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Evaluation of In vivo Anticancer Activity of *Scaevola taccada* Roxb against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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Abstract

Objective: The present study was carried out to evaluate the anticancer property of ethanol extract of *Scaevola taccada* leaves against Ehrlich ascites carcinoma in Swiss albino mice.

Materials and Methods: The leaves powder was subjected to continuous hot extraction using ethanol and cold maceration by water to get Ethanol and Aqueous extracts, respectively. Identification of the chemical constituents of plant extract was determined by standard procedures. The in vivo anticancer study was determined in mice using Ehrlich ascites carcinoma cell line. In vivo cytotoxic effect of the extract was assessed by monitoring the mean survival time, effect on hematological parameters, cell count, tumor weight and status of antioxidant enzymes such as lipid peroxidase, reduced glutathione, superoxide dismutase and catalase activities.

Results: In phytochemical screening, the crude extract revealed the presence of different chemical groups like alkaloids, saponins, steroids, flavonoids and glycosides. The extract showed a significant increase in the life span and a decrease in cancer cell number, tumor weight and tumor volume. The protective effect of the extract on the hemopoietic system at the dose level 200 and 400 mg/kg were noted. The extract effectively suppressed the elevated levels of serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase. The extract prevented lipid peroxidation and restored the antioxidant enzymes.

Conclusion: From the result it can be found that the ethanol extract of *scaevola taccada* Roxb showed significant ($p<0.001$) in vivo cytotoxic effect when compared to the tumor control group.

Key words: S*caevola taccada Roxb*, In vivo Cytotoxicity studies**,** Ehrlich ascites carcinoma (EAC) cell line.

INTRODUCTION

The Plant *Scaevola taccada* Roxb belongs to the family Goodeniaceae, in vernacular language it is known as vellamuttagam, found in sea coasts of all around India and in the Andaman Islands. The Ethno pharmacology of the leaves revealed the uses of digestive, carminative, antitumor and anti-inflammatory properties. Fruit juice internally used to induce menstruation. The roots are used for dysentery. A decoction of the leaves and the bark was reported to combat tachycardia, one of the principal symptoms of beriberi. The drug reduces the frequency of heart beat, slow down the pulse rate and stimulates the heart for normal contraction. It exhibits diuretic property by increasing the tension in the renal arteries without causing irritation of the kidney parenchyma and is used for dropsy. The phytochemical studies of aerial part of the plant revealed the presence of loganin, sylvestroside-III, cantleyoside, dimethyl acetal and its compounds [1].

The Plant *Scaevola taccada* Roxb is a spreading freely branching shrub with thick stems, up to 3m in height. Leaves opposite, short-petiolate, fleshy, glossy, light-green, obovate, variable in size, but usually about 15 cm long and 5 cm wide. Flowers are white, zygomorphic, moderate sized, 5-lobed, borne on few flowered axillary inflorescences. Fruit a white juicy, globose drupe containing 1-2 seeds [2-4].

The Plant *Scaevola taccada* Roxb is reported to have Chemical constituents of scaevolin, chlorogenicacid, saponins, glycosides, lipids and alkaloids. Liquid from the leaves is used to treat weakness after childbirth which leads to pneumonia. The roots are used to treat stomachache. A decoction of the bark and leaves is used to treat a relapse after an illness. The juice from the bark is used in treating ringworm. The roots are used to treat beri-beri, syphilis and dysentery. Parts of the plant are used to treat coughs, tuberculosis and stings from the stingray [5].

The *Scaevola* species have been used in various traditional medicines. They are usually prepared in decoction form. However some are used as an application on the surface. Different parts of the plant are used to treat various illness, diseases or wounds. The crushed fruit of S*caevola taccada* has been used by early settlers to treat tinea. It is said that the leaves were taken when having indigestion. They are also used in a poultice to cure headache. In addition there are also reports indicating the use of leaf decoction and the flesh of the seeds as a contraceptive. The juice from ripe fruit has been used to treat sores and infected eyes whereas a combination of juices from ripe fruit and stem has been used as a remedy for bites and stings. This plant has also been used as a dermatological aid in Hawaii. A mixture of pounded root bark with salt is used for cut and skin diseases. In Indonesia the root is used as an antidote when poisonous fish and crabs are consumed [6].

