Effects of *Semecarpus anacardium* L.f. ethanol extract on serum biochemical parameters and antioxidant activity of DAL induced mice

Shebriya Abdulla, Amrisa Pavithra E & Santhy K S*

Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043, India

*Email: dr.santhyanandan@gmail.com

**Abstract**

*Semecarpus anacardium* L.f. nuts is one of the best, versatile and most commonly used herbs as a household remedy, distributed in sub-Himalayan region, Tropical region, Bihar, Bengal, Orissa and central and south parts of India. In this work, the anticancer efficacy of ethanol extract of *Semecarpus anacardium* nuts (EESA) was tested in Swiss albino mice with induced Dalton’s Ascites Lymphoma (DAL). Ethanol extract of *S. anacardium* nuts was given daily for 14 days at doses of 200 mg/kg and 400 mg/kg body weight, after 24 hrs of tumor inoculation. After receiving the final dose and fasting for 18 hrs, the mice were euthanized. Serum biochemical and antioxidant characteristics were measured to determine antitumor activity. 5-Fluorouracil, a typical medication, was employed as a positive control. In comparison to the control group, ethanol extract-treated mice restored biochemical and antioxidant parameters to near-normal levels. Biochemical and antioxidant parameters in the Dalton’s Ascited Lymphoma (DAL) animal model treated with EESA exhibited similar effects as the positive control drug 5-fluorouracil. Overall, the active ingredients present in ethanol extract of *Semecarpus anacardium* nuts, modulate serum biochemical and antioxidant parameters and bring about anti-tumor activity against Dalton’s Ascites Lymphoma (DAL) in mice.

**Keywords**

Dalton’s Ascites Lymphoma, *Semecarpus anacardium*, Serum biochemical parameters, Antioxidant parameters

**Introduction**

Cancer is characterised by aberrant tissue growth in which cells divide uncontrollably and autonomously which inevitably resulting in a steady rise of the number of dividing cells (1), that spread throughout the body and may eventually cause the death of the host. The World Health Organization reports that cancer is now one of the major causes of mortality worldwide (2, 3). As per the WHO, Cancer deaths are anticipated to grow, with an expected 13.1 million deaths by 2030 (4). Diseases are caused by both exogenous (e.g., smoking) and endogenous (e.g., normal cell metabolism) activities. Excessive oxidative stress can harm DNA, causing severe base damage, strand breakage, altered gene expressions and in the worst-case scenario, mutagenesis. Breast, colon and prostate cancer are thought to be caused mostly by oxidative DNA damage (5). A variety of free radicals cause oxidative stress, which has been connected to the pathogenesis of inflammatory illnesses such as cancer, diabetes and ageing (6). Psychosocial support, surgery, radiotherapy and chemotherapy are all examples of current treatments. Depending on the type and location of cancer, the patient’s age, general health and other considerations, one of these treatments may be...
utilised. As a matter of fact, enhancing cancer treatments from plant materials and integrating them into clinical practise is a top priority right now (7, 8). Tumor models are crucial in the development of experimental anticancer drugs. Swiss albino mice are the perfect model to learn about the antitumor property among all Dalton’s Ascites Lymphoma models (8). Antioxidant-rich plants have been reported to effectively prevent cardiovascular disease, cancer, neurological illnesses, inflammation and problems associated with cell and skin ageing (9).

