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Antioxidant potential of *Drosera peltata* in Dalton Ascites Lymphoma (DAL) bearing mice

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ABSTRACT

Cancer is one of the prominent causes of death reported by World Health Organization (WHO). The purpose of this study was to measure the antioxidant status of animals treated with 250 and 500 mg/kg doses of ethanol and aqueous extract of *Drosera peltata* on Dalton Ascites Lymphoma (DAL) inoculated mice. A total of 70 mice were divided into 7 groups, each group with ten mice. The first group (negative control) received normal food and water for 14 days and was kept under normal conditions. The second group also received normal food and water for 14 days, which was used as a cancer (positive) control. The third group received 5-fluorouracil (20 mg/kg, i.p.) once a day for 14 days. The fourth and fifth group animals received 250 and 500 mg/kg of ethanol extracts of *D. peltata* (EEDP) whereas the sixth and seventh groups of mice received 250 and 500 mg/kg of aqueous extracts of *D. peltata* (AEDP), orally for 14 days. All the groups were inoculated with DAL (2×10^6 cells/mouse, i.p.) except group I, 24 hours before the commencement of the drug treatment. After the completion of treatment, blood was drawn retro-orbitally and the animals were sacrificed to isolate the liver, lungs, kidneys, and brain for observing tissue antioxidant status. The parameters analyzed were total protein (TP), catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), peroxidase (P), and glutathione (GSH) from the tissues apart, and the protein carbonyl content (PCC) also measured from the blood sample. Treatment with EEDP and AEDP significantly lowered the MDA levels from 23 to 10 mmol/ml in the blood, whereas from 28 to 4 nm/g in tissue isolates of the liver, lungs, kidneys, and brain. It also raised the TP, GSH, SOD, CAT, and P levels in the blood and in the tissue samples of the cancer cell line inoculated animals, where their levels were close to those observed in control (negative) group animals. The results proposed that both extracts of *D. peltata* ameliorated various tissue antioxidant levels in mice with DAL cancer lines comparable to the negative control.

1. Introduction

Ayurveda, the Indian medical system, treats a variety of illnesses, including cancer, primarily using herbal medicines or formulations. It is one of the oldest medical systems covering thousands of medical concepts and hypotheses. Interestingly, Ayurveda can treat many chronic diseases that modern medicine cannot treat, such as cancer, diabetes, arthritis, and asthma (Parasuraman et al., 2014). Cancer is one of the prominent causes of death reported by WHO (WHO, 2022). There are several ways to treat cancer in modern medicine. These include chemotherapy, radiation therapy, and surgery. Chemotherapy is currently considered to be the most effective way to treat cancer (Baskar et al., 2012). The high toxicity of most anticancer drugs has facilitated the development of less toxic and cheaper complements. Plants have long been used to treat cancer. An important strategy for developing effective anti-cancer drugs is the discovery of phytochemicals with anti-cancer activity from natural resources. Plant-derived

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anticancer compounds and their derivatives are effective in cancer treatment. Natural plant-derived substances such as flavonoids, terpenoids, and steroids have received a great deal of attention in recent years due to their various pharmacological actions, such as antioxidants and antitumors (Desai et al., 2008). Similarly, they have proven useful in the prevention and control of adverse pathophysiological conditions and complex diseases including cancer. One of the plants rich in therapeutically important compounds as well as used in Ayurvedic formulations is *Drosera*. The genus *Drosera* is commonly known as Sandew. It is one of the largest genera of more than 170 species of carnivorous plants belonging to the Droseraceae family. In India, three *Drosera* plants were identified namely *Drosera indica* L., *D. burmannii* Vahl., and *D. peltata* J.Sm. These include 1,4 naphthoquinone, plumbagin, ramantaseon and its glucosiderosoliside, and flavonoids such as quercetin and hyperoside. In both Plumbaginaceae and Droseraceae, a yellow color pigment was found as a main phytoconstituent, named plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone). Plumbagin is a major active ingredient with a variety of pharmacological actions, including anti-fertility, anti-malaria, anti-viral, anti-bacterial, anti-convulsant, anti-cancer, and leishmania drugs. Plumbagin and quercetin are the biologically active important phytoconstituents found in *Drosera*. Pharmacological effects are based on the number of constituents present in each plant (Asirvatam & Christina, 2018). HPTLC method was used to quantify the plumbagin and quercetin content among the three Indian *Drosera* species (Asirvatham et al., 2020). The above species are used in several clinical manifestations, such as memory loss, vision loss, infertility, general weakness of the body, the development of premature aging, and bronchial asthma. It is used as an important ingredient in Ayurveda preparations ('Swarnabhasma'-Golden ash) for rheumatoid arthritis, diabetes, and neuropathy (Asirvatham et al., 2013). Cold decoration of *D. indica* is used to remove corn and these species are mentioned in the list of endangered medicinal plants (Reddy et al., 2001). Earlier in this report, the in vitro antioxidant and anticancer potential as well as the in vivo effect of *D. peltata* have been reported for different in vitro antioxidant and anticancer models (Balaji & Asirvatham, 2015). This study aimed to determine the ability of cells to recover and retain the antioxidant enzymes with the treatment of ethanol and an aqueous extract of 250 and 500 mg/kg *D. peltata* in Dalton Ascites Lymphoma (DAL) bearing mice.