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

The leaves of the plant of *Scaevola taccada* Roxb was collected from ABS Botanical Garden, Salem District, Tamilnadu, India. The dried powdered leaves of *Scaevola taccada* Roxb was extracted with Ethanol using Soxhlet Apparatus. The extract was dried and concentrated by evaporating the solvent completely under vacuum. In phytochemical screening the crude extract was revealed the presence of different chemical groups like tannins, saponins, steroids, flavonoids and glycosides [7 and 8].

Animals

The Male Swiss albino mice weighing 25-30g were used in the present study. All the animals were kept at room temperature of 22-25ºC in the animal house. All the animals were followed the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, animals were fed with standard food for one week in order to adapt to the laboratory conditions. All animal procedures were performed after approval from the institutional ethical committee (Registration No. 1525/PO/a/11/CBCSEA).

Acute Toxicity Study

The Ethanol extracts of *Scaevola taccada* leaves were studied for acute toxicity at doses of 5mg/kg, 50mg/kg, 300mg/kg, 500mg/kg and 2000mg/kg. As per OECD 420 guideline dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 420, it is considered as a LD50 cutoff value. Doses selected for pharmacological studies by fixed dose methods are 200mg/kg and 400mg/kg [9].

Tumor Cells

The Ehrlich ascites carcinoma (EAC) cells were obtained from Amala Cancer Research Centre, Trissur, Kerala, India. The cells were maintained in vivo in Swiss albino mice by intraperitonial transplantation. The EAC cells aspirated from the peritoneal cavity of mice were washed with saline and given intraperitoneally to develop ascetic tumor.

Experimental Design

The mice were divided into five groups comprising twelve animals in each group. The entire animal was injected with EAC cells ($2X10^6$cells/mouse) intraperitoneally except for the normal group as follows:

Group I : Normal (only sodium CMC Suspension (0.1%))

Group II : Control (Induced EAC cell ($2\ X10^6$) with sodium CMC Suspension (0.1%))

Group III : Standard (Induced EAC cell ($2\ X10^6$) with 5-fluorouracil 20mg/kg body weight)

Group IV : EEST (Induced EAC cell ($2X10^6$) with ethanol extract of *Scaevola taccada* 200mg/kg body weight with sodium CMC (0.1%))

Group V : EEST (Induced EAC cell ($2X10^6$) with ethanol extract of *Scaevola taccada* 400mg/kg body weight with sodium CMC (0.1%))

All groups were given with respective drugs 24 h after the tumor inoculation, once daily for 14 days. After the last dose and 24 h fasting, six mice in each group were sacrificed. The blood was collected from the animals by retro-orbital puncher under slight anesthesia conditions and the hematological parameter such as red blood cells (RBC), white blood cells (WBC), differential count (DC), and hemoglobin (HB) were estimated by cell analyzer. The differential count of WBC was carried out in the blood smear.

The ascetic fluid was collected from the peritoneal cavity of the animals, centrifuged and divided into two parts. One part was centrifuged in a graduate centrifuge tube at 1,000 rpm for 10 min and the packed cell volume was measured. The cells in the other part of the ascetic fluid were separated by centrifugation and stained with trypan blue (0.4% in normal saline). The number of viable cells and non-viable cells was counted .The rest of the animals were kept to check average life span and change in body weight for six weeks. Percent increase in life span (ILS) was calculated [10 and 11].

Tumor Growth Response

The effect of Ethanol extracts of *Scaevola taccada* on tumor growth and host's survival time were examined by studying the following parameters such as tumor volume, packed cell volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, median survival time and increase in lifespan.

Determination of Tumor Volume

The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weighed immediately.

Tumor Packed Cell Volume

The ascetic fluid was collected from the peritoneal cavity. The packed cell volume was measured by taking it in a graduated centrifuge tube and by centrifuging at 1000 rpm for 5 min.

Tumor Viable Cell Count

The ascetic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber. 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 cells were counted in the 64 small squares.

Percentage Increase Life Span

The effect of Ethanol Extracts of *Scaevola taccada* on tumor growth was monitored by recording the mortality daily for a period of six weeks and percentage increase in average life span was calculated.

$\%\ ILS = (A / B) - 1 \times 100$

A- Life span of treated group

B - Life span of controlled group

ILS - Increase in average life span

Body Weight Analysis

Body weights of the experimental mice were recorded both in the treated and control groups at the beginning of the experiment (day 0) and sequentially on every 5^{th} day during the treatment period and calculated on 15^{th} day.