*S. anacardium* is found in the sub-Himalayan, tropical, Bihar, Bengal, Odisha and central and southern parts of India, and is one of the best, most adaptable and most widely used herbal as a domestic cure. Fruits of *S. anacardium* mature from December to March and the plant blooms in June and then yields fruit from then on. For millennia, it has been freely utilised throughout India. 'Ballataka' or 'Bhilwa' are two prevalent names for it and it is one of the most widely used therapeutic plants in Ayurveda (10). Reports are on the presence of flavonoids, phenolic compound, bhihlanol, anacardic acid, semecarpol, bhlawanol, monolefin-1, dilefin-2, tetrahydromentoflavone, tetrehydrobustafavlon, jeedifavonone, semecarpufavanone and gullufavanone, oleic acid, linoleic acid, palmitic acid, stearic acid and arachidic acid in *S. anacardium* (11). Seed oil is primarily employed in the medical field. Seeds are usually boiled in milk and then consumed with the milk. The seed oil is used in the lowest dose possible, usually blended with food or mustard oil. The external wound is treated with *S. anacardium* seed oil to reduce the formation of pus and to help wounds recover more quickly (12). Several biological activities of nut milk extract have been documented, including anti-arthritis, antispermatogenic, antibacterial and mutagenic characteristics (13). Studies on the antitumor activity of *S. anacardium* have been scanty so far. Hence, the present study focused on investigating the *S. anacardium* against Dalton’s ascites lymphoma tumor model.

### Materials and Methods

#### Collection of plant materials

*S. anacardium* L.f. nuts were collected from villages in the Western Ghats hillocks of Tamilnadu and Kerala, such as Chavadi, Kadambara and Mukkali. The nuts were identified in the Department of Botany at Sree Narayana College in Kannur and the reference specimen is herbarium-preserved and sent to the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. Fresh nuts were left to dry in shade and pulverised with an electrical grinder. The powder was saved in clean, airtight containers for subsequent 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) and Ascorbic acid were procured from SDFCL (Biosar), India. All the other solvents and chemicals were obtained in the systematic reagent category.

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

#### Experimental animals

The study employed healthy Swiss albino mice weighing 20 ± 5 gm. Animal House, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore provided the animals. Mice were kept in a lab environment with sawdust bedding in a cage made of polypropylene. The diet consisted of standard pellets, and water was given ad-lib. Mice were given a week to acclimate to lab conditions before beginning the experiment (14). The tests were conducted in accordance with the rules for the proper care and use of laboratory animals, as approved by the Institution's Animal Ethical Committee (AIW: IAEC.2019:ZOO:04).

#### Treatment procedure

The animals were grouped into five of six each. One group was used as a control, while the other four were given Dalton’s ascites lymphoma cells (1 x 10⁶ cells per animal) to initiate tumour development. The experimental drugs were delivered intraperitoneally 24 hrs after the tumour was injected and extended for 14 days.

#### Experimental design

The following were the animal groups' designations and treatment details:

- **Group I → Normal control**
- **Group II → DAL control**
- **Group III → DAL + Positive control (5-Fluoro Uracil: 10 mg/kg)**
- **Group IV → DAL + EESA (100mg /kg)**
- **Group V → DAL+EESA (200mg /kg)**

#### Estimation of Serum biochemical parameters

Using a semi-automated analyzer (Photometer 5010 v5+) and standard enzymatic kits, the isolated serum sample had been used to determine Alkaline Phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), total protein, urea, uric acid, BUN, total cholesterol and triglycerides.

#### Estimation of Antioxidant parameters

Ohkawa et al’s approach was utilised to calculate the level of lipid peroxidation (LPx) (15). Using a Sigma Aldrich kit, the activity of enzyme antioxidants such as catalase (CAT) and superoxide dismutase (SOD) was assessed (St Louis, MO). The standard method was used to determine the activity of GPx (16). Eilman’s method was used to determine the amount of reduced glutathione (GSH) in the body (17).

#### Statistical analysis

With the statistical software package SPSS 17, one-way analysis of variance (ANOVA) and Duncan’s multiple comparison test were used to analyse differences across groups. The value of *p*<0.01 was used as a statistically significant value.
Results

The ALP level in the control group was determined to be 89.58 ± 0.52 IU/l. The maximal ALP level in DAL carrying mice was found to be 101.59 ± 1.46 IU/l, which was considerably (p<0.01 and p<0.05) lowered in EESA (200 and 400 mg/kg) treated mice (89.38 ± 1.17 and 90.66 ± 1.72 IU/l). When compared to the DAL control group (47.09 ± 1.96 IU/l), SGOT levels were observed to be considerably lower in all treatment groups. In comparison to the greater dose of EESA (400 mg/kg), the low dose of EESA (200 mg/kg) was considered to be effective (42.69 ± 1.28 IU/l).

