



RESEARCH ARTICLE

PHARMACOLOGY

**ANTIOXIDANT POTENTIAL OF ETHYLACETATE FRACTION OF *CYNODON DACTYLON* AGAINST ELA IMPLANTED SWISS ALBINO MICE****M.SAROJA<sup>1</sup>, R.SANTHI\*<sup>2</sup> AND S.ANNAPOORANI<sup>1</sup>**<sup>1</sup>Department of Biochemistry, Avinashilingam University, Coimbatore, India<sup>2</sup>School of Biotechnology, Dr. G.R. Damodaran College of Science, Coimbatore**R.SANTHI**

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**ABSTRACT**

The study was carried out to determine the level of enzymic and non enzymic antioxidants in Ehrlich's Lymphoma Ascite (ELA) transplanted mice treated with ethylacetate fractions of *Cynodon dactylon*. The levels of enzymic antioxidants like super oxide dismutase, glutathione peroxidase and catalase and non enzymic antioxidants like reduced glutathione, vitamin A and vitamin E were decreased in ELA induced mice due to the liberation of free radicals from the liver. Administration of ethylacetate extracts showed increased levels of enzymic and non enzymic antioxidants in ELA transplanted mice. The result suggests that ethylacetate fractions possessed lipid peroxidation inhibiting activity. Thus it could be concluded that ethylacetate fractions possess antioxidant activity.

## KEY WORDS

*Cynodon dactylon*, Ehrlich's Lymphoma Ascite, Ethylacetate fractions

## INTRODUCTION

Medicinal plants, since time immemorial have been in use for treatment of various diseases all over the world. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems<sup>1-3</sup>. Natural antioxidants like phenolics and flavanoids are safe and bioactive. Phenolic compounds with antioxidant activity are widely distributed in many fruits and vegetables. Phenolic compounds show antioxidant, antimutagen, antitumour, anti-inflammatory, and anticarcinogenic properties<sup>4</sup>. In general deep coloured vegetables and fruits are good sources of phenolic compounds.

The *Cynodon dactylon* has been shown to possess variety of medicinal properties. The *Cynodon dactylon* (Family; Poaceae), commonly known as 'doob'(Hindi), 'aroogum pillo'(Tamil), is called creeper in India. Recent studies showed that aqueous and ethanolic extracts of *Cynodon dactylon* contain hypoglycemic as well as antidiabetic potential<sup>5</sup>. Recently, it has been reported by our research group that *Cynodon dactylon* leaf protein has antioxidant activity against ELA implanted Swiss albino mice<sup>6</sup>. In the present study to determine the antioxidant potential of ethylacetate fraction of *Cynodon dactylon* against ELA implanted Swiss albino mice.

## MATERIALS AND METHODS

### **Plant material:**

Fresh leaves of *Cynodon dactylon* was collected in area free of pesticides and other contaminants from the area surrounding of Coimbatore, Tamilnadu. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the flavonoid fraction preparation.

### **Preparation of flavonoid fraction:**

In the preliminary screening, the direct ethyl acetate extract of *Cynodon dactylon* with powdered magnesium + conc.HCl developed an orange to magenta color indicated the presence of flavonoid showed a characteristic color reaction in Shinoda test. The color is due to the reductive conversion of the flavone into the corresponding anthocyanin pigment<sup>7</sup>.

Knowing the presence of flavonoid in ethyl acetate extract, the extraction was undertaken with 20g of powdered plant material and 200ml. of light petroleum ether (b.p. 40°-60°C) in a Soxhlet apparatus for 18 hours to remove the chlorophyll, non flavonoid components and lipid de waxing<sup>8</sup>. The treated material was dried and extracted with ethyl acetate using Soxhlet apparatus<sup>9</sup>. This fraction is referred as Cdff.

### **Animals:**

Seven to eight weeks old Swiss albino male mice weighing about 25-30g were brought from small animals breeding station, Thrissur, Kerala. The animals were acclimatized for 15 days under standard laboratory conditions and fed with standard diet with water ad libitum. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee prior to the beginning of the project work (889/ac/05/CPCSEA).

### **Propagation of ELA cell lines:**

Ehrlich's Lymphoma Ascites (ELA) tumor cell lines were procured from Amala Cancer Research Centre, Thrissur, Kerala. The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation of  $1 \times 10^6$  cells in 100 $\mu$ l of PBS. After 15 days, the cells were drawn from the intraperitoneal cavity and used for the *in vitro* cytotoxic studies by trypan blue exclusion method<sup>10</sup>. *In vitro* cytotoxic studies were carried out to find out the 50% effective dose (ED<sub>50</sub>) of *C. dactylon* ethylacetate extract

which was 80µg/100µl determined by trypan blue exclusion method. The fraction which showed minimum ED<sub>50</sub> was selected for the *in vivo* studies.

### **Grouping of animals:**

The animals were divided into 5 groups and each group consisted of 6 mice. The Group I received 0.1 ml of Dimethyl sulphoxide (DMSO) every day intraperitoneally and served as a vehicle control for the experimental groups 2 to 4. Group 2: Positive control group fed with (0.18 mg/kg body weight) standard antioxidant silymarin. Group 3: received 1x10<sup>6</sup> ELA tumor cells, intraperitoneally and treated as ELA control. Group 4: received ED<sub>50</sub> of ethylacetate extract of *C. dactylon* (80 µg in 100 µl of DMSO) intraperitoneally. Group 5: *C. ethylacetate* extract and ELA tumour cells were administered on the same day and Ethylacetate Extract administration was continued for 15 days.