$C = (a - b / a) \times 100$

a - Wt. of animal on day 0

b - Wt. of animal on day 15

C – % increase in body weight

Hematological Parameters

The collected blood was immediately used for the estimation of HB content, RBC and WBC. WBC differential count was carried out from Leishman stained blood smears [12].

Study of Biochemical Parameters

The remaining blood was centrifuged and serum was used for the estimation of hepatoprotective parameters like Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate Pyruvate transaminase (SGPT), alkaline phosphatase (ALP). The antioxidant parameters, tissue lipid peroxidation levels (LPO), Glutathione peroxidase (GSH), Superoxide dismutase (SOD) and Catalase (CAT).

Statistical Analysis

All the values were expressed as mean ± standard error of mean (S.E.M) and analyzed for significance by ANOVA and groups were compared by Tukey-Kramer multiple comparison test.

RESULTS AND DISCUSSION

In phytochemical screening the Ethanol Extracts of *Scaevola taccada* (EEST) showed the presence of alkaloids, saponins, steroids, flavonoids and glycosides. The animals of the tumor control group inoculated with EAC survived for a period 17.66±1.52 days. The treatment with EST at 200 and 400 mg/kg body weight increased the average life span of animals by 26±1.0 and 31.66±6.02 days, respectively, which is comparable to the standard drug (5-FluoroUracil) at the dose of 20mg/kg with the survival period of 35.33±5.85 (Table. 1 and Figure. 3). The increases in life span at 200 and 400 mg/kg body weight were found to be significant. The extracts at the 400 mg/kg body weight dose was found to be more potent in inhibiting the proliferation of EAC with the percentage increases in life span of 75.39%. The average increase in body weight of the EAC tumor control group was found to be 12.47±0.32%. Treatment at the doses of 200 and 400 mg/kg significantly inhibited the average increase in body weight (5.7±0.53**,** 4.86±8.88) when compared to the tumor control ($p<0.001$) (Table 1 & Fig. 1). The tumor volume (Table 1 & Fig. 2), packed cell volume, viable tumor cell count ($\times 10^6$ cells/ml) (Table 1 & Fig. 4) and total WBC (x10/mm) (Table. 2 and Figure. 6) were found to decreases significantly in animal treated with the extract at almost all the doses tested when compared to EAC tumor control which indicating the antitumor nature of the ethanol extract of *Scaevola taccada*.

Table 1. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Tumor Parameters.

Parameter	Control	Standard (20mg/kg)	EEST (200 mg/kg)	EEST (400 mg/kg)
Survival Time (Days)	17.66±1.52	35.33±5.85***	26±1.0*	31.66±6.02***
% Increase Life span	-	84.12**	61.9**	75.39**
Body Weight (g)	12.47±0.32	2.0±0.50*	5.7±0.53	4.86±8.88
Tumor volume (ml)	10.23±0.85	1.73±0.40***	4.2±0.20***	3.53±0.40***
Packed Cell Volume (mm)	6.83±0.28	1.16±0.28***	4.33±0.28***	3.5±0.50***
Viable cells ($\times 10^6$ cells/ ml)	8.24±0.28	1.21±0.20***	2.98±0.10***	2.28±0.21***
Non viable cells ($\times 10^6$ cells/ ml)	0.32±0.06	3.59±0.12***	1.91±0.07***	2.34±0.09***

n=6 animals in each group, Values are represented as mean ± SEM of six animals. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ between disease control and treated groups. (Analysed by ANOVA Tukey-Kramer multiple comparison test).

