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ANTIOXIDANT STATUS IN THE MICE TREATED WITH THE PROTEIN FRACTION OF THE GRASS *CYNODON DACTYLON*

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ABSTRACT

Cells are equipped with enzymatic and non enzymatic antioxidative mechanism that play an important role in elimination of free radicals SOD, Catalase, Glutathione peroxides and reductase involved in the clearance of superoxide and H_2O_2.Hence the present study was carried out to evaluate the antioxidant levels and observed catalase (60.21U/mg protein), superoxide dismutase (4.49U/mg protein), glutathione peroxidase (3.3U/mg protein), glutathione reductase (10.8U/mg protein)and glutathione -s- transferase (2083.32U/mg protein) in the liver of the experimental mice treated with protein fraction of *Cynodon dactylon*. The results were found to be statistically significant when compared to their controls.

Key words : *Cynodon dactylon* Mice, Catalase, SOD, Glutathione peroxides, Reductase, GST.

INTRODUCTION

Free radicals play an important role in a number of biological processes, some of which are necessary for life. Free radicals due to environmental pollutants, radiation, chemicals, toxins, as well as physical stress, cause depletion of immune system, antioxidants, change in gene expression and induce abnormal proteins. Free radicals such as superoxide radical, hydroxyl radical, peroxyl radical and singlet O_2 play an important role in the genesis of various diseases (Agostini *et al* 2002).

Natural plant products have been used since ancient times and a tendency is emerging today for their increased use. Studies have investigated the potential of plant products to serve as antioxidants against various diseases induced by free radicals. The present study was aimed to explore the antioxidative profile of the protein fraction of the medicinal plant *Cynodon dactylon* in the experimental mice.

MATERIALS AND METHODS

Plant material : The fresh plant material *Cynodan dactylon* (Family Poacaeae) 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 out the ED50 to be administered to the experimental mice.

Experimental animals : 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 acclimated 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 : Mice 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 Ed_{50} concentration of protein (40 µg).

Silymarin was used as a standard antioxidant. Paraffin oil and PBS served as the vehicle control for the silymarin and protein fraction respectively.

Mice were treated for 21 days and then sacrificed on 22nd day, liver was excised, washed with ice - cold saline, 10% liver homogenate was prepared and used for the assay of enzymic antioxidants, CAT (Luck 1974), SOD (Misra &Fridovich 1972), GPx (Rotruck *et al* 1973), GR (David & Richard 1983) and GST(Habig *et al* 1974). The different parameters studied were subjected to the statistical analysis with the student 't' test.

RESULTS AND DISCUSSION

Liver is the main organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation. The present study focused on the hepatic antioxidant status of the animals treated with the protein fraction of the *Cynodan dactylon* in the control and experimental groups (Table 1).

Table 1 Activities of enzymic antioxidants in the liver of control and experimental groups of mice.

Treatment	CAT [a](U/mg protein)	SOD [b](U/mg protein)	GPx [c](U/mg protein)	GR [d](U/mg protein)	GST [e](U/mg protein)
PBS	34.33	3.06	2.02	4.70	347.20
Paraffin oil	35.71	3.12	2.09	4.35	390.60
Silymarin	47.21 @	3.68 @	3.02 @	6.57 @	1171.85 @
Protein fraction	60.21 @,$	4.49 @,$	3.30 @,$	10.80 @,$	2083.32 @,$
CD (α=0.05)	1.178	0.162	0.0987	0.204	283.423

Values are mean of six mice in each group

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

a - 1 Unit is defined as the amount of enzyme required to decrease absorbance by 0.05 units at 240 nm.

b - 1 Unit is defined as the amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 min.

c - 1 Unit is defined as the nano moles of GSH oxidized / min.

d - 1 Unit is defined as the milli moles of NADPH oxidized / min.

e - 1 Unit is defined as the nano moles of CDNB conjugated / min.

Catalase (CAT): Catalase (EC 1.11.1.6) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and liver. It is the key component of antioxidant defense system. CAT decomposes H_2O_2 and protects the tissue from highly reactive hydroxyl radicals. Therefore reduction in the activity of these enzymes may result in a deleterious effects due to the accumulation of superoxide radicals and H_2O_2 (Prakash *et al* 2001).

The present study showed the increased CAT activity in liver of the mice administered with the protein fraction of *Cynodon dactylon* to a statistically significant level when compared to control and silymarin groups.

Raja *et al* (2007) have shown that the administration of hydrochloric extract of *Cystisus scoparius* enhanced the activities of CAT and SOD in the liver of rats. The methanolic extract of *Phyllanthus niruri* was found to increase the activities of CAT, SOD and levels of GSH in streptazotocin induced diabetic rats (Mazunder *et al* 2005). In consistence with these reports, the administration of protein fraction of *Cynodon dactylon* also has significantly increased the activities of CAT and thus it protects tissues from free radical induced damage.

A study by Ng *et al* (2005) showed significant increase in CAT activity by administration of Rose (*Rose rugosa*) flower extract to 6-month old Swiss albino mice.

Another study by Manna *et al* (2006) showed increased level of CAT treated with aqueous extract of *Terminalia arjuna* to CCl4 induced mice.

Super oxide dismutase (SOD): Super oxide dismutase (EC 1.15.1.1) is an ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress and provides the first line of defence against free radical damage(Folz *et al* 1997). It catalyzes the dismutation of the highly reactive superoxide anion to O_2 and H_2O_2, which has to be eliminated by GPx and or catalase. Superoxide radicals are one of the most important reactive O_2 free radicals constantly produced in living cells (Winterbourn & Kettle 2003). Superoxide anion is the first reaction product of O_2 which is measured in terms of inhibition of generation of O_2. (Kamalakannan & Stanley Mainzn Prince 2003) .