2. Materials and methods

2.1. Plant material and extraction

The whole plant of *D. peltata* (Figure 1) was collected from Munnar, Kerala, India (10.0889° N, 77.0595° E) in December 2008. Air-dried, coarsely powdered whole plants (350 g) were defatted with petroleum ether (60-80 °C), and followed the merc was extracted with ethanol solvent in a Soxhlet extractor for 72 hours. The obtained ethanol extract was concentrated and allowed to dry under a controlled temperature (40-50 °C). The obtained merc was soaked in water containing chloroform for 2 days, filtered, concentrated, and allowed dry to get the aqueous extract (Asirvatham et al., 2013). Ethanol extract of *D. peltata* (EEDP) and aqueous extract of *D. peltata* (AEDP) were reconstituted with distilled water for animal study.

2.2. Experimental animal and study approval

The treatment protocol (A. Raju 0903PH2254/JNTUH 2009) was initially presented to the Institutional Animal Ethical Committee. After reviewing the procedure, the committee had permitted to conduct the study. Adult male and female Swiss albino mice weighing approximately 20 to 25 g were acclimated to experimental conditions for approximately 2 weeks before subjecting to the experimental procedure. Tumor cell line-Dalton Ascites Lymphoma (DAL) cells-were obtained from the Amala Cancer Institute in Thrissur, Kerala, India. DAL cells were maintained in mice by weekly intraperitoneal (i.p.) inoculation of 2 x 10^6 cells/mouse.

2.3. Treatment protocol

It was a 14-day study, in which a total of 70 mice were divided into seven groups containing ten animals in each (Christina et al., 2004). All the mice were inoculated with DAL cells (2 × 10^6 cells/mouse, i.p.) 24 hours before the commencement of the drug treatment, except mice belonging to group I (negative control).

Group I: The animals received normal food and water for 14 days
Group II (DAL control): The animals received normal food and water for 14 days
Group III (DAL cells): The animals received 5- fluorouracil (20 mg/kg, i.p) for 14 days
Group IV (DAL cells): The animals received EEDP (250 mg/kg, p.o.) for 14 days
Group V (DAL cells): The animals received EEDP (500 mg/kg, p.o.) for 14 days
Group VI (DAL cells): The animals received AEDP (250 mg/kg, p.o.) for 14 days
Group VII (DAL cells): The animals received AEDP (500 mg/kg, p.o.) for 14 days

On the 15th day, blood was collected by retro-orbital puncture and was allowed to stand for 45 minutes at room temperature. Serum was collected after centrifugation at 2500 rpm at 30 °C for 15 minutes to estimate antioxidant enzyme levels (Demirci et al., 2011) in serum such as malondialdehyde (MDA), catalase (CAT), glutathione (GSH), superoxide dismutase (SOD), and protein carbonyl content (PCC).

After the collection of blood, mice were sacrificed with excessive anesthesia, and the liver, lung, kidney, and brain samples were removed for the measurement of tissue antioxidant status. To estimate the content of antioxidants in the tissue, the isolated organ was divided into two parts to prepare tissue homogenates (Vani et al., 1990). The first homogenate (10%, w/v) was prepared with potassium chloride (KCl, 0.15 M) and the content was centrifuged at 8000 rpm for 10 minutes, and the supernatant was used to measure total protein (TP), peroxidase (P), catalase (CAT), and malondialdehyde (MDA). Similarly, the second homogenate (10%, w/v) was prepared with sucrose phosphate buffer (5M, pH 7.4) and the test tube content was centrifuged at 8000 rpm for 10 minutes, and the supernatant was used for the measurement of glutathione peroxidase (GSH) and superoxide dismutase (SOD). The determination of the levels of mentioned antioxidant enzymes from tissue homogenates was done by COBAS MIRA PLUS–S autoanalyzer (Roche, Switzerland) using antioxidant estimation assay kits from Agappe Diagnostics, India (Raju et al., 2022).