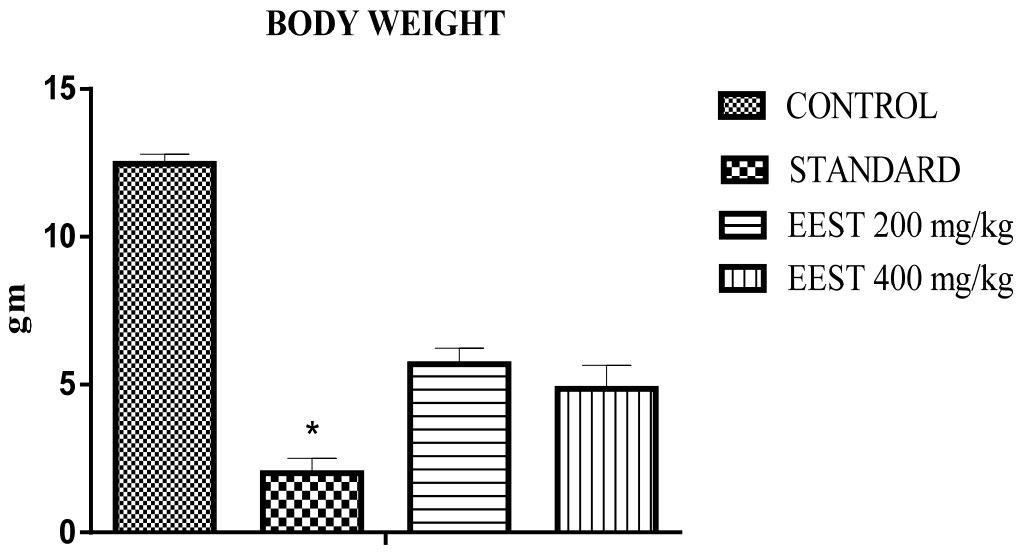

Figure 1. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on body weight in EAC bearing mice.

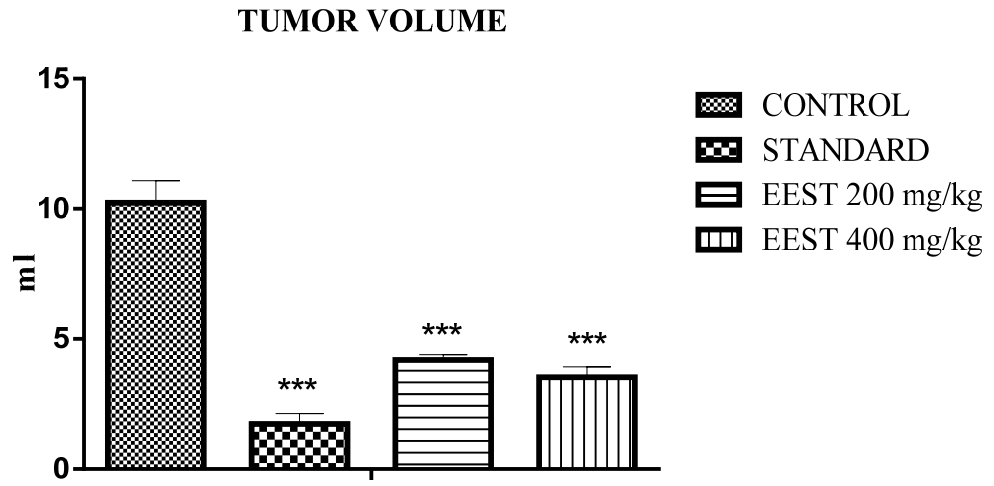

Figure 2. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on tumor volume in Ehrlich ascites carcinoma (EAC) bearing mice.

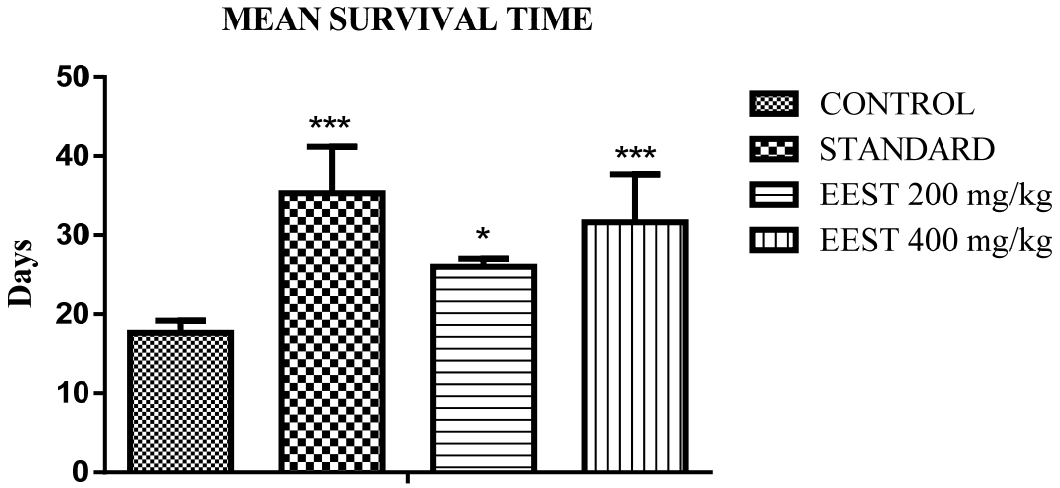

Figure 3. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on mean survival time in Ehrlich ascites carcinoma (EAC) bearing mice.

