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## Cynodon Dactylon Effect as Hepatoprotective on CCl<sub>4</sub> Induced Experimental Mice

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### Abstract

Medicinal plants having diverse pharmacological properties including cytotoxic and cancer chemopreventive effects can be developed as novel drugs for cancer chemoprevention. One of the best approaches in search for anticancer agents from plant resources is the selection of plants based ethno medical leads and testing the selected plants efficacy and safety in light of modern science. The present study aims in assessing the hepatoprotective activity of protein fraction of Cynodon dactylon on CCl<sub>4</sub> induced mice. Fresh leaves were homogenized with phosphate buffered saline (PBS) at 4°C to obtain 20 % homogenate. The supernatant obtained was used for the ammonium sulphate fractionation. Hepatoprotective role was evaluated in the liver of mice administered with and without protein fraction. Paraffin oil and PBS serves as the vehicle control for Silymarin and protein fraction respectively. Silymarin was used as the standard antioxidant. CCl<sub>4</sub> acts as a hepatotoxin. The activity of enzymic antioxidants Catalase (CAT), Superoxide dismutase (SOD) and Peroxidase (Px) were determined in the liver homogenate of the control and experimental mice. These effects were found to be more significant than that of Silymarin, the standard antioxidant and the CCl<sub>4</sub> the hepatotoxin. Administration of Cynodon dactylon plus CCl<sub>4</sub> significantly decreases the levels of liver marker enzymes, hepatic enzymic and non enzymic antioxidants. The observed increased levels of lipid peroxides and decreased levels of enzymic and non enzymic antioxidants are the indications of liver damage due to high oxidative stress in CCl<sub>4</sub> induced mice when compared to the respective vehicle controls. The findings of the present study revealed that the Cynodon dactylon had the potent hepatoprotective activity due to its antioxidant property against CCl<sub>4</sub> induced liver damage in mice.

**Keywords:** Protein fraction, Cynodon dactylon, enzymic antioxidants, lipid peroxides, marker enzymes, CCl<sub>4</sub>

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## 1. Introduction

Cynodon dactylon commonly referred to as Arugampul, is a valuable medicinal plant and is used as a curative for various ailments. This medicinal herb has a renowned position in Indian Systems of Medicine. The medicinal plant Cynodon dactylonis traditionally being used in diabetes, jaundice, kidney problems, urinary diseases, GI disorders constipation and abdominal pains. It has been reported to possess antimicrobial, wound healing, antioxidant and ant mutagenic activities. The constituents reported in this plant are cynodin, hydrocyanic acid, tritacin, proteins, carbohydrates, beta-carotene and minerals like calcium, phosphorus, iron and potassium. The present investigation explores the hepatoprotective effect of the protein fraction of the Cynodon dactylon on CCl<sub>4</sub>, the hepatotoxin induced experimental animals.

## 2. Materials and Methods

### Plant

The fresh plant material Cynodon dactylon (Arugampul) 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. DPPH assay was carried out by the method of Mensor et al. (2001) for finding ED<sub>50</sub> so that this dosage (40 µg) was used for administrating to experimental animals.

### Animal

Swiss albino mice of 5 to 7 weeks of (20 to 25 g) were obtained from animal breeding station. The mice were acclimatized to laboratory conditions for 15 days before the commencement of experiments. All procedures described were reviewed and approved by the University Animals Ethical Committee.

### Experimental Design

Hepatoprotective role was evaluated in the liver of mice administered with and without protein fraction. Paraffin oil and PBS serves as the vehicle control for Silymarin and protein fraction respectively. Silymarin was used as the standard antioxidant. CCl<sub>4</sub> acts as a hepatotoxin. The administrations were carried out for 21 days. The mice were divided into seven groups of six each.