2.4. Statistical analysis

The results obtained after statistical analysis were expressed in terms of mean ± SEM. To present the results, the data were evaluated using a one-way ANOVA followed by Newman Keul's multiple comparison tests.

3. Results and discussion

Ayurvedic treatment is very effective, but the proper pharmacovigilance, mechanism of action, pharmacokinetics, and other important aspects of Ayurvedic drugs are not yet fully understood. Moreover, due to the lack of evidence, the basic ideologies in Ayurveda are not scientifically acceptable. With the help of advanced research methodologies, validated research, and advanced technology, the Western medicine system has been almost at the forefront of the medical system. Therefore, there is an urgent need for research methodologies to validate the fundamental concept of Ayurveda treatment because a perfect health restoration including maintenance is possible in Ayurvedic drug treatment (Parasuraman et al., 2014).

Figure 1. *D. peltata* (from https://flowersofindia.net/catalog/)

The generation of free radicals is the basic etiology of most human diseases condition, and there is an increasing need to develop techniques for measuring free radicals and their responses in vivo. These free radicals are so reactive that they have a short lifespan because of that, are not suitable for direct assays, and are determined by indirect methods where various end products of the target molecule like lipids, proteins, and DNA are measured quantitatively (Sara et al., 2015). The end product formed from the reactions of free radicals and biomolecules is more stable than free radicals. Measurements of these end products concerning the oxidation target are an important aspect of the treatment modulation. MDA and PCC are the by-products of lipid peroxidation and oxidized proteins respectively (Ahmad et al., 2008).

Medicinal plants have various phytoconstituents which are an excellent source of lead compounds to develop therapeutic drugs including anticancer agents. Therefore, many studies have been conducted on herbs for various ethnobotany reasons (Newman & Cragg, 2009) The antitumor properties of the extracts from these plant species have already been reported (Raju et al., 2012).

Table 1 shows the status of various blood antioxidants in DAL cancer cell line-bearing mice. In this study, MDA levels were increased significantly ($p < 0.001$) in DAL control mice (positive control) when compared to negative controls. After treatment with EEDP and AEDP at the doses of 250, and 500 mg/kg, levels of MDA were reduced, and the other free radical protective enzymes were slightly increased when compared to the positive control group. Similarly, serum PCC was raised in DAL-controlled group mice (positive control), but 14 days of extract treatment at doses of 250, 500 mg/kg EEDP and AEDP significantly re-established PCC ($p < 0.001$) like the negative control group mice. Inoculation of DAL cells also caused a significant increase in the level of MDA in tissues of the liver, brain, lungs, and kidneys when compared to the negative control animals, and simultaneous significant ($p < 0.001$) reductions in SOD, TP, GSH, P, and CAT levels were also observed in the above tissue samples. 14 days of continuous treatment with EEDP (250 and 500 mg/kg) brought back the alteration of internal antioxidant status to normal levels (Tables 2-5). Most of the parameters were returned to normal levels upon the administration of a high dose of EEDP and AEDP. Almost the same results like restoration of SOD, TP, GSH, P, and CAT and reduction of MDA were observed with 5-fluorouracil-treated mice. EEDP at 250 mg/kg and AEDP at 250 mg/kg treatment showed a non-significant ($p > 0.05$) effect on the restoration of P in the liver and kidney.

This study was conducted to estimate the antioxidant status in blood and various organs and the effectiveness of EEDP and AEDP on the restoration of these parameters in cancer-bearing animals. Significantly increased levels of MDA and SOD in serum, liver, brain, lung, and kidney were found in the tumor-bearing animal, which causes tissue damage and loss of functional completeness of cell membranes (Gupta et al., 2004). Lipid peroxidation is an autocatalytic chain reaction initiated by free radicals which affect the pathological state of cells. MDA, the marker compound of lipid peroxidation, is found at a high concentration in cancerous tissues than in normal organs (Yagi, 1987). GSH, an effective inhibitor of neoplastic cell processes, and a part of the endogenous antioxidant system plays an important role in cell proliferation. It is biosynthesized especially in high concentrations in the liver and is known to have important functions in the protection process against free radicals (Shaik & Mehvar, 2006). Excessive production of free