In our study the level was found to be increased to a more significant level when compared to control and silymarin groups. The SOD activity was significantly elevated in the liver of mice administered with protein fraction of *Cynodon dactylon* when compared with the control and Silymarin treated groups The SOD dismutases superoxide radicals O_2- into H_2O_2 plus O_2, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity (Gupta *et al* 2004). Koneri *et al* (2008) have shown that the ethanolic extract of roots of *Momordica cymbalaria* as well as silymarin increased the levels of antioxidant markers like GSH, SOD and CAT in CCl_4 induced hepatic damage in rats.

The aqueous extract of *Eucalyptus globulus* were found to elevate the activities of SOD in rat liver (Arise *et al* 2009). In line with these reports, a significant increase in SOD activity was caused by the administration of protein fraction of *Cynodon dactylon* and thus the protein fraction reduces superoxide radical induced oxidative damage to liver.

Glutathione peroxidase(GPx): Glutathione per oxidase (EC 1.11.1.7) is also considered to be an important H_2O_2 removing enzyme in mammalian cells and is more important than catalase for removing H_2O_2. GPx is involved in the defense mechanism against oxidative damage, it reduces the H_2O_2 and hydroperoxide levels. GPx offers protection to the cellular and subcellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxide (Meena *et al* 2008).

In the present study, an increase in hepatic GPx activity was noticed in mice treated with the protein fraction of *C. dactylon* when compared to that of control groups and silymarin administered groups. The induction of GPx, which is the central importance in the detoxification of peroxides and hydroperoxides, was measured in the hepatic cytosol where these processes have fundamental importance.

Administration of extracts of *P. aculeata* and silymarin were found to enhance the hepatic activities of GPx., GST, SOD and CAT in CCl_4 intoxicated rats. Alanivel *et al* (2008) have shown increase of antioxidants GPx, SOD and GSH by extracts of *Sargassum polycystan* in liver of rats with D galactosamine indued hepatitis. In the present study, a similar kind of increase in hepatic GPx activity was noticed in mice treated with the selected protein fraction of *C. dactylon*.

Glutathione Reductase (GR) : Glutathione Reductase (EC 1.6.4.2) is concerned with the maintenance of cellular level of GSH (especially in the reduced form) by effecting fast reduction of oxidized glutathione to reduced state (Balamurugan& Muthusamy 2008). The administration of protein fraction of *Cynodon dactylon* afforded a significant increase in GR activity in the liver of mice in comparison with control groups and silymarin administered groups. The methanolic extract of *Urtica pilulifera* was found to greatly enhance the activities of enzymes like GR, GPx and GST in liver of mice (Mahmoud 2006). In accordance to this report, the protein fraction of *C. dactylon* enhances the activity of GR in liver of mice.

Glutathione-S-Transferase (GST) : Glutathione-S-Transferase(EC 2.5.1.18) plays an essential role in liver by eliminating toxic compounds by conjugating them with GSH. GST has a direct role in the neutralization of hydroperoxides derived from LPO processes, GST catalyzes the conjugation of glutathione with many environmental and electrophilic molecules - including metabolites of mutagens and carcinogens to form less toxic and water soluble substances which can readily be excreted. Increases in GST activities are probably related to the oxidative stress caused by periodontal inflammatory process.

The GST activity in the liver of mice administered with protein fraction of *C. dactylon* was found to be significantly higher than those of control groups and silymarin groups of mice. The GST enzyme family serves as catalyst for the reaction of electrophilic compounds with GSH, generally resulting in detoxification and facilitated elimination. Studies by Venkateswara and Pari (2002) had shown that administration of *Phaseoulus vulgaris* pod extract increased the activities of SOD, CAT, GST and GPx in liver of streptazotocin induced diabetic rats. Hur *et al* (2007) showed that the methanol extract of *Aliama orientale* rhizome enhanced the activity of GST in liver of rats treated with Bromobenzene. A similar kind of enhancememt of the activity of GST in the liver of protein fraction treated mice was observed and thus the protein fraction facilitates the elimination of free radicals.

An enhancement of the activity of GST suggests an increase in the hosts ability to detoxify xenobiotics including carcinogens. Thus any substances that can increase activity of GST and other detoxifying enzymes may be a potential anticarcinogen which act as potential inhibitors of chemically induced tumorigenesis. Likewise, any compound that induce the

activity of GST - detoxifying enzyme system may act as potential inhibitors for chemically induced tumorigenesis and can be used as a method for detecting potential inhibitors of carcinogenesis.

Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Antioxidants are intimately involved in the preventions of cellular damage - the common pathway for cancer, aging and a variety of disease. An attempt was made in our lab to explore the antioxidant status in liver of the mice treated with the protein fraction of *Cynodon dactylon*.The enhanced levels of SOD, CAT, GPx, GR and GST are involved in xenobiotic metabolism and maintaining antioxidant status of liver are suggestive of anti cytotoxic efficacy of protein fraction

To conclude, the antioxidative potential might be responsible for protein fraction of *Cynodon dactylon* (Arugampul) to be anticytotoxic. Hence, it can be recommended as a natural, easily available antioxidative agent for preventing free radical induced toxicity in chronic diseases such as cancer, arthritis, diabetes and aging.The knowledge or results obtained from these studies will be useful for future applications in nutraceutical and pharmaceutical research.

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