Antioxidant Potential of *Drosera indica* L in Presence of Dalton Ascites lymphoma (DAL) Tumor in Mice

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ABSTRACT

The aim of the present study was to evaluate the antioxidant role of *Drosera indica* L. in Dalton Ascites lymphoma (DAL) bearing Swiss albino mice. The effect of ethanol and aqueous extracts of *D. indica* (EEDI and AEDI) were administered at a 250 and 500mg/kg once a day for 14 days, 24h after the inoculation of tumor cell line. After the treatment period, blood was collected from the animals and subsequently the animal were sacrificed for isolation of liver, brain, kidney and lungs for the observation of antioxidant status level. The parameters analyzed were catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), peroxidase (P), total protein (TP) and protein carbonyl content (PCC). Treatment with EEDI and AEDI significantly reduced the levels of MDA and increased the levels of GSH, SOD, CAT, P and TP in cancer induced animal and are similar as that of normal mice. The results suggest that the ethanol and aqueous extract of *Drosera indica* L. possess significant antioxidant effects in DAL bearing mice.

Keyword: *Drosera indica* L, Antioxidant, Dalton Ascites lymphoma, Malondialdehyde, Protein carbonyl content

INTRODUCTION

Oxidative stress is characterized by an increased level of reactive oxygen species (ROS) that disrupts the intracellular reduction-oxidation (redox) balance and has been implicated in various diseases including cancer. Various studies reported that an increase in oxidative stress and decrease in antioxidant status in cancer (Fatima *et al.*, 2011). Antioxidants can inhibit or delay the oxidation of oxidizable substrates and this appears to be very important in the prevention of oxidative stress which is suggested as the leading cause of many oxidation related diseases (Sara *et al.*, 2012). Phytochemicals such as phenolics, carotenoids and flavonoids, have been shown to possess functional properties such as antimicrobial, antimutagenic, and free radical scavenging activity. These phytoconstituents acts as a natural antioxidant and prevent the free radical formation (Anil *et al.*, 2013). Under the natural category one of the flavonoid rich plant is *Drosera*.

Drosera is an insectivorous plant and consists of approximately 170 species. In India, *Drosera indica* L., *Drosera burmannii* Vahl and *Drosera peltata* J.E.Sm. have been reported at different locations. These species are used as

vital components in an Ayurvedic preparation called 'Swarnabhasma' (Golden ash) which has been used in several clinical manifestations including loss of memory, defective eyesight, infertility, overall body weakness, incidence of early aging, bronchial asthma, rheumatoid arthritis, diabetes mellitus, nervous disorders (Raju *et al.*, 2013a). Macerated *D. indica* is used to remove corns and this species has been categorized under the vulnerable medicinal plants list (Reddy *et al.*, 2001). The *in vitro* antioxidant potential against various models has been reported (Raju *et al.*, 2013b). This study was aimed to determine the levels of oxidative stress and antioxidant defense in mice bearing with DAL cell line and the restoring capacity of cellular antioxidants by plant extracts (ethanol and aqueous) of *D. indica* L.

MATERIAL AND METHODS

Plant material and extraction

The whole plant of *D. indica* L. was collected from the forests of Savanadurga, The plant material was identified and authenticated by Dr. S.N.Yoganarasimhan, Taxonomist and Research Coordinator at M. S. Ramaiah College of Pharmacy, Bangalore, Karnataka, India. The shade dried and coarsely powdered was

extracted successively with petroleum ether and ethanol in a Soxhlet extractor for 72h. The ethanol extract was concentrated to dryness under reduced pressure and controlled temperature (40–50°C). The resultant marc was extracted by maceration with chloroform water (2.5mL of chloroform in 1000mL of water) for 24h followed by filtration and concentration to dryness. The aqueous extract for animal studies was prepared using distilled water.

The ethanol extract of *D.indica* (EEDI) and aqueous extract of *D.indica* (AEDI) were completely dried and used for the pharmacological screening.