radicals leads to oxidative stress and damage to macromolecules which affects many functions of important organs, especially in the liver, kidneys, brain, and lungs, even if the tumor site does not directly interfere with these organs (DeWys, 1982). The generated oxygen radicals destruct the cell membranes by lipid peroxidation and can lead to tissue and/or organ damage (Koca et al., 2005) followed by the reduction of other enzyme systems such as SOD, CAT, and GSH that counteract the harmful effects of reactive oxygen species (Ichimura et al., 2004). The reduction in SOD, CAT, GSH, and P in the DAL group is due to the loss of Mn^{2+} in mitochondria (Sun et al., 1989). Tumor growth also reduces the level of SOD and CAT and arrests the functional activity in cancer-bearing mice.

Table 1. Effect of EEDP and AEDP on blood antioxidant status of DAL bearing mice

Blood	CAT U/ml serum	SOD U/ml serum	P U/ml serum	GSH U/l	MDA nmol/ml	PCC nmol/mg protein
Normal	14.4 ± 0.13	7.5 ± 0.67	34.5 ± 1.25	73.98 ± 0.51	8.65 ± 0.17	1.9 ± 0.8
DAL (control)	4.48 ± 0.19	2.63 ± 0.11	21.35 ± 0.54	43.2 ± 0.4	21.65 ± 0.15	8.6 ± 1.6
DAL + 5FU (20 mg/kg)	12.63 ± 0.23	6.68 ± 0.11	33.13 ± 0.72	73.5 ± 0.15	7.68 ± 0.18	2.2 ± 0.7
DAL + EEDP (250 mg/kg)	10.85 ± 0.13[a]	4.6 ± 0.24[a]	29.08 ± 0.61[a]	63.95 ± 0.58[a]	11.63 ± 0.11[a]	4.3 ± 1.1[a]
DAL + EEDP (500 mg/kg)	13.3 ± 0.11[a]	5.68 ± 0.25[a]	33.88 ± 0.31[a]	73.15 ± 0.42[a]	8.68 ± 0.18[a]	2.1 ± 0.54[a]
DAL + AEDP (250 mg/kg)	5.73 ± 0.18[a]	4.55 ± 0.1[a]	23.63 ± 0.51[b]	49.15 ± 0.84[a]	17.83 ± 0.15[a]	6.5 ± 1.5[a]
DAL + AEDP (500 mg/kg)	9.53 ± 0.2[a]	4.58 ± 0.18[a]	28.23 ± 0.54[a]	56.9 ± 1.77[a]	14.75 ± 0.12[a]	5.5 ± 0.6[a]

The data were expressed as mean ± SEM, n = 10. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: $p < 0.001$. All the data were compared with cancer control group.

Table 2. Effect of EEDP and AEDP on the liver antioxidant status of DAL bearing-mice

Liver	TP mg/dl	CAT U/mg tissue	SOD U/mg tissue	P nm/100mg tissue	GSH nm/100mg tissue	MDA nm/g protein
Normal	12.25 ± 0.66	10.45 ± 0.1	4.35 ± 0.06	22.78 ± 0.29	53.05 ± 0.21	4.65 ± 0.06
DAL (control)	5.37 ± 0.15	1.83 ± 0.02	14.93 ± 0.36	16.7 ± 0.22	43.05 ± 0.21	24.85 ± 0.27
DAL + 5FU (20 mg/kg)	11.55 ± 0.29	9.13 ± 0.3	5.2 ± 0.44	21.9 ± 1.21	50.95 ± 3.66	5.7 ± 0.14
DAL + EEDP (250 mg/kg)	11.97 ± 0.27[a]	8.28 ± 0.38[a]	5.6 ± 0.2[a]	21.27 ± 0.43[a]	54.1 ± 0.48[a]	5.5 ± 0.18
DAL + EEDP (500 mg/kg)	12.55 ± 0.28[a]	9.75 ± 0.15[a]	3.2 ± 0.1[a]	23.9 ± 0.56[a]	56.4 ± 0.35[a]	3.6 ± 0.12[a]
DAL + AEDP (250 mg/kg)	9.95 ± 0.14[a]	7.75 ± 0.25[a]	8.07 ± 0.43[a]	19.95 ± 0.33[b]	51.07 ± 0.51[b]	13.2 ± 0.46[a]
DAL + AEDP (500 mg/kg)	11 ± 0.36[a]	9 ± 0.19[a]	6.17 ± 0.16[a]	21.83 ± 0.73[a]	53.15 ± 0.54[a]	7.5 ± 0.15[a]

The data were expressed as mean ± SEM, n = 10. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: $p < 0.001$, b: $p < 0.01$. All the data were compared with cancer control group.