SGPT level shown in Table 1 revealed a significantly higher level of enzyme in DAL treated mice (41.83 ± 1.86 IU/l) when compared to the normal animals (32.28 ± 0.98 IU/l). EESA treated groups differ significantly from DAL treated mice (31.01 ± 1.52 and 34.45 ± 0.88 IU/l). The total protein content was determined in all the treated and control mice. In the control group, total protein content was found to be 5.96 ± 0.71 IU/l. DAL-treated mice’s total protein content increased to 11.46 ± 0.63 IU/l, whereas EESA-treated mice’s total protein content (200 mg/kg and 400 mg/kg) was found to be elevated to 20.82 ± 0.22 mg/dl in DAL-treated mice, whereas it was dramatically decreased in EESA-treated mice, 200 mg and 400 mg/kg respectively (18.39 ± 0.97 and 17.85 ± 0.73 mg/dl) (Table 1). Among antioxidant parameters, SOD level recorded in DAL induced mice was significantly decreased (1.51 ± 0.11 U/mg) in comparison with control mice (2.53 ± 0.60 U/mg). SOD levels were observed to be elevated to 2.48 ± 0.45 and 2.47 ± 0.51 U/mg in DAL mice treated with EESA extract (200 mg/kg and 400 mg/kg) as shown in Table 2.

CAT level was decreased significantly in DAL induced mice (45.65 ± 1.69 U/mg) as compared to control mice (66.44 ± 3.60 U/mg). When DAL induced mice were administered EESA (200 mg/kg and 400 mg/kg), the CAT levels were significantly elevated to 63.88 ± 1.36 and 64.17 ± 0.86 U/mg. In this study, the DAL-treated group had a drastic decline in GPx (21.78 ± 2.44 U/mg) when compared with normal control mice (36.16 ± 0.92 U/mg). When compared to the DAL-treated group, both EESA treated group (200 and 400 mg/kg) demonstrated a significant increase in GPx (36.15 ± 1.59 and 35.87 ± 0.57 U/mg). A significant decrease in GSH (6.16 ± 0.55 and 6.09 ± 0.42 IU/l respectively), decreased considerably (P<0.05).

All of the animals in the treatment groups had their cholesterol levels measured. The cholesterol content in the control group was determined to be 76.29 ± 1.50 mg/dl. The cholesterol level of DAL-treated mice increased to 93.35 ± 3.05 mg/dl, but EESA-treated animals, 200 and 400 mg/kg, had considerably lower cholesterol content (88.38 ± 2.26 and 86.73 ± 2.97 mg/dl respectively). When comparing the DAL-treated animals to the EESA-treated animals, the amount of uric acid was shown to be significantly lower in both groups (Table 1). In terms of uric acid, the EESA alone treated group (200 mg/kg) did not exhibit any significant differences when compared to the normal control group. The treatment of EESA considerably decreased the triglyceride level in 200 and 400 mg/kg treated groups (149.26 ± 2.68 and 151.96 ± 4.92 mg/dl) when compared with DAL treated animals (177.39 ± 4.03 mg/dl). The effect of lower dose was found to be significantly higher than the higher dose EESA. The BUN content was analysed in all the treated and control mice. In the untreated mice, BUN content was determined to be 17.52 ± 0.46 mg/dl. BUN level was recorded in DAL induced mice (0.36 ± 0.04 μg/mg) in comparison to normal mice (1.48 ± 0.91 μg/mg). In the case of DAL induced mice, the GSH level of both the EESA treated groups (200 and 400 mg/kg) was found to be increased to 1.31 ± 0.53 and 1.21 ± 0.64 μg/mg respectively. With the administration of EESA to the DAL treated mice, the level of LPx was observed to be considerably decreased. In both the treatment groups of EESA, 200 mg/kg (0.84 ± 0.05 μg of MDA/mg) and 400 mg/kg (0.82 ± 0.05 μg of MDA/mg) LPx was found to be decreased (Table 2).