Tumor cell lines

Dalton Ascites lymphoma (DAL) cells were obtained from Amala Cancer Research Center, Thrissur, India. They were maintained by weekly intraperitoneal (i.p) inoculation of 2×10^6 cells/per mouse.

Animals

Male and female adult Swiss albino mice (20-25g) were procured from KM College of Pharmacy, Madurai, Tamil Nadu, India. They were acclimatized to the experimental conditions for about 2 weeks before subjecting them to experimental procedures and were fed with standard pellet diet and water *ad libitum*.

Treatment protocol

Study protocol was approved by the Institution Animal Ethical Committee of K M College of Pharmacy, Madurai, Tamil Nadu (Protocol. No: A. Raju 0903PH2254/JNTUH 2009). Swiss albino mice were divided in to seven group of ten each (Raju *et al.*, 2013c). All the animals in groups 2- 6 were injected with DAL cells (2×10^6 cells per mouse/i.p.), and the remaining one group is a normal control group. Group 1 served as the normal control. Group 2 served as the DAL control. Group 1 and 2 received normal diet and water. Group 3 served as the positive control and was treated with injection 5- Fluorouracil (5FU) at 20mg/kg body weight, intraperitoneally. Group 4 served as the treatment control, which was treated with ethanol extract of *D. indica* L (EEDI) at 250mg/kg body weight orally. Group 5 served as the treatment control, which was treated with

EEDI at a dose of 500mg/kg body weight orally. Group 6 served as treatment control which was treated with aqueous extract of *D. indica* L (AEDI) at 250mg/kg of body weight, through orally. Group 7 served as treatment control which was treated with AEDI at 500mg/kg of body weight orally. In this study, drug treatment was given after the 24h of inoculation, once daily for 14d.

Estimation of blood antioxidant enzyme

After 14d treatment, blood samples were collected by retro-orbital puncturing and allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15min and used for the estimation of serum antioxidant parameters such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) (Senem *et al.*, 2011). In addition to that protein carbonyl content (PCC) also estimated from blood (Levine *et al.*, 1994).

Estimation of various tissue antioxidant enzymes

After collection of blood samples, the mice were killed by excess anesthesia and the brain, lungs, kidney and liver samples were isolated. All the tissue preparations were frozen on dry ice and then transferred to a -80°C freezer. The isolated organs were divided in to 2 parts for the preparation of homogenates.

One part was used for the preparation of 10%w/v homogenate in potassium chloride (0.15M). It was centrifuged at 8000rpm for 10 min and the supernatant thus obtained was used for estimation of total proteins (TP) (Lowry *et al.*, 1951; Sapan *et al.*, 1999), peroxidase (P) (Alexander *et al.*, 1962), catalase (CAT) (Catherine *et al.*, 1987; Aebi *et al.*, 1974) and malondialdehyde (MDA) (Ohkawa *et al.*, 1979). The second part was used for preparation of 10% w/v homogenate in 0.25%w/v sucrose in phosphate buffer (5M, pH 7.4) and was centrifuged at 8000rpm for 10min. The supernatant thus obtained was used for estimation of superoxide dismutase (SOD) (Kakkar *et al.*, 1954) and glutathione (GSH) (Beutler *et al.*, 1963). All the estimations were done according to the manufacturer manual of markedly available reagent kit. (Agappe, Kerala, India)

Statistical analysis

The results are expressed as mean $\pm$ S.E.M. The evaluation of the data was done using one way ANOVA followed by Newman-Keul's multiple comparison tests.

RESULTS AND DISCUSSION

The levels of various blood antioxidants (Figure 1). In the present study, the levels of MDA were significantly ($p<0.001$) increased in DAL control animals when compared with normal control animals. After treatment with the doses of 250, 500mg/kg of EEDI and AEDI was significantly increased the free radical defense enzyme level more or less like normal groups when compared with DAL control mice. The PCC content in hemolysate was increased in DAL control mice. Treatment with doses of 250, 500mg/kg of EEDI and AEDI restored the PCC significantly ($p<0.01$) as that of normal group, whereas 250mg/kg of AEDI was less significant ($p<0.01$) to PCC.

