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# Antioxidant efficacy of *Cynodon dactylon* leaf protein against ELA implanted swiss albino mice

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## ABSTRACT

The levels of enzymic and non enzymic antioxidants were determined in Ehrlich's Lymphoma Ascite (ELA) transplanted Swiss albino mice treated with the protein fraction of *Cynodon dactylon* (Cdpf). ELA induced mice showed significant decrease in the level of enzymic antioxidants like catalase (73.43 to 55.03), superoxide dismutase (4.49 to2.47) and glutathione peroxidase (48.48 to 36.98) in units/mg protein and non enzymic antioxidant like vitamin A (7.18 to 3.25), vitamin E (8.55 to 3.18) in µg/g protein and reduced glutathione (2.38 to 1.65) in nmoles/g protein which indicated the production of free radicals in the liver. Administration of protein fraction to the ELA induced mice showed enhanced level of enzymic antioxidants (69.18, 4.11 and 49.39 units/mg protein) and non enzymic antioxidants (5.63, 5.20 µg/g protein and 3.43 nmoles/g protein) respectively which proved their protective action against the free radical damage caused by ELA tumor cells and maintains the normal function of the organ and confirmed their antioxidant activity.

***Keywords:*** *Cynodon dactylon*, Antioxidant properties, ELA, Swiss albino mice

## INTRODUCTION

Medicinal plants play a key role in the human health care. About eighty percent of the world populations rely on the use of traditional medicine, which is predominantly based on plant materials [1]. A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic properties [2]. Many plants have been reported to exhibit properties that combat oxidative stress through their active constituents [3]. The majority of the active antioxidant compounds in plants are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins[4]. The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years. Thus, recent studies have investigated the potential of plant products to serve as antioxidants to protect against various diseases induced by free radicals [5].

Proteins also have excellent potential as natural antioxidant additives in foods because they can inhibit lipid oxidation through multiple pathways including inactivation of reactive oxygen species, scavenging free radicals, chelation of pro oxidative transition metals, reduction of hydroperoxides, and alteration of the physical properties of food systems [6]. Plants and herbal formulations are frequently considered to be less toxic and more free from side effects than synthetic ones [7]. *Cynodon dactylon* (Family: Poaceae) is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. Increasing evidence indicates that *Cynodon dactylon* (arugampul) extract has significant application in dropsy and secondary syphilis [8]. The objective of the present study is to evaluate the Antioxidant Efficacy of *Cynodon dactylon* Leaf Protein against ELA Implanted Swiss Albino Mice

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## MATERIALS AND METHODS

### Plant material

Fresh leaves of *Cynodon dactylon* were collected in an area free of pesticides and other contaminants from the surrounding of Tiruchengode. The collected leaves were washed thoroughly and blotted dry with filter paper and used for the protein preparation.

### Preparation of proteins

Using Phosphate Buffered Saline (PBS), 20% extract of *Cynodon dactylon* fresh leaves were prepared and centrifuged at 5000 rpm for 10 minutes. The supernatant was subjected to the ammonium sulphate fractionation using 10-100% saturation of ammonium sulphate and the precipitates were dissolved in a known amount of PBS. Dialysis was done to desalt the *Cynodon dactylon* protein fractions (Cdpf).

### Animals

Swiss albino mice weighing 25-30g of either sex were used in this study. They were procured from Perundurai Medical College,

Perundurai. The animals were acclimatized for 15 days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C. They were fed with standard mice feed and water *ad libitum* was provided. The litter in the cages was renewed thrice a week to ensure hygeinicity and maximum comfort to animals. 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).

### Tumor cell lines maintenance

Ehrlich's Lymphoma Ascites (ELA) tumor cells were procured from Amala Cancer Research Centre, Thrissur. The mice were acclimatized for two weeks and cells were propagated by intraperitoneal transplantation of 1 x $10^6$ cells in 100 µl of PBS. After 10-15 days, the cells were drawn from the intraperitoneal cavity and used for the *in vitro* cytotoxic studies by trypan blue exclusion method [9].*In vivo* studies were carried out using 60% ammonium sulphate fractionation of *Cynodon dactylon* protein.

### Grouping of animals

The animals were divided into 6 groups and each group consisted of 6 mice.**Group 1** received (i.p) 0.1 ml of PBS every day and served as a vehicle control for the experimental groups. **Group 2** received (i.p) 0.1 ml of paraffin oil, which constituted the vehicle control for the standard antioxidant silymarin group. **Group 3** received (i.p) 25mg standard antioxidant silymarin in 100 µl of paraffin oil / kg body weight.**Group 4** received (i.p) $ED_{50}$ of Cdpf in 100µl of PBS).**Group 5** received Cdpf and 1x$10^6$ ELA tumor cells on the same day and Cdpf administration was continued for 15 days. **Group 6** received 1x$10^6$ ELA tumor cells (i.p) that served as control.

