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NON ENZYMIC ANTI OXIDANTS (VITAMIN A, E & C) IN THE LIVER OF THE MICE TREATED WITH PROTEIN FRACTION OF *CYNODON DACTYLON*

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ABSTRACT

Protein fraction of *Cynodon dactylon*, a plant with various medicinal properties, has been investigated for its non enzymic anti oxidants - vitamin A, vitamin E and vitamin C in the Swiss Albino mice. Results depicted that there is a statistically significant increased levels. The non enzymic antioxidants like vitamin A, E and C content were found to be 19.75 µg / g tissue, 24.25 mg / g tissue and 3.08 mg /g tissue respectively in the liver of the experimental mice when compared to their corresponding vehicle controls. These observations clearly indicate that these non enzymic anti oxidants may be one of the factors responsible for the anti carcinogenic effects.

Key words : *Cynodon dactylon*, Non enzymic anti oxidants, Mice, Liver.

INTRODUCTION

Cancer is a major health problem worldwide which is likely to assume alarming proportions in the coming decades. Conventional therapies cause serious side effects and at the best, merely extend the patient's lifespan by few years. In the last few years, there has been an increase in the attention directed towards the association of non-nutritive component in foods and protection against chronic diseases including some form of cancer and ageing.

Keeping this into view, research is being carried out to evaluate the potential anticancer activities of various vegetables, fruits and other plant parts. In this study, the antimutagenic activity in the plant *Cynodon dactylon* has been studied.. The present study was aimed to explore the bioactivity of the non enzymic antioxidants (Vitamins A , E & C) in the liver of the mice treated with protein fraction of medicinal plant *Cynodon dactylon* (Arugampul).

MATERIALS AND METHODS

Plant material : The fresh plant material *Cynodan dactylon* (Family Poaceae) was collected from the pesticide free area, washed thoroughly to remove the dust particles, blotted dry between filter paper. The leaves were homogenized with phosphate buffered saline (PBS) at 4°C to obtain 20 % homogenate. The homogenate was strained through 8 layers of cotton gauze and centrifuged at 5000 rpm for 10 minutes at 4° C. The supernatant obtained was used for the ammonium sulphate fractionation.

Ammonium sulphate protein fractionation and estimation of protein content : Ten to hundred percentage ammonium sulphate fractionations of proteins was carried out and the precipitate obtained by this method was dissolved in 0.01 M PBS by the method of Jayaraman (1981). The protein content of all the fractions of the precipitate obtained after dialysis was estimated using the method of Shakir *et al* (1994). DPPH assay was carried out by the method of Mensor *et al* (2001), to find ED50 for administering to the animals.

Experimental anlmals : Swiss Albino mice of 5 to 7 weeks of (20 to 25 g) were obtained from animal breeding station, Kerala Agricultural University, Thrissur. The mice were acclimatised to laboratory conditions for 15 days before the commencement of experiments. All procedures described were reviewed and approved by the University Animals Ethical Committee (Reg. No.623/02/b/CPSCSEA).

Experimental design : Animals were divided in to 4 groups of 6 individuals each.

Group I : Intraperitonially injected with 100 µl paraffin oil.

Group II : Intraperitonially injected with 100 µl paraffin oil containing 500 µg of silymarin.

Group III : Intraperitonially injected with 100 µl of PBS

Group IV : Intraperitonially injected with 100 µl of PBS containing ED50 concentration of protein (40 µg).

Silymarin was used as a standard antioxidant. Paraffin oil and PBS serves as the vehicle control for the silymarin and protein fraction respectively. Animals were treated for 21 days and then sacrificed on 22nd day, liver removed, washed with ice - cold saline. 10% liver homogenate was prepared and used for the assay of vitamin A according to the method of Bayfield and Cole (1980), Vitamin E according to the method of Rosenberg (1992) and Vitamin C according to the method of Roe &Keuther (1943).

The different parameters studied were subjected to the statistical analysis with the student 't' test.

RESULTS AND DISCUSSION

Anti oxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infection and degenerative diseases. Realizing this fact, the present study was carried out to evaluate the non enzymic antioxidants-Vitamin A, E and C in the Swiss albino mice treated with the protein fraction of *Cynodon dactylon*. The results of these non enzymic antioxidants are depicted in the Table 1.

Table 1 Effect of non enzymic antioxidants in liver of the mice treated with protein fraction of *Cynodon dactylon.*

Treatment	Vitamin A (μg / g tissue)	Vitamin E (mg / g tissue)	Vitamin C (mg / g tissue)
PBS	6.83	4.46	1.55
Paraffin oil	7.13	3.57	1.65
Silymarin	11.42 @	16.24 @	2.13 @
Protein fraction	19.75 @,$	24.25 @,$	3.08 @,$
C D (α = 0.05)	0.331	0.806	0.342

Values are mean of six mice in each group

@ P<0.001 compared to control groups; $ P<0.001 compared to silymarin groups

Vitamin A : Vitamin A (retinol), a fat-soluble vitamin, is an essential nutrient for the normal functioning of the visual system, epithelial cell integrity and growth, immunity and reproduction (Maciel *et al* 2007). Liver contained the highest mean concentrations of vitamin A, followed by epidermis and serum (Rosa *et al* 2007). In the present study the levels of vitamin A in the protein fraction treated group was found to be significantly increased in comparison with the control groups and silymarin treated group.

Crespy and Williamson (2004) had showed a similar elevation in the level of Vitamin A on treatment of green tea in cardiovascular diseases containing animal models. Bhaya and Saini (2008) reported that the supplementation of *Aloe* to irradiated mice, lowered lipid peroxidation in liver, which was due to the enhancement of concentrations of antioxidants (Vitamin A, C & E) by the supplement. Skrzydlewska *et al* (2002) also reported a significant reduction in the levels of vitamin C, E and A caused by the alcohol intoxication in the liver and blood serum of rats were reverted by the administration of the green tea.

Vitamin E : Among lipid soluble antioxidants, α-tocopherol (Vitamin E) plays a central role in antioxidant defense system as it controls radical-induced lipoprotein lipid peroxidation (Ramesh *et al* 2006). Vitamin E has more important molecular properties, such as the scavenging of reactive oxygen and nitrogen species with consequent prevention of oxidative damage associated with many diseases, or the modulation of signal transduction and gene expression in antioxidant and non-antioxidant manners (Zing 2007).

Vitamin E has anti tumorigenic, photoprotective, skin barrier stabilizing properties and functions as a lipophilic chain-breaking antioxidant that prevents lipid peroxidation (Thiele & Mudiyanselage 2007). Vitamin E prevents lipid peroxidation by involving the oxidative deterioration of polyunsaturated fatty acids (PUFA) that may distrupt the structure and function

The antioxidant status A, E and C which play a vital role in the free radical mechanism is found to be provoked to a statistically significant level and may be one of the active factors responsible for the anti carcinogenic activity of *Cynodon dactylon*

It can be concluded that plant extract itself has the capacity to protect against the carcinogenesis by generating the reactive intermediates in the mechanism of carcinogenesis.

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