The data were expressed as mean $\pm$ S.E.M. $n=10$. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test. Serum enzyme levels were expressed in different units, PCC-nmol/mg protein, CAT-U/ml serum, SOD-U/ml serum, P-U/ml serum, GSH-U/L, MDA-nmol/ml units.

The levels of various blood antioxidants (Figure 1). In the present study, the levels of MDA were significantly ($p<0.01$) increased in DAL control animals when compared with normal control animals. After treatment with the doses of 250, 500mg/kg of EEDI and AEDI was significantly increased the free radical defense enzyme level more or less similar to normal groups when compared with DAL control mice. The PCC content in hemolysate was increased in DAL control mice. Treatment with doses of 250, 500mg/kg of EEDI and AEDI restored the PCC significantly ($p<0.01$) as that of normal group, whereas 250mg/kg of AEDI was less significant ($p<0.05$) to reduction of PCC.

The data were expressed as mean $\pm$ S.E.M. $n=10$. The data analyzed by one-way

analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test. a- ($p<0.001$), b- ($p<0.01$) compared to the DAL control group.

Effect of EEDI and AEDI on liver antioxidant enzymes level in DAL bearing mice (Table I). In the present study, MDA and SOD level in liver were significantly increased in DAL control animals when compared with normal control animals whereas TP, CAT, P and GSH levels in liver tissue were significantly ($p<0.001$) reduced in cancer control group. After continuous 14d treatment with the doses of 250, 500mg/kg of EEDI and AEDI was significantly increased the free radical defense enzyme thereby increased level of TP, CAT, P and GSH to more or less similar to normal group and significant reduction found on MDA and SOD where the results were compared with DAL control mice. Low dose of AEDI showed less significant ($p<0.01$) on the restoration of GSH level in DAL bearing mice.

The data were expressed as mean $\pm$ S.E.M. $n=10$. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test. a- ($p<0.001$) compared to the DAL control group.

Effect of EEDI and AEDI on lungs antioxidant enzymes level in DAL bearing mice (Table II). In the present study, the levels of MDA in lungs were significantly increased ($p<0.001$) in DAL control animals when compared with normal control mice whereas TP, CAT, P, SOD and GSH levels in lung tissues were significantly ($p<0.001$) reduced in cancer bearing mice. After continuous 14d treatment of oral administration of the doses of 250,500mg/kg of EEDI and AEDI, significantly increased the free radical defense enzyme thereby increased level of TP, CAT, P, SOD and GSH to more or less similar to normal group and also significant reduction of MDA was found with DAL bearing mice, the results were compared with DAL control mice.

The data were expressed as mean $\pm$ S.E.M. $n=10$. The data analyzed by one-way analysis of variance (ANOVA) followed

Figure 1. Effect of EEDI and AEDI on blood antioxidant status of DAL bearing mice

by Newman-Keul's multiple comparison test. a- ($p < 0.001$) compared to the DAL control group.

Effect of EEDI and AEDI on kidney antioxidant enzymes level in DAL bearing mice (Table III). In the present study, the levels of MDA in kidneys were significantly increased ($p < 0.001$) in DAL control animals when compared with normal control mice whereas TP, CAT, P, SOD and GSH levels in kidney tissues were significantly ($p < 0.001$)

reduced in cancer bearing mice. After continuous 14d treatment of oral administration of the doses of 250, 500mg/kg of EEDI and AEDI, significantly increased the free radical defense enzyme thereby increased level of TP, CAT, P, SOD and GSH to more or less similar to normal group and also significant reduction of MDA was found with DAL bearing mice, the results were compared with DAL control mice.