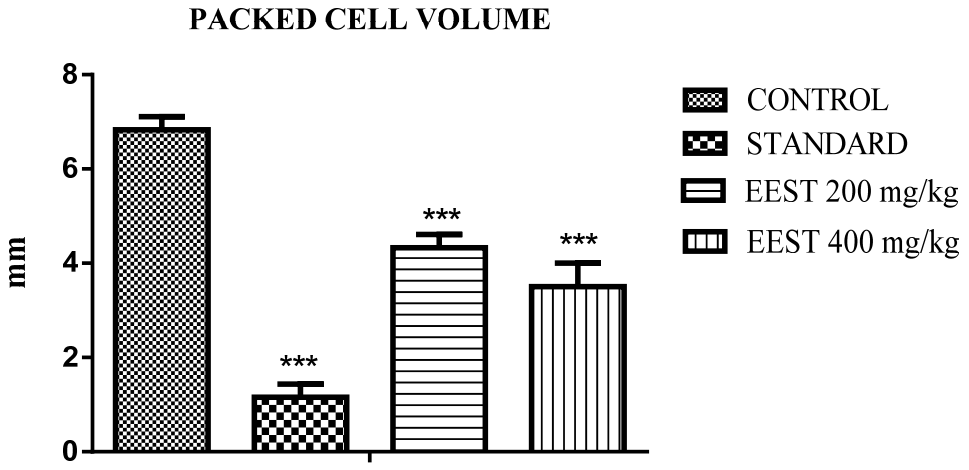

Figure 4. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Packed Cell Volume in Ehrlich ascites carcinoma (EAC) bearing mice.

Similarly, RBC count, hemoglobin content, and lymphocytes count, which were decreased after EAC inoculation, were found to be significantly returned to the normal levels in the animals treated with the EST at all the two doses. The neutrophile count, which was increased in EAC tumor control animals, was found to be decreased towards the normal by the extracts significantly ($p< 0.001$) at all the doses (Table 2 & Fig 5). The standard 5-FU treatment at 20 mg/kg body weight produced better results than the extract treatment in all these parameter.

Table 2. Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Hematological Parameters.

Hematological parameters		Normal	Control	Standard (20 mg/kg)	EEST (200 mg/kg)	EEST (400 mg/kg)
Hb (g %)		14.13±1.25	4.8±0.9	12.23±1.0***	9.86±1.0*	11.4±0.40***
RBC (million/mm^3)		5.33±0.75	2.3±0.1	5.16±0.25***	3.16±0.11	4.3±0.43*
WBC (10^3 cells/mm^3)		6.7±0.62	14.26±0.70	9.66±0.55***	12.3±0.43	11.5±0.62*
Differential Count (%)	Lymphocytes	70.1±0.40	32.4±0.70	66.26±0.50***	48.96±0.65***	57.93±0.65***
	Neutrophils	31.33±0.92	35.93±0.25	29.7±0.20***	33.43±0.47*	31.96±0.30***
	Monocytes	2.03±0.15	4.4±0.20	28.7±0.20***	3.83±0.15	2.9±0.26***

n=6 animals in each group, Values are represented as mean ± SEM of six animals. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ between disease control and treated groups. (Analysed by ANOVA Tukey-Kramer multiple comparison test)

