ANTITUMOR ACTIVITY OF *ALOE VERA* AGAINST EHRLICH ASCITIS CARCINOMA (EAC) IN SWISS ALBINO MICE

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ABSTRACT

Antitumor activity of 50% ethanol extract (100 mg/kg) of *Aloe vera* was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. After 24 h of tumor inoculation, the extract was administered daily for 14 days. After administration of the last dose followed by 18 h fasting, mice were sacrificed for observation of antitumor activity. The effect of *Aloe vera* on the growth of transplantable ascites tumor, body weight of EAC bearing hosts and simultaneous alterations in the hematological profile, serum (ALT, AST, LDH, ALP and glucose) and liver biochemical parameters (lipid peroxidation, GSH and antioxidant enzymes) were estimated. The *Aloe vera* showed decrease in abdominal circumference and body weight of EAC tumor bearing mice. Hematological profile reverted towards normal levels in extract treated mice. Treatment with *Aloe vera* restored the serum biochemical parameters towards normal levels and decreased the levels of lipid peroxidation and increased the levels of reduced glutathione and other antioxidant enzymes (SOD, CAT and GPx). The 50% ethanol extract of *Aloe vera* exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

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KEY WORDS
Aloe vera, antineoplastic agents, antioxidants, Ehrlich ascites carcinoma

INTRODUCTION
Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [1]. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development [2]. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. *Aloe vera* (*Sottrukattalai*, Tamil) is a perennial succulent belonging to the Liliaceae family and is called the healing plant or the silent healer. *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids [3]. Aloe gel has demonstrated wound healing [4], anti-inflammatory [5], antiviral [6], spermicidal [7], gastroprotective [8] and immune-stimulating [9] properties.

Plant derived natural products such as flavonoids, terpenoids, and steroids *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [10,11]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infection and degenerative diseases. From this viewpoint, the present study was carried out to evaluate the antitumor activity and antioxidant status of 50% ethanol extract of *Aloe vera* against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

MATERIALS AND METHODS
Materials
The dried leaf powder of *Aloe vera* was extracted (1:1 weight/volume) using 50% ethanol and kept overnight at room temperature. The residue was removed by filtration and the extract was centrifuged at 3214g for 10 min and the supernatant was collected and freeze dried. The freeze dried extract was stored at 4^0C and used for all further experiments. All reagents and chemicals used were of analytical grade. 5-fluorouracil was purchased from Himedia, Mumbai. Ehrlich Ascites Carcinoma (EAC) cell line was obtained from National Centre for Cell Sciences, Pune, India. Studies were carried out using Swiss albino mice of both sex weighing 20±2 g which were obtained from the Department of Laboratory Animals Medicine, Madhavaram, Chennai. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee.

Methods
Swiss albino mice were divided into 4 groups (*n*=6). All the groups were injected with EAC cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. From the first day, *Aloe Vera* extract (100mg/kg) and 5-FU (25mg/kg) were administered intraperitoneally for 14 days to groups 3 and 4 respectively. After the administration of last dose followed by 18 h fasting, all the mice were sacrificed for the study of antitumor activity, serum biochemical, hematological and liver biochemical parameters. Antitumor effect of *Aloe vera* was assessed by observation of changes with respect to body weight and abdominal circumference.

(i) Haematological studies:
Red blood cell (RBC), white blood cell (WBC) counts and haemoglobin levels were determined by routine clinical laboratory techniques. Differential leukocyte count

(DLC) was carried out from Leishman stained blood smears [12].

(ii) Serum biochemical parameters:

Serum glucose was estimated in semiauto analyzer system by glucose oxidase/peroxidase method by using standard kit (Agappe diagnostics). Serum enzymes, ALT, AST, LDH and ALP were estimated in semi auto analyzer system by using standard kits.

(iii) Liver biochemical parameters:

Immediately after sacrificing the animals, the liver was isolated and washed in ice cold normal saline to remove blood and it was blotted dry and stored at -20^{0}C for further analysis. Liver was crushed in tissue homogenizer (Heidolph, Germany) and 10% w/v liver homogenate was prepared in 0.05 M phosphate buffer (pH 7.4) and was used for the estimation of lipid peroxidation[13] (LPO) and reduced glutathione[14] (GSH). The rest of the homogenate was centrifuged at 15,000g for 1 hour at 4^{0}C and the supernatant thus obtained was used for the estimation of superoxide dismutase[15] (SOD), catalase[16] (CAT) and glutathione peroxidase[17] (GPx).