Table I. Effect of EEDI and AEDI on liver antioxidant capacity in DAL bearing mice

Parameters 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	11.93±0.31	7.78±0.38	2.35±0.06	20.45±0.32	51.1±0.22	2.7±0.09
DAL Control	4.35±0.17	1.25±0.06	13.18±0.55	14.73±0.19	41±0.21	23.3±0.39
DAL+5FU (20mg/kg)	10.4±0.2	6.95±0.29	3.28±0.38	19.98±1.18	48.98±3.75	3.8±0.38
DAL + EEDI250	9.55±0.19 ^a	6.63±0.14 ^a	3.73±0.25 ^a	19.35±0.4 ^a	52.23±0.39 ^a	3.5±0.18 ^a
DAL + EEDI500	10.63±0.29 ^a	7.08±0.09 ^a	2.6±0.13 ^a	22.03±0.62 ^a	54.33±0.36 ^a	2.9±0.29 ^a
DAL + AEDI250	7.95±0.14 ^a	5.63±0.23 ^a	6.38±0.51 ^a	18.03±0.31 ^a	49.35±0.39 ^b	11.2±0.6 ^a
DAL + AEDI500	9±0.26 ^a	6.83±0.08 ^a	4.18±0.17 ^a	20.03±0.63 ^a	51.18±0.47 ^a	6.2±0.49 ^a

Table II. Effect of EEDI and AEDI on lungs antioxidant capacity in DAL bearing mice

Parameters Lungs	TP mg/ dL	CAT U/ mg tissue	SOD U/ mg tissue	P nm/100mg tissue	GSH nm/ 100mg tissue	MDA nm/ g protein
Normal	24.78±0.25	18.55±0.28	11.52±0.26	40.7±0.33	61.03±0.48	6.05±0.34
DAL Control	13.83±0.65	4.7±0.11	2.68±0.11	28.63±0.33	21.33±0.48	20.9±0.46
DAL+5FU (20mg/kg)	22.03±0.29	18.05±0.23	10.7±0.19	36.28±0.42	58.7±0.09	5.1±0.33
DAL+ EEDI250	21±0.52 ^a	16.8±0.19 ^a	9.92±0.32 ^a	38.7±0.23 ^a	57.67±0.25 ^a	4.07±0.05 ^a
DAL+ EEDI500	26.6±0.81 ^a	18.8±0.2 ^a	7.3±1.34 ^a	41.48±0.06 ^a	61.62±0.34 ^a	6.15±0.06 ^a
DAL+ AEDI250	22.17±0.57 ^a	16.08±0.29 ^a	8.53±1.65 ^a	37.2±0.6 ^a	52±0.67 ^a	9.1±0.34 ^a
DAL+ AEDI500	24.23±0.42 ^a	18.35±0.29 ^a	10.57±0.26 ^a	39.97±1.99 ^a	59.8±0.25 ^a	4.55±0.1 ^a

The data were expressed as mean± S.E.M. n=10. The data analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test. a- (p<0.001) compared to the DAL control group.

Effect of EEDI and AEDI on brain antioxidant enzymes level in DAL bearing mice (Table IV). In the present study, the levels of MDA in brain were significantly increased (p<0.001) in DAL control animals when

compared with normal control mice whereas TP, CAT, P, SOD and GSH levels in brain tissues were significantly (p<0.001) reduced in cancer bearing mice. After continuous 14d treatment of oral administration of the doses of 250, 500mg/kg of EEDI and AEDI, significantly increased the free radical defense enzyme thereby increased level of TP, CAT, P, SOD and GSH to more or less similar to normal group and also significant

Table III. Effect of EEDI and AEDI on kidney antioxidant capacity in DAL bearing mice