After 15 days the mice were sacrificed after an overnight fasting. The liver was dissected, washed with PBS at pH 7.2 and homogenate was prepared using PBS and used for the determination of catalase<sup>11</sup>, superoxide dismutase<sup>12</sup>, glutathione peroxidase<sup>13</sup> and the non enzymic antioxidants such as vitamin C<sup>14</sup>, vitamin E<sup>15</sup>, reduced glutathione<sup>16</sup> and lipid peroxidation<sup>17</sup>. The results were presented as the mean ± standard deviation of 6 animals.

## **RESULT AND DISCUSSION**

Table I shows the levels enzymic antioxidants Catalase, Superoxide dismutase and Glutathione peroxidase in the liver homogenate of control and experimental groups. The levels of CAT, SOD, GPx were decreased in ELA induced mice when compared to control groups. Free radicals play an important role in development of tissue damage and pathogenesis of many diseases and these free radicals are eliminated by an enzymic and non enzymic antioxidants<sup>18-20</sup>. The enzyme CAT, SOD, GPx plays a team to defense against ROS.

The oral feeding *Cynodon dactylon* extract on diabetes rats showed increased activity of enzymic antioxidants CAT, SOD, GPx<sup>21</sup>. Increased CAT, SOD, GPx were reported (6) in *Cynodon dactylon* leaf protein against ELA implanted mice.

The CAT, SOD and Gpx activity was found to be significantly increased in ELA induced mice on administration with ethylacetate fraction of *Cynodon dactylon* compared to ELA control mice. The CAT, SOD and Gpx activity also increased when administered with Silymarin.

Table II shows the levels of non enzymic antioxidants such as vitamin A, vitamin E, reduced glutathione and lipid peroxidation in the liver homogenate of control and experimental groups. The levels non enzymic antioxidants Vitamin A, Vitamin E and Reduced Glutathione were decreased in ELA induced mice when compared to control groups. The level of lipid peroxidation was increased in ELA induced mice when compared to control groups.

Non enzymic antioxidants Vitamin A, Vitamin E and Reduced Glutathione were decreased and lipid peroxidation was increased in ELA induced mice. Glutathione peroxidase, which utilises GSH as substrate, is a major mechanism for the removal of H<sub>2</sub>O<sub>2</sub> and organic hydroperoxides<sup>22</sup>. Cell depleted of glutathione are more prone to membrane damage due to oxidative stress. Vitamin E and vitamin C, potent quenchers of free radicals, prevent the oxidation of glutathione. Both ascorbate and GSH reduce vitamin E, prolonging the antiperoxidative activity of the latter<sup>23</sup>. Non Enzymic antioxidants Vitamin A, Vitamin E and Reduced Glutathione were increased and lipid peroxidation was decreased in ELA induced mice administered with phenol content of *Cynodon dactylon* when compared to control mice. In ELA alone induced mice antioxidant activity decreased. The non enzymic antioxidants activity also increased when administered with Silymarin. The results from our study showed that ethylacetate fraction of *Cynodon dactylon* extract enhanced enzymic and non enzymic antioxidants in ELA induced Swiss albino mice

**Table 1**  
**Activities of enzymic antioxidants in the liver of control and experimental Swiss albino mice**

Control and Experimental Groups	CAT (U/mg Phenol) (a)	SOD (U/mg Phenol) (b)	GPx (U/mg Phenol) (c)
PBS	6.41 ± 0.102	2.12 ± 0.077	0.27 ± 0.021
Paraffin oil	6.16 ± 0.148	2.02 ± 0.114	0.26 ± 0.016
DMSO	6.31 ± 0.170	2.16 ± 0.149	0.22 ± 0.015
SILYMARIN	6.65 ± 0.184	2.30 ± 0.180	0.34 ± 0.041
Cynodon	8.54 ± 0.330	2.69 ± 0.150	0.49 ± 0.098
Cynodon + ELA	6.06 ± 0.230	1.96 ± 0.080	0.41 ± 0.032
ELA	3.47 ± 0.240	0.85 ± 0.060	0.05 ± 0.010

The values are the means and standard deviation of six animals.

Where,

a – n moles H<sub>2</sub>O<sub>2</sub> decompse / seconds / mg phenol.

b – amount of enzyme that gives 50% inhibition of extent of NBT reduction

c – micrograms of GSH utilized per minute per milligram phenol.

**Table 2**  
**Activities of non enzymic antioxidants in the liver of control and experimental Swiss albino mice**

Control and Experimental Groups	Vitamin A (a)	Vitamin E (b)	GSH (Reduced Glutathione) (c)	LPO (d)
PBS	0.67±0.30	2.18±0.12	10.11±0.15	0.21±0.10
Paraffin oil	0.73±0.04	2.23±0.09	10.17±0.21	0.20±0.00
DMSO	0.70 ± 0.08	2.24 ± 0.15	10.25±0.45	0.20±0.01
SILYMARIN	0.80 ± 0.12	3.31±0.31	11.54±0.26	0.17±0.01
Cynodon ethylacetate extract	0.95 ± 0.08	3.56±0.21	11.97±0.20	0.19±0.02
Cynodon ethylacetate extract+ ELA	0.73 ± 0.09	3.43±0.12	10.16±0.30	0.20±0.01
ELA	0.63 ± 0.07	2.04±0.27	8.76±0.20	0.25±0.01

The values are the means and standard deviation of six animals.

a -  $\mu\text{g} / \text{g tissue}$

b -  $\mu\text{g} / \text{g tissue}$

c- n moles /g tissue

d - n moles /g tissue

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