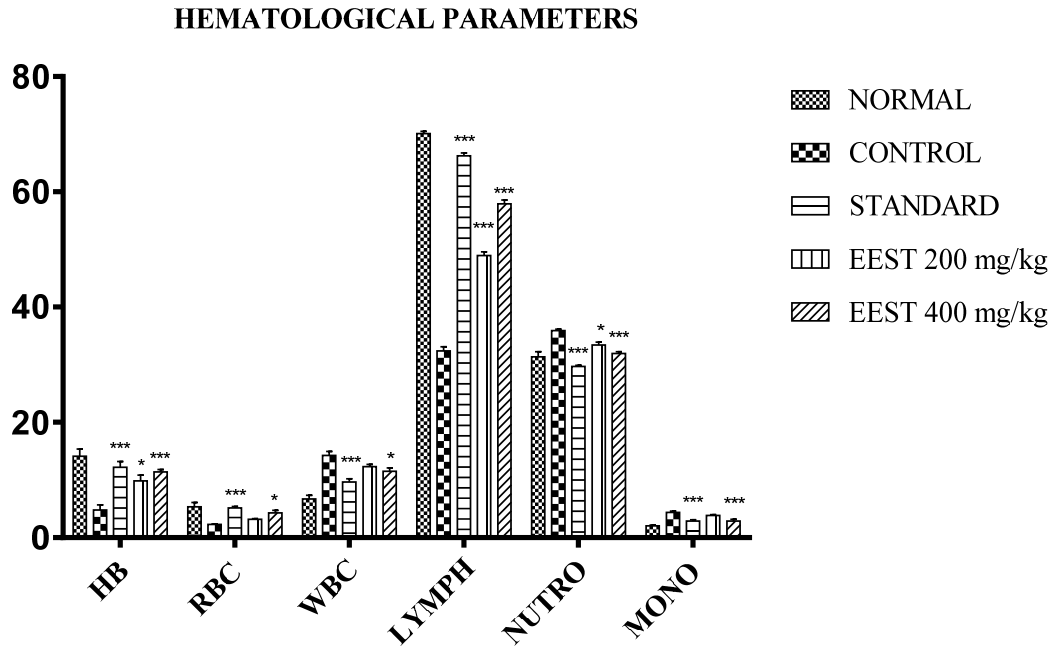

Figure 5. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Hematological Parameters.
HB: Hemoglobin, **RBC**: Red blood cells, **WBC**: White blood cells, **LYMPH**: Lymphocytes **NUTRO**: Neutrophils **MONO**: Monocytes.

Table 3. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Biochemical Parameters.

Design of treatment	Normal	Control	Standard (20 mg/kg)	EEST (200 mg/kg)	EEST (400 mg/kg)
Proteins (g %)	8.16±0.47	11.48±0.17	7.28±0.30	9.86±0.05***	7.96±0.12*
SGPT (U/L)	27.18±2.09	57.732±3.69***	32.95±1.10	43.54±1.87***	36.55±0.811*
SGOT (U/L)	33.48±1.18	62.88±3.09***	40.16±1.36	45.77±2.29**	37.73±2.13
ALP (U/L)	79.45±2.71	119.72±4.33***	81.31±1.48	99.42±2.59**	86.45±4.26
LPO (mol MDA/mg protein)	0.76 ± 0.031	2.96± 0.039***	0.97± 0.041***	2.12 ± 0.075**	1.03 ± 0.015***
GSH (mol/ g. wet tissue)	2.05 ± 1.532	0.61± 1.333	1.79± 0.977	1.47 ± 1.872	1.81 ± 0.931
SOD (U/mg protein)	4.19 ± 3.74	1.26 ± 1.01	3.32 ± 3.17	2.17 ± 4.00	3.48 ± 8.32
CAT (U/mg protein)	26.1 ± 0.023	9.37± 0.114	21.6± 0.011	19.1 ± 0.055	21.0 ± 0.063

n=6 animals in each group, Values are represented as mean ± SEM of six animals. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ between disease control and treated groups. (Analyzed by ANOVA Tukey-Kramer multiple comparison tests).