Parameters 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	23.45±0.43	12.65±0.25	5.53±0.19	30.88±0.35	70.95±0.33	6.55±0.17
DAL Control	3.28±0.11	2.58±0.27	2.58±0.38	18.55±0.33	40.77±0.37	19.6±0.30
DAL +5FU (20mg/kg)	9.55±0.29	11.05±0.21	4.7±0.19	31.08±0.23	71.68±0.11	5.75±0.19
DAL + EEDI250	9.93±0.25 ^a	8.6±0.22 ^a	4.38±0.27 ^a	26.28±0.4 ^a	62.28±0.81 ^a	9.5±0.11 ^a
DAL + EEDI500	10.5±0.27 ^a	12.6±0.21 ^a	5.97±0.18 ^a	32.5±0.49 ^a	73.45±0.37 ^a	6.13±0.44 ^a
DAL + AEDI250	7.95±0.14 ^a	8.48±0.15 ^a	3.9±0.32 ^a	27.7±0.63 ^a	63.8±0.62 ^a	11.15±.62 ^a
DAL + AEDI500	8.88±0.14 ^a	11.02±0.23 ^a	5.75±0.12 ^a	29.7±0.77 ^a	68.98±0.68 ^a	8.75±0.41 ^a

Table IV. Effect of EEDI and AEDI on brain antioxidant capacity in DAL bearing mice

Parameters 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	23.45±0.43	12.65±0.25	5.53±0.19	30.88±0.35	70.95±0.33	6.55±0.17
DAL Control	3.28±0.11	2.58±0.27	2.58±0.38	18.55±0.33	40.77±0.37	19.6±0.30
DAL +5FU (20mg/kg)	9.55±0.29	11.05±0.21	4.7±0.19	31.08±0.23	71.68±0.11	5.75±0.19
DAL + EEDI250	9.93±0.25 ^a	8.6±0.22 ^a	4.38±0.27 ^a	26.28±0.4 ^a	62.28±0.81 ^a	9.5±0.11 ^a
DAL + EEDI500	10.5±0.27 ^a	12.6±0.21 ^a	5.97±0.18 ^a	32.5±0.49 ^a	73.45±0.37 ^a	6.13±0.44 ^a
DAL + AEDI250	7.95±0.14 ^a	8.48±0.15 ^a	3.9±0.32 ^a	27.7±0.63 ^a	63.8±0.62 ^a	11.15±.62 ^a
DAL + AEDI500	8.88±0.14 ^a	11.02±0.23 ^a	5.75±0.12 ^a	29.7±0.77 ^a	68.98±0.68 ^a	8.75±0.41 ^a

reduction of MDA was found with DAL bearing mice, the results were compared with DAL control mice.

Reactive oxygen species (ROS) were formed via enzymatic reactions or non-enzymatic reactions. ROS were neutralized / deactivated by the antioxidant defence system, which composed of SOD, GSH, and CAT (Battisti *et al.*, 2008). Certain molecular products formed from the reaction of free radicals with biomolecules are generally considered and are more stable than free radicals themselves (Raju Asirvatham and Joshila Akhil *et al.*, 2017). These products are tracked by measuring stable

metabolite concentrations of their oxidation target products, including malondialdehyde (MDA), a by-product of lipid peroxidation, and protein carbonyl (PC) as product of oxidized proteins (Rizwan *et al.*, 2008).

Increased levels of protein carbonyl groups have been observed in various diseases and are oxidative stress markers as oxidised proteins. Generally, are more stable. The increase in protein carbonyls not only reflects oxidative stress but also protein dysfunction caused by the disease (Dalle *et al.*, 2003). Several studies have observed increased levels of plasma protein carbonyl levels in patients with

Hodgkin's lymphoma (Morabito *et al.*, 2004). Carbonyl modifications are not repaired and the extent of carbonylation depends upon factors that influence oxidant status. Thus, the plasma protein carbonyl biomarker captures the net of pro-oxidant exposures and antioxidant status. The most commonly oxidized amino acids are Arg, Lys, Pro, Thr, and the plasma protein, fibrinogen, is highly susceptible to free radical attack (Dalle *et al.*, 2003). In our study also found that plasma protein carbonyl levels was significantly increased in cancer control mice and normalized by extract treatment.