(iv) Histopathological examination:

A piece of liver, spleen and abdominal muscle samples were fixed in 10% formalin for histopathological examination. The thin sections were cut and then stained by haematoxylin and eosin and observed under light microscope[18].

(v) Statistical analysis:

The results were expressed as mean ± S.E. All analyses were carried out using the SPSS statistical program. The effect of treatments was determined by analyzing the data using one way-ANOVA followed by Duncan's multiple comparison test.

RESULTS

1. Effect of Aloe vera on body weight of EAC bearing mice :

There was a significant (P<0.01) increase in the body weight of EAC-bearing mice from 8th day onwards during a growth period of 14 days as compared to normal group and treatment with *Aloe vera* significantly decreased the body weight of tumor bearing mice from 10th day onwards (Table-1).

TABLE – 1

Effect of drugs on body weight (g) of EAC bearing mice

Measurement	NC	TC	AV	FU
Day 0	20.3^{a1} ± 0.3	21.8^{ab1} ± 0.7	22.7^{b1} ± 0.3	21.7^{ab12} ± 0.4
Day 2	21.7^{a12} ± 0.6	23.5^{ab12} ± 1.0	25.3^{ab1} ± 0.2	24.5^{ab3} ± 0.4
Day 4	23.2^{a23} ± 0.5	24.2^{a12} ± 0.5	24.7^{a1} ± 0.6	24.5^{a3} ± 0.4
Day 6	23.2^{a23} ± 1.1	25.3^{a23} ± 0.3	24.0^{a1} ± 0.8	23.5^{a23} ± 0.9
Day 8	23.8^{ab23} ± 0.3	26.3^{b234} ± 0.8	24.0^{ab1} ± 0.8	21.8^{a12} ± 0.7
Day 10	23.7^{b23} ± 0.4	27.8^{c34} ± 0.6	23.5^{b1} ± 1.0	20.3^{a1} ± 0.6
Day 12	24.2^{b3} ± 0.2	27.8^{c34} ± 0.8	22.7^{b1} ± 0.7	20.0^{a1} ± 0.5
Day 14	25.0^{b3} ± 0.3	29.0^{c4} ± 0.6	22.3^{a1} ± 0.9	20.0^{a1} ± 0.4

(Mean ± SE, n=6) (P<0.01)

Means bearing different superscripts (a,b,c) between the drug treatments differ significantly
Means bearing different superscripts (1,2,3,4) between the days differ significantly

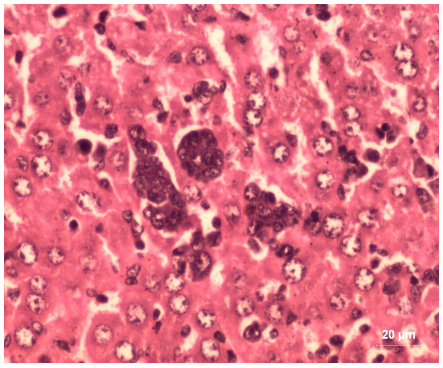


PLATE 1: TC – Liver showing multifocal infiltration of neoplastic cells in sinusoids

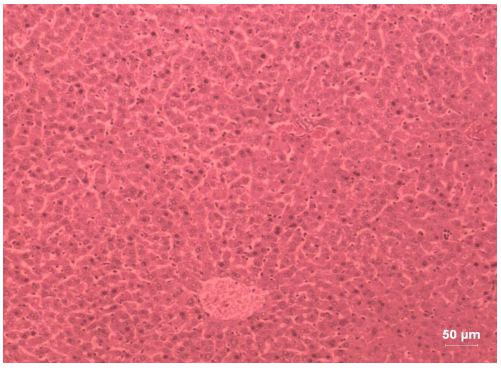


PLATE 2: AV - Liver showing normal architecture with no signs of infiltration

2. Effect of Aloe vera on abdominal circumference of EAC bearing mice :

There was a significant (P<0.05) increase in the abdominal circumference of EAC-bearing mice from 6th day onwards during a growth period of 14 days as compared to normal group and treatment with *Aloe vera* significantly decreased the abdominal circumference from 8th day onwards (Table-2).

