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Evaluation of Antitumor Properties of *Semecarpus anacardium* Nuts using Daltons Ascites Lymphoma Bearing Swiss Albino Mice

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A B S T R A C T

Herbal concoctions have recently been investigated for various chronic and complicated ailments as effective therapeutics. In this study, a preliminary analysis of the *Semecarpus anacardium* nuts, has been investigated as a potent anti tumour agent against Daltons Ascites Lymphoma in mice by evaluating the tumour growth, toxicity and hematological parameters. The results of acute toxicity study of EESA have no mortality or change in body weight was observed in rats at a dose level of EESA500mg/kg body weight. These observations indicated that the calculated LD50 value (Dixon's likelihood method) for the oral doses of the EESA was found to be more than 2000 mg/kg body weight, accordingly 200 and 400 mg/kg body weight were taken as low and high dose of EESA for the experiment. After 24 hours of tumor inoculation, ethanol extract at doses of 200mg/kg and 400 mg/kg body weight was administered daily for 14 days. After administration of the last dose and 18hour fasting, the mice were sacrificed. Antitumor activity was assessed by monitoring tumor growth parameters and hematological parameters. The standard drug, 5- Fluoro Uracil, was used as a positive control. The ethanol extract of *Semecarpus anacardium* (EESA) was observed to restore the hematological parameters as compared with the DAL bearing mice in a dose dependent manner indicating a significant anti-tumour activity, thus indicating *Semecardium anacardium* nuts to be a potent ethnomedicine.

***Key words*:** Dalton's Ascites Lymphoma, *Semecarpous anacardium*, Tumor growth parameters, Toxicity, Hematological parameters

Conventional use of plant-based medications for treatment of various ailments is widely prevalentin both developed and developing countries. It has been estimated that around 60% of the world's population relies on plants for medications. This quantity raises to more than 80% due to the increase of populations in developing world, easy access and increasing drug expenses [1]. Thus, plants remain the chief provider of active drugs from natural sources [2]. Flora contains pharmacologically active components which are quite safe and often considered to be less toxic and free from side effects than synthetic ones. Plant derived components play a vital role in world health and have long been known to have biological activity [3]. Thirty percent of all recent drugs are derived from plants. According to the World Health Organization about 80% of the world's population living in developing countries relies basically on plants for primary health care [4].

Most importantly, these herbal medication methods are used in most disease conditions over a long period of time without proper dosage monitoring and consequently toxic effects maybe produced from such prolonged traditional practice. The danger associated with the potential toxicity of such herbal therapies used over a long period of time results in occurrences of renal and hepatic toxicities, as a consequence of intake of these medicinal herbs [5]. Thus, in modern medicine, animal toxicity studies are also required to establish the potential adverse effect of newly plant derived drugs [6].

METERIALS AND METHODS

Plant materials

The *Semecarpus anacardium* nuts, were collected from Kannur district of Kerala, India. Identification of the nut's material was done in the Department of Botany, Sree Narayana College, Kannur and voucher specimen is preserved as herbarium and submitted to the Department of

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Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. Fresh nuts used for extraction were shade dried and powdered using a mechanical grinder. The powder was collected in two clean, air tight containers for further use.

Chemicals

The chemicals used were 2,2-diphenyl-1-picrylhydrazyl which was acquired from sigma aldrich. 2,2'-anizo-bis (3- ethylbenzothiazoline-6-sulfonic acid. Ascorbic acid was procured from SDFCL (Biosar), India. All the other solvents and chemicals were obtained in systematic reagent category.

In vivo anticancer activity on Dalton's Ascites Lymphoma (DAL) induced cancer in mice

Experimental animals

Healthy Swiss albino mice (20±5 gm) were used for the study. The animals were obtained from Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. Animals were kept in polypropylene cages with sawdust bedding and maintained in laboratory conditions. Standard pellets were given as diet and water was provided adlibitum. The animals were acclimatized to laboratory conditions for about one week before commencement of the experiment. The experiments were performed after the approval from the institutional Animal Ethical Committee and in accordance with the recommendation for the proper care and use of the laboratory animal.

Acute toxicity study

Healthy Swiss albino mice (Group I – IV), starved overnight, were orally fed with Ethanol extract of *Semicorpus anacardium* (EESA) in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 gm/kg body weight, while group V (untreated) served as control. The animals were under continuous observation for two hours for changes in behavior, autonomic profiles and effects were observed intermittently for 72 h for death. IC_{10} and IC_{50} values of the extract were tested for acute toxicity and *in-vivo* experiments.