Table 3. Effect of EEDP and AEDP on the kidney antioxidant status of DAL bearing mice

Kidney	TP mg/dl	CAT U/mg tissue	SOD U/mg tissue	P nm/100mg tissue	GSH nm/100mg tissue	MDA nm/g protein
Normal	22.68 ± 0.27	11.48 ± 0.14	23.55 ± 0.4	77.5 ± 3.23	101.85 ± 0.61	12.87 ± 0.67
DAL (control)	16.6 ± 0.22	4.92 ± 0.32	12.83 ± 0.08	55.65 ± 1.88	50.75 ± 0.06	22.03 ± 0.11
DAL + 5FU (20 mg/kg)	21.53 ± 0.05	10.35 ± 0.13	20.75 ± 0.2	72.18 ± 0.94	96.82 ± 0.79	10.73 ± 0.86
DAL + EEDP (250 mg/kg)	22.4 ± 0.16[a]	10.5 ± 0.01[a]	19.53 ± 0.11[a]	75.98 ± 0.94[a]	87.87 ± 0.75[a]	10 ± 0.24[a]
DAL + EEDP (500 mg/kg)	21.75 ± 0.06[a]	10.35 ± 0.06[a]	22.05 ± 0.59[a]	79.62 ± 0.39[a]	94.23 ± 0.69[a]	11 ± 0.4[a]
DAL + AEDP (250 mg/kg)	21.2 ± 0.34[a]	10.9 ± 0.15[a]	19.85 ± 0.06[a]	66.75 ± 0.59[b]	63.63 ± 1.26[a]	14.65 ± 0.19[a]
DAL + AEDP (500 mg/kg)	21.63 ± 0.33[a]	10.93 ± 0.35[a]	16.05 ± 0.42[a]	71.23 ± 0.39[a]	71.95 ± 1.26[a]	15.72 ± 0.35[a]

The data were expressed as mean ± SEM, n = 10. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: $p < 0.001$, b: $p < 0.01$. All the data were compared with cancer control group.

Table 4. Effect of EEDP and AEDP on the brain antioxidant status of DAL bearing mice

Brain	TP mg/dl	CAT U/mg tissue	SOD U/mg tissue	P nm/100mg tissue	GSH nm/100mg tissue	MDA nm/g protein
Normal	41.82 ± 2.83	22.1 ± 0.59	33.3 ± 0.58	34.66 ± 0.66	82.48 ± 0.94	8.48 ± 0.38
DAL (control)	9.82 ± 0.28	8.4 ± 1.67	15.64 ± 0.39	10.36 ± 0.33	38.16 ± 0.18	27.06 ± 0.66
DAL + 5FU (20 mg/kg)	35.84 ± 1.35	21.22 ± 0.37	30.64 ± 0.77	29.04 ± 0.69	79.68 ± 1.25	10.08 ± 0.32
DAL + EEDP (250 mg/kg)	26.22 ± 0.58[a]	16.58 ± 0.77[a]	25.06 ± 0.49[a]	23.42 ± 0.89[a]	69.44 ± 0.34[a]	15.92 ± 0.9[a]
DAL + EEDP (500 mg/kg)	40.62 ± 0.92[a]	21 ± 0.46[a]	31.68 ± 0.62[a]	31.92 ± 1.2[a]	80.92 ± 0.72[a]	9 ± 0.32[a]
DAL + AEDP (250 mg/kg)	24.78 ± 0.41[a]	14.28 ± 0.53[a]	22.74 ± 1.1[a]	20.98 ± 2.15[a]	57.28 ± 1.28[a]	16.92 ± 0.9[a]
DAL + AEDP (500 mg/kg)	33.08 ± 0.69[a]	17.56 ± 1.04[a]	27.68 ± 0.57[a]	29.48 ± 0.71[a]	64.3 ± 1.55[a]	15.06 ± 0.98[a]

The data were expressed as mean ± SEM, n = 10. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: $p < 0.001$. All the data were compared with cancer control group.