TABLE – 2
Effect of drugs on abdominal circumference (cm) of EAC bearing mice

Measurement	NC	TC	AV	FU
Day 0	$6.62^{a1} \pm 0.22$	$6.65^{a1} \pm 0.25$	$6.68^{a1} \pm 0.22$	$6.65^{a123} \pm 0.18$
Day 2	$6.75^{a1} \pm 0.22$	$7.08^{a1} \pm 0.26$	$7.42^{a23} \pm 0.18$	$7.32^{a34} \pm 0.19$
Day 4	$6.95^{a1} \pm 0.17$	$7.72^{b2} \pm 0.21$	$8.00^{b3} \pm 0.30$	$7.57^{ab4} \pm 0.27$
Day 6	$6.87^{a1} \pm 0.20$	$8.27^{b23} \pm 0.25$	$8.03^{b3} \pm 0.12$	$7.33^{a34} \pm 0.20$
Day 8	$7.07^{a1} \pm 0.18$	$8.60^{b34} \pm 0.19$	$7.55^{a3} \pm 0.19$	$7.20^{a34} \pm 0.17$
Day 10	$7.10^{a1} \pm 0.23$	$8.95^{b4} \pm 0.19$	$7.40^{a23} \pm 0.15$	$7.02^{a234} \pm 0.24$
Day 12	$7.07^{a1} \pm 0.16$	$9.12^{b4} \pm 0.21$	$6.78^{a12} \pm 0.24$	$6.42^{a12} \pm 0.23$
Day 14	$7.03^{b1} \pm 0.19$	$9.23^{c4} \pm 0.19$	$6.38^{a1} \pm 0.25$	$6.07^{a1} \pm 0.22$

(Mean ± SE, n=6) (P<0.05)
Means bearing different superscripts (a,b,c) between the drug treatments differ significantly
Means bearing different superscripts (1,2,3,4) between the days differ significantly

2. *Effect of Aloe vera on serum glucose level of EAC bearing mice :*

Inoculation of EAC to mice significantly (P<0.05) decreased the serum glucose level in the EAC group (53.50 ± 4.97mg %) when compared to normal group (84.5 ± 2.85mg %) and treatment with *Aloe vera* significantly increased the glucose level as compared to EAC group but not to the level of normal group (Table-3).

TABLE – 3

Effect of drugs on tumor marker enzymes in the serum of EAC bearing mice

Groups	ALT** (IU/L)	AST** (IU/L)	LDH** (IU/L)	ALP* (IU/L)	Glucose* (mg %)
NC	22.8^{a}± 0.87	43.8^{a}± 1.51	148.20^{a}± 2.73	21.28^{a}± 2.26	84.5^{c}± 2.85
EAC	45.54^{b}± 2.75	66.84^{c}± 3.55	229.59^{b}± 12.64	45.67^{c} ± 3.28	53.50^{a}± 4.97
AV	27.06^{a} ± 2.08	54.9^{b}± 1.46	153.47^{a} ± 2.17	38.52^{bc}± 3.96	65.12^{b}± 3.96
FU	24.60^{a} ± 1.33	49.98^{ab} ± 2.98	150.80^{a} ± 2.51	28.78^{ab}± 3.55	68.09^{b} ± 3.19

Means bearing different superscripts between the drug treatments differ significantly
* (P<0.05), ** (P<0.01)

3. *Effect of Aloe vera on serum biochemical enzymes of EAC bearing mice :*

There was a significant (P<0.01) increase in serum ALT, AST, LDH and ALP (45.54 ± 2.75 IU/L), (66.84 ± 3.55 IU/L), (229.59 ± 12.64 IU/L) and (45.67 ± 3.28 IU/L) activity of EAC group as compared to normal group (22.8 ± 0.87 IU/L), (43.8 ± 1.51 IU/L), (148.20 ± 2.73 IU/L) and (21.28 ± 2.26 IU/L) respectively and treatment with *Aloe vera* significantly decreased the enzyme activity as compared to EAC group (Table-4).

TABLE – 4

Effect of drugs on hematological parameters of EAC bearing mice (Mean ± SE, n=6)

Groups	RBC* (x 10^6/µl)	WBC** (x 10^3/µl)	Hb* (g %)	Monocyte* (%)	Neutrophil* (%)	Lymphocyte* (%)
NC	4.2^{b} ± 0.2	7.3^{a} ± 0.2	13.8^{d}± 0.3	1.7^{a} ± 0.3	18.4^{a} ± 0.4	79.9^{c}± 0.3
EAC	2.9^{a}± 0.2	21.0^{c}± 0.6	8.2^{a}± 0.2	1.1^{a} ± 0.2	63.8^{c}± 2.6	35.0^{a}± 2.6
AV	4.0^{b} ± 0.2	10.7^{b}± 0.5	11.0^{c}± 0.2	1.6^{a} ± 0.3	43.0^{b} ± 3.4	55.4^{b} ± 3.2
FU	3.4^{a} ± 0.2	7.0^{a} ± 0.2	9.9^{b}± 0.1	1.7^{a} ± 0.4	40.5^{b} ± 1.9	57.8^{b} ±1.8

Means bearing different superscripts between the drug treatments differ significantly
*(P<0.05), ** (P<0.01).