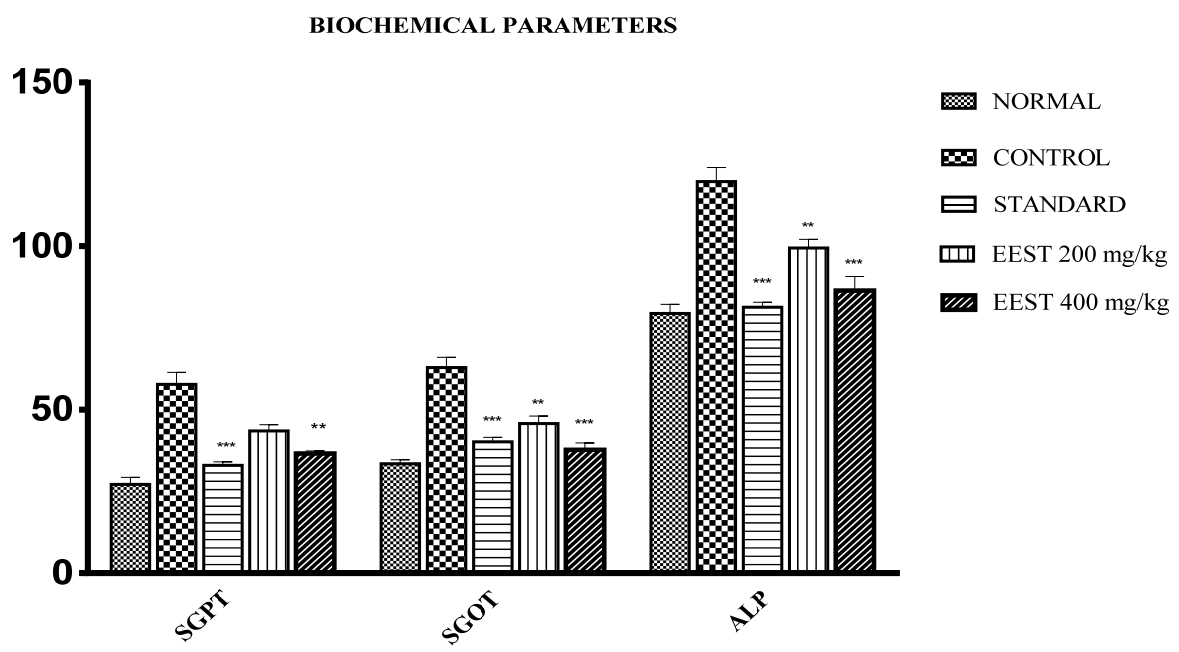

Figure 6. The Effect of Ethanol Extract of *Scaevola taccada* (EEST) on Serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase and alkaline phosphatase of Ehrlich ascites carcinoma (EAC) bearing mice.

The total WBC count was found to be increased significantly in the EAC bearing control group when compared to normal control. Administration of extract in EAC bearing mice significantly reduced the WBC count as compared with EAC bearing control group. The extract effectively suppressed the elevated levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphatase (ALP) (Table 3 & Fig. 6). The extract prevented lipid peroxidation and restored the antioxidant enzymes catalase, superoxide dismutase; glutathione peroxidase and glutathione-s-transferase in the liver were noted as compared with the tumor induced control mice. Tumor growth normally affects various haematological parameters and the anticancer activity is generally assessed by restoration of the changes in these parameters to normal and most significantly in decreased WBC and increased RBC, Lymphocyte and haemoglobin content as compared to control group.

Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. Plant derived compounds in particular have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use includes paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor from water soluble derivatives. Obviously natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development [15]. The present investigation was carried out to evaluate the anticancer activity of the ethanol extract of *Scaevola taccada* leaves extract using EAC tumor in mice.

The reliable criteria for judging the potency of any anticancer drug is the prolongation of the life span of tumor bearing animals. There is also a significant increase in the body weight in the EAC tumor bearing mice due to the regular rapid increase in the ascetic tumor volume [16]. The mice bearing EAC tumor when orally administered with the ethanol extract of *Scaevola taccada* leaves extract showed a significant increase in the life span and also a significantly prevented the increase in bodyweight that was observing in the EAC control mice.

Treatment with the ethanol extract of *Scaevola taccada* leaves extract also showed a significant decrease in the tumor volume, packed cell volume and viable tumor cell count, thereby indicating the anticancer nature of *Scaevola taccada* leaves extract. These results indicate cytotoxic effect on the tumor cells of *Scaevola taccada* leaves extract.

In cancer chemotherapy the major problem are myelosuppression and anemia [17]. The anemia encountered in the tumor bearing mice is due to the reduction in the RBC count or hemoglobin percentage and this may occur due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with *Scaevola taccada* leaves extract restored the hematological profiles as compared to EAC mice. This indicates the ethanol extract of *Scaevola taccada* leaves extract possess protective action on the hemopoietic system.

CONCLUSIONS

From the above findings it could be concluded that the leaves of ethanol extract of *Scaevola taccada* exhibited potent anticancer activity against EAC induced tumor in mice. Extensive research is needed to determine the individual component responsible for the anticancer activity and molecular mechanism responsible for the same.

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