Table 5. Effect of EEDP and AEDP on the lung antioxidant status of DAL bearing mice

Lung	TP mg/dl	CAT U/mg tissue	SOD U/mg tissue	P nm/100mg tissue	GSH nm/100mg tissue	MDA nm/g protein
Normal	26.55 ± 0.39	20.55 ± 0.28	13.63 ± 0.28	42.18 ± 0.51	62.73 ± 0.35	7.65 ± 0.17
DAL (control)	16.03 ± 0.73	6.48 ± 0.15	4.68 ± 0.11	30.63 ± 6.33	22.95 ± 1.11	23.05 ± 0.22
DAL + 5FU (20 mg/kg)	23.5 ± 0.48	20.05 ± 0.23	12.7 ± 0.18	38.15 ± 0.31	60.65 ± 0.19	6.75 ± 0.36
DAL + EEDP (250 mg/kg)	22.9 ± 0.44[a]	18.8 ± 0.19[a]	11.93 ± 0.32[a]	40.75 ± 0.27[a]	59.63 ± 0.25[a]	6.28 ± 0.08[a]
DAL + EEDP (500 mg/kg)	28.65 ± 0.92[a]	20.8 ± 0.21[a]	10.5 ± 0.2[a]	43.6 ± 0.11[a]	63.95 ± 0.12[a]	8.35 ± 0.12[a]
DAL + AEDP (250 mg/kg)	24.18 ± 0.49[a]	18.07 ± 0.29[a]	10.52 ± 0.17[a]	38.87 ± 0.54[a]	54.2 ± 0.89[a]	10.53 ± 0.25[a]
DAL + AEDP (500 mg/kg)	26.33 ± 0.4[a]	20.35 ± 0.29[a]	12.57 ± 0.26[a]	48.8 ± 2.18[a]	60.88 ± 0.32[a]	6.63 ± 0.14[a]

The data were expressed as mean ± SEM, n = 10. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where a: $p < 0.001$. All the data were compared with cancer control group.

The PCC of blood has increased significantly in the positive control group mice. The carbonyl content of proteins has been reported to be a sensitive and early marker of oxidative stress on tissues compared to lipid peroxidation (Rajesh et al., 2004). Elevated levels

of PCC have been reported in patients with brain tumors. Elevated values of PCC strongly suggest that oxidative stress (OS) may play a vital role in the carbonylation of protein in the brain which is an oxidation reaction mediated by Fe^{2+} and Cu^{2+}. Free radicals bind to the cation binding site of a protein in presence of H_2O_2 or O_2, which converts the side chain of the amino acid to a carbonyl group. Accumulation of carbonyl groups in a protein causes many chemical modifications to form the oxidation product of the protein (Levine et al., 2000). In this study, a significant increase was determined in PCC levels in DAL-inoculated mice. The PCC levels were recovered after the administration of two doses of EEDP and AEDP. Administration of EEDP and AEDP significantly increased the SOD and CAT levels.

Plant extracts containing antioxidative components such as plumbagin have been reported to be cytotoxic to tumor cells (Ruby et al., 1995) and have antitumor activity in experimental animals (Liu et al., 1998). Induction of apoptosis and cell cycle arrest during the G_2/M phase are the two main mechanisms behind the antioxidative and antitumor activity. The effects of free radicals on the initiation of cancer are well documented (Maity et al., 2000). Mice treated with 250, and 500 mg/kg EEDP and AEDP showed inhibition of lipid peroxidation, reflected as a reduction in MDA and PCC. Furthermore, EEDP and AEDP administration also restored the antioxidant enzyme systems of the blood, liver, brain, kidney, and lungs to almost normal levels.

4. Conclusions

Natural antioxidants may enhance the endogenous antioxidant defenses against ROS disruption and restore optimal balance by neutralizing reactive species. They are becoming more and more important because of their important role in disease prevention. Ethanol extracts of *D. peltata* showed significantly higher activity than the aqueous extracts. The possible mechanism of the antitumor activity of *D. peltata* may be due to the antioxidant activity of its ethanol extract. Maintaining endogenous antioxidant status during the treatment of cancer has the advantage of minimization of serious adverse effects and reducing the treatment course period. A medicinal plant, *D. peltata*, that has anticancer and antioxidant potential, may help expect a better outcome from the disease condition.

Acknowledgments

None.

Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

This study required permission from the “Institutional Animal Ethical Committee (IAEC)”. The study, treatment and handling of the animals was submitted to the IAEC prior to the start of the study and the committee approved the proposal number as A. Raju 0903PH2254/JNTUH 2009.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Raju Asirvatham: Conceptualization, Investigation, Data curation, Writing - original draft
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Supplementary File

None.

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