Treatment procedure

Animals were divided in to five groups each comprising six animals. One group served as the control while the remaining four groups were injected with Dalton's ascites lymphoma (1×10^6 cells/ mouse) to induce tumor. The treatments were given intraperitoneally at 24 h after the tumor inoculation and continued for 14 consecutive days.

Experimental design

The designation of the animal groups and treatment details were as follows:

Group I → Normal control
Group II → DAL control
Group III → DAL + Positive control (5-Fluoro Uracil: 10 mg/kg)
Group IV → DAL + EESA (200 mg /kg)
Group V → DAL+EESA (400 mg /kg)

General body weight observation and tumour characteristics evaluation

All the mice were weighed for every five days, after tumor inoculation. Average gain in body weight was determined and recorded. The percentage decrease in body weight was calculated by the formula:

$$\text{Percent decrease in body weight} = \frac{\text{Decrease in body weight}}{\text{Initial body weight}} \times 100$$

Haematological studies

Blood was withdrawn by cardiac puncture from *Semecarpus anacardium* treated and untreated DAL bearing mice. Different haematological parameters analysed were red blood cell count (RBC), Haemoglobin (Hb), White blood cell count (WBC), and differential leucocyte count by standard procedures.

Statistical analysis

Statistical data analysis differences between groups using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons with least significance difference test were made by statistical software package S.P.S.S. 17. A value of $p < 0.01$ was considered statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study

The results of acute toxicity study of EESA are presented in (Table 1). No mortality or change in body weight was observed in rats at a dose level of EESA500mg/kg body weight. Some clinical signs such as tremors, pilo erection and abdominal breathing were observed after the oral dosing of 1000 and 2000 mg/kg but no mortality or change in body weight was observed. These observations indicated that the calculated LD50 value (Dixon's likelihood method) for the oral doses of the EESA was found to be more than 2000 mg/kg body weight, accordingly 200 and 400 mg/kg body weight were taken as low and high dose of EESA for the experiment.

Tabel 1 Clinical signs of toxicity observed during acute oral toxicity study of EESA

Dose of EESC (mg/kg b.wt)	Latency	Symptoms
500	-	None
1000	-	Piloerection, abdominal breathing
1500	-	Piloerection, abdominal breathing
2000	-	Tremor, Piloerection, abdominal breathing

Effect on body weight and tumor growth analysis

Throughout the experimental period all the animals gained body weight. After tumor induction, at the end of 7th day, when compared with tumor bearing group (24.17 ± 1.67 µg/mg), the treatment groups (positive control and Ethanol extract of *Semicorpus anacardium* (EESA) 200

mg/kg) showed decrease in body weight (22.80 ± 1.03 and 24.14 ± 1.50 µg/mg) respectively. At the end of 14th day, when compared with Dalton's Ascites Lymphoma (DAL) bearing mice (30.32 ± 1.47 µg/mg) all the treated groups showed a decrease in body weight (23.40 ± 1.59, 27.17 ± 0.67 and 24.34 ± 1.67 µg/mg) (Table 2).

Table 2 Effect of Ethanol extract of *Semicorpus anacardium* (EESA) on body weight

Experimental group	Before induction	After induction	
		On 7th day	On 14th day
Normal control	19.60 ± 0.66	-	-
DAL control	21.50 ± 1.09	24.17±1.67	30.32±1.47
DAL + 5FU	18.73 ± 1.44	22.80±1.03	23.40±1.59
DAL + EESA (200 mg/kg)	20.25 ± 1.44	24.14±1.50	27.17±1.67
DAL + EESA (400 mg/ kg)	19.44 ±0.87	25.72±0.91	24.34±1.67
F- test	3.7896*	4.1025*	18.6881**
SED	0.3829	0.4925	0.7361
CD (P< 0.05)	1.0990	1.4136	2.1123