In cancer conditions we found significantly lower products of oxidative damage to DNA (DNA breaks with oxidized purines or pyrimidines) in peritoneal fluid analysis (Raju *et al.*, 2013). End products of lipid peroxidation, are malondialdehyde (MDA) and 4-hydroxynonenal. MDA react with DNA to form several pro-mutagenic lesions (Niedernhofer *et al.*, 2003). The probable reason for the elevated level of serum lipid peroxide in cancer may be due to defective antioxidant system which leads to the accumulation of lipid peroxides in cancer tissue which are released into the blood stream (Kumaraguruparan *et al.*, 2002).

In this study, mice with cancer exhibited higher levels of MDA, both in tissues and serum compared to the normal group. The difference in significance of MDA levels between blood and tissue may be attributed to the fact that tissue is a more precise site of free radical generation and hence more accurate measurement can be obtained when compared to plasma values. From these observations, it can be concluded that MDA levels play an important role in assessing the outcome of cancer (Sinha *et al.*, 2009). Similar observations have previously been stated in different studies related to free radical levels in EAC and DAL bearing mice (Raju *et al.*, 2013c; Raju *et al.*, 2013d).

Cellular antioxidant enzymes such as SOD, GSH, P and CAT protect the cell against cellular and molecular damage caused by the ROS. SOD and CAT are considered primary antioxidant enzymes, since they are involved in direct elimination of reactive oxygen metabolites. They also act as anti-carcinogens and inhibitors at initiation and promotion/transformation stage in

carcinogenesis. These enzymes (SOD and CAT) acts by DNA strand scission caused by xanthine/xanthine oxidase and chromosomal aberration caused by hypoxanthine/xanthine oxidase respectively (Sinha *et al.*, 2009). It also prevents the onset of spontaneous neoplastic transformation in mouse fibroblast and epidermal keratinocytes (Jones *et al.*, 1985). In our study, SOD and CAT levels were found to be lower in DAL control mice. Lower CAT activity in our study may be the effect of higher production of ROS which support the oxidative stress hypothesis in carcinogenesis (Ray *et al.*, 2000). After 14d extract treatment at the doses of 250, 500mg/kg of EEDI and AEDI brought back this changes into normal.

Several studies supported that elevated levels of GSH in cancer treatment can able to protect the other cell from violent ROS (Balendiran *et al.*, 2004). Its synthesis is upregulated during oxidative stress and inflammation (Angel *et al.*, 2011). In our research also showed that extract treatment group showed an elevation of GSH in blood, liver, kidney, lungs and brain. Hypoproteinemia is a condition at which there is a decrease in albumin content. Albumin, which is considered as a sacrificial antioxidant, via its thiol groups, fight against free radicals. Moreover, hypoproteinemia in cancer may be an expression of cachexia, representing homeostatic derangement in which the utilization and destruction of albumin by the tumor cannot be compensated by the organism, especially by the liver (Anita *et al.*, 2010). Total protein (TP) level was significantly reduced in DAL control mice and was brought back to normal by the administration 250, 500mg/kg doses of EEDI and AEDI.

CONCLUSION

In recent years much research has been dedicated in identifying the plant components which contribute in combating the oxidative stress and free radical induced damage and protection of biological system from the OS induced disease such as cancer. Plants with phytoconstituents such as flavonoids, terpenoids and phenolics showed their diverse pharmacological properties including antioxidant and antitumor activity. Improved antioxidant status helps to minimize oxidative

damage, and thus can delay or prevent pathological changes. This suggests the possible utility of antioxidant-based dietary strategies for lowering the risk of chronic age-related, free radical induced diseases. Our study already reported that both the extracts of *D. indica* protect hematological system, lipid profile, liver enzyme and hormone level in cancer bearing mice. The possible mechanism of anticancer effect may be due to its antioxidant effect. This antioxidant property is again confirmed from blood and various tissue level antioxidant enzyme analysis. It may be possible that the natural antioxidants strengthen the endogenous antioxidant defense from ROS destroy and restore the optimal balance by neutralizing the reactive species.

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