Discussion

When it comes to cancer, cell organelles, notably hepatocytes, undergo differentiation, resulting in the release of enzymes, which affects the plasma membrane’s permeability (18). In tumor-induced DAL mice, researchers found a rise in tumor volume as well as higher levels of SGOT, SGPT and ALP. Alkaline phosphatase aids in the transport of metabolites across the cell membrane, protein synthesis, secretory functions, and glycogen metabolism. Any changes to alkaline phosphatase, which is a membrane-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholesterol (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>ALP (IU/L)</th>
<th>Total protein (gm/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Blood Urea (mg/dl)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>76.29 ± 1.50b</td>
<td>2.52 ± 0.46b</td>
<td>89.58 ± 0.52b</td>
<td>5.96 ± 0.71b</td>
<td>134.40 ± 3.84b</td>
<td>17.52 ± 0.46b</td>
<td>40.62 ± 1.34b</td>
<td>32.28 ± 0.98b</td>
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<tr>
<td>DAL control</td>
<td>93.35 ± 3.05a</td>
<td>5.82 ± 0.22a</td>
<td>101.59 ± 1.46a</td>
<td>11.46 ± 0.63a</td>
<td>177.39 ± 4.03a</td>
<td>20.82 ± 0.22a</td>
<td>47.09 ± 1.96a</td>
<td>41.83 ± 1.86a</td>
</tr>
<tr>
<td>DAL + (5-FU, 10 mg/kg)</td>
<td>79.92 ± 3.33b</td>
<td>4.88 ± 0.62b</td>
<td>88.51 ± 1.34b</td>
<td>6.13 ± 0.56b</td>
<td>142.03 ± 3.03b</td>
<td>17.68 ± 0.41b</td>
<td>42.21 ± 1.47b</td>
<td>36.08 ± 1.15b</td>
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<tr>
<td>DAL + EESA 200 mg/kg</td>
<td>88.38 ± 2.26a</td>
<td>3.98 ± 0.28a</td>
<td>89.38 ± 1.17b</td>
<td>6.16 ± 0.55b</td>
<td>149.26 ± 2.68b</td>
<td>18.39 ± 0.95b</td>
<td>42.69 ± 1.28b</td>
<td>35.01 ± 1.52b</td>
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<tr>
<td>DAL + EESA 400 mg/kg</td>
<td>86.73 ± 2.97b</td>
<td>3.70 ± 0.46b</td>
<td>90.66 ± 1.72b</td>
<td>6.09 ± 0.42b</td>
<td>151.96 ± 4.92b</td>
<td>17.85 ± 0.73b</td>
<td>42.90 ± 1.41b</td>
<td>34.45 ± 0.88b</td>
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<tr>
<td>F-test</td>
<td>**</td>
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<tr>
<td>SEd</td>
<td>1.713</td>
<td>0.275</td>
<td>0.830</td>
<td>0.371</td>
<td>2.395</td>
<td>0.389</td>
<td>0.958</td>
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<td>2.382</td>
<td>1.067</td>
<td>6.875</td>
<td>1.117</td>
<td>2.751</td>
<td>2.422</td>
</tr>
</tbody>
</table>

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.
bound enzyme, are likely to disrupt metabolite transport and reduce membrane permeability (19).

In the current work, biochemical analysis of EESA-induced Dalton's ascites lymphoma (DAL) in mice revealed a significant increase in ALP, SOD, and SGP, demonstrating the tumor's hepatotoxic effect. Many tissues, including the bone, liver, gut and placenta, produce serum alkaline phosphatase, which is excreted in the bile. After treatment with EESA, the up-regulation of serum hepatic marker enzymes was dropped to near-normal levels. Treatment with this nut extract also balanced the lipid profile and protein profile. The findings were compared to those of the conventional medication 5-fluorouracil (10 mg/kg). As a result, the current work demonstrates that EESA extract can regulate increased levels of ALP in EESA-induced Dalton's ascites lymphoma (DAL) in mice, implying that this extract may have the potential to recover the plasma membrane.