After 15 days, the mice were sacrificed and their liver was used for the experiment. From the liver extract enzymatic antioxidants such as Catalase [10], superoxide dismutase [11] and glutathione peroxidase [12] and the non enzymic antioxidants such as Vitamin A [13], vitamin E [14] and reduced glutathione [15] were determined.

**Table 1. Level of enzymic antioxidants on ELA induced mice treated with and without *Cynodon dactylon* protein fraction (Cdpf).**

| Sl. No | Group | Catalase | Superoxide dismutase | Glutathione Peroxidase |
|---|---|---|---|---|
| | | (U/mg protein) | (U/mg protein) | (U/mg protein) |
| 1 | PBS control | 75.35±0.55 | 4.49±0.04 | 38.28±0.23 |
| 2 | Paraffin oil | 70.35±0.70 | 4.51±0.03 | 38.92±0.26 |
| 3 | Silymarin | 75.10±0.08 | 4.98±0.03 | 50.37±0.42 |
| 4 | Cdpf | 75.63±0.28 | 5.38±0.04 | 56.04±0.23 |
| 5 | Cdpf+ELA | 69.18±0.07 | 4.11±0.34 | 39.[illegible] |
| 6 | ELA | 55.03±0.70 | 2.47±0.03 | 36.[illegible] |

**Table 2. Level of non enzymic antioxidants on ELA induced mice treated with and without *Cynodon dactylon* protein fraction (Cdpf).**

| Sl. No | Group | Vitamin A (µg/g protein) | Vitamin E (µg/g protein) | Reduced Glutathione (µg/g protein) |
|---|---|---|---|---|
| 1 | PBS control | 7.48±0.19 | 8.55±0.24 | 2.[illegible] |
| 2 | Paraffin oil | 7.45±0.13 | 8.78±0.17 | [illegible] |
| 3 | Silymarin | 8.05±0.32 | 9.53±0.21 | 2.[illegible] |
| 4 | Cdpf | 8.61±0.28 | 10.91±0.24 | 5.[illegible] |
| 5 | Cdpf+ELA | 5.63±0.13 | 5.70±0.08 | [illegible] |
| 6 | ELA | 3.25±0.22 | 3.18±0.16 | [illegible] |

### Statistical Analysis

Results were expressed as mean±SD. Statistical analysis was performed with one way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

The enzymic antioxidants catalase, superoxide dismutase and peroxidase play an important role in protecting the tissues from the action of lipid peroxidation. Hence, the effect of the protein fraction on the level of enzymic antioxidants in Swiss albino mice implanted with and without ELA tumor cells were studied.

The levels of catalase, superoxide dismutase and peroxidase in the liver of mice treated with Cdpf alone and along with ELA tumor cells were shown in Table 1. Catalase, superoxide dismutase and peroxidase activity was found to be significantly reduced in mice induced with ELA cells when compared to that of the control mice and was increased in the mice treated with the Cdpf in the presence and absence of ELA induced mice might be due to the enhanced production of hydroperoxides. The levels of enzymic antioxidants were increased by the administration of Cdpf when compared to the standard antioxidant Silymarin.

Decreased catalase activity was reported by [16] in ethanolic extract of *Momordica dioica* leaves treated mice. The increased peroxidase level in the liver might be responsible for the increased inhibition of *in vivo* lipid peroxidation. SOD played an important role as protective enzyme against lipid peroxidation in tissues[17]. Decreased activity of peroxidase in liver tissue when compared to CCl4 treated rats by the administration of ethanol extract of *Pisonia aculeate* was reported by [18].The increased level of enzymic antioxidants on treatment with the Cdpf showed their protective effect by preventing the formation of unwanted free radicals and maintains the organ in normal architecture.

The present study has shown the antioxidant activity of the protein fraction by enhancing the catalase, peroxidase and SOD activities when administered to the ELA induced mice.

Table 2 shows the level of the liver non enzymic antioxidants in the ELA induced mice. The levels of reduced glutathione, vitamin A and vitamin E were found to be significantly decreased in the liver of mice treated with ELA cells and this might be due to the increased rate of lipid peroxidation. In contrast, the Cdpf alone and the Cdpf to the ELA induced mice showed a significant increase in the levels of vitamin A,E and reduced glutathione indicating their protective function against ELA tumor cells.

Vitamin A has been associated with a decreased risk of human cancer and has protective effects in animal models of carcinogenesis. A significant increase in the levels of vitamins E and A in the liver and kidney of rats induced with ammonium metavanadate toxicity by the prior treatment with green tea [19]. [20] also reported a similar result that GSH showed a significant increase with their levels close to the normal control upon treatment of *Desmodium gangeticum* in arthritic rats.

The results of the present investigation revealed that the protein fraction of *Cynodon dactylon* are the rich sources of antioxidants, which offer a future study for the development of drug designing and also justifies the medicinal values of the plants.

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