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant

Tabel 3 Effect of EESA on hematological parameters

Parameters	Haemoglobin (g/dl)	RBC ($x10^6$ cells/µl)	WBC ($x10^3$ cells/µl)	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)	Basophils (%)	Platelets ($x10^3$ cells/µl)
Normal control	14.32 ± 1.56	4.83 ± 1.60	8.45 ± 1.86	25.68 ± 1.36	2.66 ± 1.13	54.48 ± 1.26	6.17 ± 0.55	0.23 ± 0.04	9.19 ± 1.64
DAL control	7.17 ± 1.67	2.48 ± 0.76	21.77 ± 2.17	21.68 ± 1.57	0.93 ± 0.58	66.57 ± 1.26	3.68 ± 1.57	0.10 ± 0.04	15.68 ± 1.61
DAL +5 FU	11.34 ± 1.69	3.50 ± 1.15	9.48 ± 1.21	22.39 ± 1.79	1.72 ± 0.99	57.40 ± 1.37	4.19 ± 1.64	0.10 ± 0.06	7.07 ± 1.77
DAL + EESA (200 mg/kg)	10.51 ± 1.77	3.30 ± 1.01	10.40 ± 1.63	23.53 ± 1.36	1.32 ± 0.96	58.5 ± 1.75	4.30 ± 1.64	0.12 ± 0.05	10.39 ± 1.82
DAL + EESA (400 mg/kg)	9.60 ± 1.29	2.78 ± 0.97	11.34 ± 1.69	23.86 ± 1.53	1.74 ± 0.60	60.45 ± 1.68	4.27 ± 1.69	0.10 ± 0.06	4.27 ± 1.69
F- test	13.15**	3.18*	47.88**	4.98**	2.59NS	36.51**	2.07NS	0.0009NS	16.98**
Sed. CD	0.731	0.364	0.863	0.666	0.221	0.788	0.624	0.002	0.859
(p<0.05)	2.100	1.046	2.476	1.913	0.636	2.263	1.792	3.117	2.467

Values are expressed as the mean ± S.E.M (n=6); Statistical significance (p) calculated by one way ANOVA followed by Duncan's multiple range test. Significant levels were ** $p < 0.01$; * $p < 0.05$; NS –Non significant

Effect on hematological parameters

Hemoglobin (Hb) indicates that EESA does not affect the erythropoiesis, morphology, or osmotic fragility of the red blood cells. The level of RBC that has been observed to decline during the progression of tumour has been found to improve in mice treated with EESA 200 and 400 mg/kg (3.30 ± 1.01 and 2.78 ± 0.97 µg/mg) with regard to Hb content all the treatment groups showed significant increase when compared to DAL control (7.17± 1.67 µg/mg). The treatment with EESA 200 mg and 400 mg to DAL bearing mice enhanced Hb content to (10.51±1.77 and 9.60 ± 1.29 µg/mg) respectively. Total WBC count was found to be increased in DAL control group (21.77 ± 2.17 µg/mg) when compared with normal control animals (8.45 ± 1.86 µg/mg). Administration of EESA at the dose of 200 mg and 400 mg/kg reduced the WBC count to (10.40±1.63 and 11.34±1.69 µg/mg) (Tabel 3). In a differential count of WBC, a significant (p<0.05) decrease in monocyte, neutrophil, basophils and eosinophil and an increase in lymphocyte and platelets count in DAL control mice was observed [7]. Treatment with EESA at different doses changed these altered parameters more or less to the normal values (Table 3). Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity when the data are translated from animal studies [8]. Myelosuppression and anaemia are the foremost difficulties encountered in the cancer chemotherapy. The anemia encountered in tumor bearing mice is mainly due to the reduction in RBC or Hemoglobin percentage and this may occur either due to deficiency due to hemolytic or myelopathic conditions. Similar results were observed in the present study. Treatment with EESA brought back the hemoglobin and RBC count more or less to normal levels. This indicates that EESA possess protective action in the hemopoietic system. Leukocytes are the first line of cellular defence that

responds to infectious agents, tissue injury, or inflammatory process. In a differential count of WBC the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval EESA treatment could change those altered parameters to near normal. These results are in accordance with the reports of Chitra *et al.* [9] indicating the possibility of EESA as a potential agent in the area of cancer chemotherapy.

CONCLUSION

The present study indicates *Semecarpus anacardium* nuts could be a promising anti-tumour agent against cancer for a malignant DAL carcinoma in induced Swiss albino mice. Additional investigations are required to understand the mechanism of the anti-tumour action of *Semecarpus anacardium* nuts in the other cancer celllines.

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