When compared to normal tissue, tumor tissue has more cholesterol. Specific changes in plasma cholesterol are characterized by changes in intracellular cholesterol (20). Hence, the present study report that the EESA significantly restores cholesterol level to near normal value. The liver produces triglycerides that may change to cholesterol. In this study, EESA treatment in both doses (200 and 400 mg/kg) could restore the total protein content and lipid to a near-normal level suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. Uric acid is a biomarker of oxidative stress as well as a purine metabolic end product. It can operate as a pro-oxidant at high concentrations. In the current investigation, a drop in uric acid levels in the EESA-treated groups indicated that renal function had returned to normal (21).

The development of numerous metabolic, chronic diseases or malignancies has been linked to oxidative stress (22, 23). The goal of this study was to identify how ethanol extract of S. anacardium affected biochemical and antioxidant parameters of Dalton's ascites lymphoma (DAL) in mice. This extract possessed certain amounts of antioxidant potential by scavenging free radicals. A study reported that Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) from the enzymic antioxidant system, scavenge ROS and lipid peroxidation (24).

SOD acts as a radical scavenger and facilitates the partitioning of the superoxide anion radical into molecular oxygen, water or hydrogen peroxide in practically all living cells exposed to oxygen. When SOD isn't controlled, it causes a variety of cell destructions (24). We found a significant reduction in SOD and catalase activity in our study, which could be attributable to an increase in circulating lipid peroxides. An antioxidant enzyme, catalase present within cells that aids in the removal and conversion of hydrogen peroxide (H₂O₂) to molecular oxygen (O₂) and water. CAT activity is directly regulated by the build-up of H₂O₂ in the tissues (25). Hydroxyl radicals and singlet oxygen are neutralised by GPx. It protects cells from free radical damage when present in high concentrations in the cells. GSH, a non-protein thiol, protects cells from cytotoxic and carcinogenic chemicals when coupled with GPx and GST. By donating a hydrogen atom, GSH serves as a direct antioxidant, neutralising the hydroxyl radical. GSH protects the body from free radicals, functioning as a cofactor for enzymatic antioxidants and speeding up the elimination of xenobiotics (26). The decrease in GSH levels in this study may also be due to a reduction in the substrate obtainable for GSH synthesis. According to a study, mammmary lipid peroxidation, which is the oxidative degradation of polyunsaturated fatty acids (PUFA) in biological membranes, was found to be higher in breast cancer-bearing rats, resulting in impaired structural integrity, decreased membrane fluidity, and inactivation of several membrane-bound enzymes and functions (27, 28). As a result, it's fair to believe that carcinogen exposure causes PUFA peroxidation, which leads to cellular degeneration in breast tissue (29). In EESA, similar results were found using the DAL model. This was in line with the findings in which higher levels of lipid peroxides in cancer-bearing animals' serum and lungs (30).

**Conclusion**

Effects of *Semecarpus anacardium* L.f. ethanol extract on serum biochemical parameters and antioxidant activity of DAL induced mice was investigated in this work. The extract treatment at the 200 mg/kg and 400 mg/kg body weight inhibited the tumor activity by restoring the serum biochemical and antioxidant parameters. The normalization of these parameters indicates the antitumor activity of *Semecarpus anacardium* nuts extracts. Based on the facts
above, the ethanol extract of S. anacardium has the potential to be used as a natural agent in cancer treatment.

Acknowledgements

This work was supported by Department of Zoology, Avinashilingam Institute for Homoeoscience and Higher Education for Women, Coimbatore.

Authors contributions

SA carried out the biochemical and antioxidant studies, drafted the manuscript. APE participated in the design of the study and performed the statistical analysis. SKS conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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