4. Haematological parameters :

Haemoglobin content and RBC count were significantly (P<0.05) decreased and total WBC count was significantly (P<0.01) increased in the EAC group as compared to the normal group. Treatment with *Aloe vera* significantly restored the RBC and haemoglobin levels towards the normal.

In the differential count of WBC, the neutrophil count increased, while the lymphocyte count decreased in the EAC group as compared to the normal group. Treatment with *Aloe vera* significantly restored the altered parameters towards the normal values (Table-5).

5. Liver antioxidants assay :

5a. Effect of drugs on lipid peroxidation in the liver tissue of EAC bearing mice:

The level of lipid peroxidation in liver tissue was significantly (P<0.05) increased in the EAC group (515.26 ± 14.7) as compared to the normal group (95.65 ± 3.02). After treatment with *Aloe vera*, the level of lipid peroxidation was significantly (P<0.05) reduced in comparison to the EAC group (Table-6).

5b. Effect of drugs on glutathione content of the liver:

Inoculation of EAC significantly (P<0.05) decreased the GSH content in the EAC group (7.57 ± 0.73) when compared with the normal group (14.67 ± 0.77). The decreased amount was restored towards the normal levels in *Aloe vera* treated group (Table-6).

5c. Effect of drugs on liver antioxidant enzymes (SOD, CAT and GPX):

There was a significant (P<0.05) decrease in SOD, CAT and GPx activities in EAC group (2.80 ± 0.31, 0.19 ± 0.02 and 4.23 ± 0.32) when compared to normal group (5.27±0.27, 0.46±0.02 and 7.87±0.35). *Aloe vera* treatment significantly restored the liver antioxidant enzymes levels towards normal (Table-6).

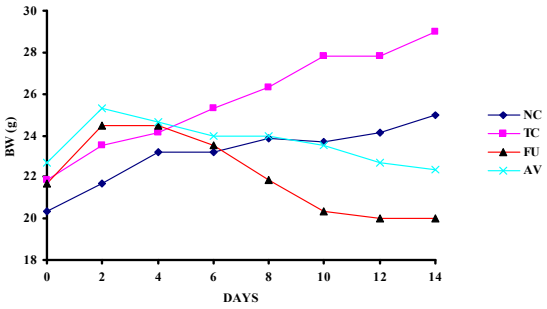
6. Histopathological examination:

The metastasis and invasion of neoplastic cells into liver, spleen and abdominal muscle were observed in EAC-bearing mice indicating the metastatic property of tumor. However, treatment with *Aloe vera* exerted a significant inhibition of metastasis in liver and spleen indicating their antimetastatic activity which could be comparable to that of fluorouracil, the standard drug used for comparison. However, *Aloe vera* was found less efficient in preventing metastasis at abdominal muscle when compared to fluorouracil(shown in Plate 1 to 6).

LPO	-	Lipid peroxidation	-	µM of MDA/g tissue
GSH	-	Reduced glutathione	-	mg of reduced GSH/g tissue
SOD	-	Superoxide dismutase	-	Units/mg protein
CAT	-	Catalase	-	µM of H_2O_2 utilized/min/mg protein
GPx	-	Glutathione peroxidase	-	µM of GSH utilized/min/mg protein

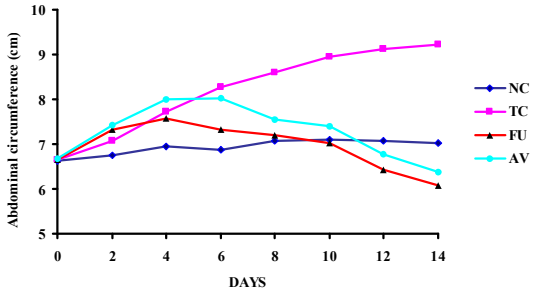
Graph. 1

Effect of drugs on body weight of EAC bearing mice



Graph. 2

Effect of drugs on abdominal circumference of EAC bearing mice



DISCUSSION

The present study was carried out to evaluate the antitumor activity of 50% ethanol extract of *Aloe vera* on EAC bearing mice. The extract treatment at the dose of 100 mg/kg inhibited the increase in body weight and abdominal circumference and also brought back the serum biochemical and hematological parameters towards normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD, CAT and GPx in tumor bearing mice to near normal levels.

In cancer chemotherapy the major problem are of myelosuppression and anemia [19,20]. 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 [21]. Treatment with *Aloe vera* brought back the hemoglobin content, RBC and WBC cell count near to normal values. This indicates that *Aloe vera* possess protective action on the haematopoietic system.

The free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactions of superoxide and hydrogen peroxide. Sun *et al* [22] reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn-SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported [23]. Similar findings were observed in the present investigation with EAC bearing mice. The administration of *Aloe vera* increased the SOD and CAT levels, which may indicate the antioxidant and free radical scavenging property of *Aloe vera*.

Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor

cells [24] and antitumor activity in experimental animals [25]. The lowering of lipid peroxidation and increase in levels of GSH, SOD and catalase in *Aloe vera* -treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress.

In addition, treatment with *Aloe vera* exerted a significant inhibition of metastasis in liver and spleen indicating their antimetastatic activity which is also supported by its antitumor and hepatoprotective activities. Thus, the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, *etc*, present in *Aloe vera* could be responsible for its potent antitumor activity.

CONCLUSION

The present study was carried out to evaluate the antitumor activity of 50% ethanol extract of *Aloe vera* on EAC bearing mice. The extract treatment at the dose of 100 mg/kg inhibited the increase in body weight and abdominal circumference and also brought back the serum biochemical and hematological parameters towards normal levels. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD, CAT and GPx in tumor bearing mice to near normal levels.

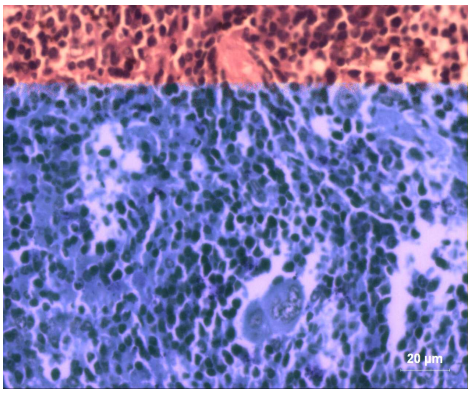


PLATE 3: TC – Spleen showing infiltration of multi nucleated cells

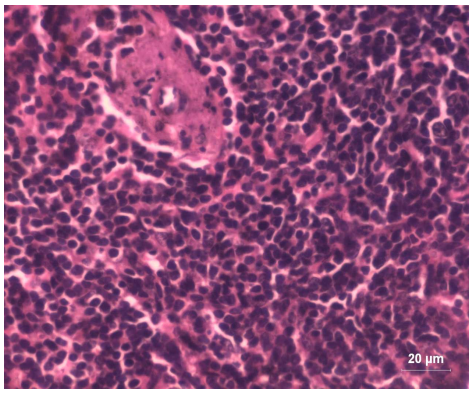


PLATE 4: AV – Spleen showing normal red and white pulp areas

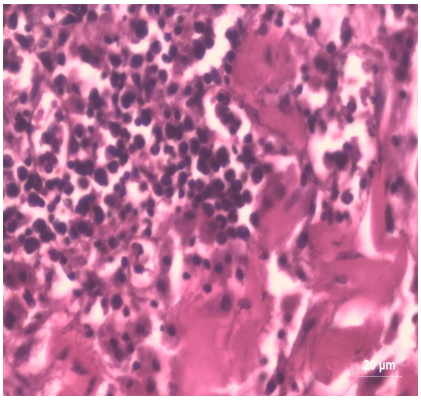


PLATE 5: TC – Abdominal muscle showing invasion of neoplastic cells into the muscle

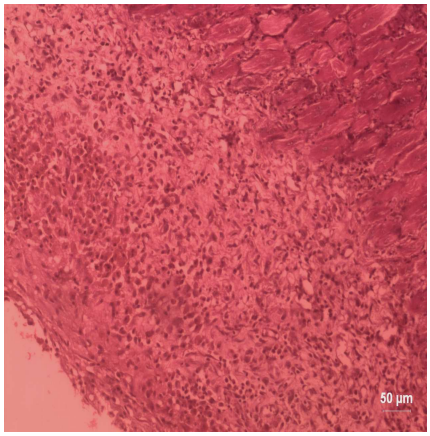


PLATE 6: AV – Abdominal muscle showing moderate infiltration of neoplastic cells

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