**Group I:** PBS (intraperitoneally injected with 100µl).

**Group II:** Paraffin oil (intraperitoneally injected with 100µl)

**Group III:**

Silymarin (intraperitoneally injected with 100µl paraffin oil containing 500µg Silymarin, i.e. 25 mg/kg b.w)

**Group IV:** Protein fraction (intraperitoneally injected with 100µl PBS containing 40µg (ED<sub>50</sub>) of protein)

**Group V:** CCl<sub>4</sub> (intraperitoneally injected 100µl with CCl<sub>4</sub>)

**Group VI:**

CCl<sub>4</sub> + Silymarin (intraperitoneally injected with 100µl of paraffin oil containing 500µg of Silymarin and 100µl of CCl<sub>4</sub>)

**Group VII:** CCl<sub>4</sub> +Protein fraction (intraperitoneally injected with 100µl of CCl<sub>4</sub>containing 40µg (ED<sub>50</sub>) of protein).

### Assessment of the enzymic activity and Antioxidative role

The activity of enzymic antioxidants Catalase (CAT), Superoxide dismutase (SOD) and Peroxidase (Px) were determined in the liver homogenate of the control and experimental mice (Luck 1974). Apart from enzymic antioxidants, a spectrum of non-enzymic antioxidants namely retinol, ascorbic acid, tocopherol and reduced glutathione, are important in cellular system in curtailing ROS. The levels of these antioxidants were also assessed in the liver homogenate of the control and experimental mice. Liver marker enzymes Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase were assayed in the serum of the control and experimental mice. Knowing the Antioxidative role of protein fraction, the antilipid peroxidative effect also was evaluated.

### Statistical analysis:

The data presented here are means of 6 mice each group. The data were statistically analyzed by one-way ANOVA followed by Dunnet's method.

## 3. Results and Discussion

The hepatoprotective potential of protein fraction of Cynodon dactylon leaves was evaluated by assessing the activity of enzymic antioxidants, the levels of non enzymic antioxidants, the levels of lipid peroxides and the liver marker enzymes in the experimental mice.

**Activity of enzymic antioxidants:**

The activities of enzymic antioxidants like catalase, superoxide dismutase, and glutathione peroxidase were analyzed using the liver homogenate and the findings are depicted in Table 1. The catalase activity of CCl<sub>4</sub> group was found to be significantly lower than that found in control groups. Catalase activity in Cynodon dactylon treated group was found to be significantly increased when compared to vehicle control groups. In Cynodon dactylon plus CCl<sub>4</sub> administered group the enzyme activity was found to be more significant than that of Silymarin plus CCl<sub>4</sub> treated group. The SOD activity in CCl<sub>4</sub> group was found to be lower than that of other experimental groups. SOD activity of Cynodon dactylon plus CCl<sub>4</sub> group was found to be higher than that of Silymarin plus CCl<sub>4</sub> treated group. Activity of GPX in liver was significantly decreased in CCl<sub>4</sub> treated group as compared to control mice. The intraperitoneal administration of Cynodon dactylon to CCl<sub>4</sub> induced mice afforded a significant increase in GPx activity in the liver when compared to the mice treated with PBS, paraffin oil and Silymarin plus CCl<sub>4</sub>.

**Table 1. Enzymic and non-enzymic antioxidants in liver of the experimental animals treated with protein fraction of Cynodon dactylon**

Treatment	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Catalase 1 (u/mg protein)	5.16	5.22	6.10	16.3	1.83 <sup>f</sup>	6.02 <sup>abc</sup>	7.6 <sup>bcd</sup>
SOD <sup>2</sup> (u/mg protein)	3.04	2.10	3.58	4.42	5.42 <sup>f</sup>	5.51 <sup>abc</sup>	6.41 <sup>bd</sup>
Gpx <sup>3</sup> (u/mg protein)	2.0	2.01	3.00	3.20	1.03 <sup>f</sup>	3.39 <sup>ac</sup>	7.19 <sup>bcd</sup>
Vitamin A (µg / g tissue)	6.73	7.11	11.32	19.65 <sup>abc</sup>	3.5 <sup>f</sup>	23.86 <sup>abc</sup>	43.47 <sup>bc</sup>
Vitamin C (mg / g tissue)	1.45	1.55	2.11	3.04 <sup>abc</sup>	0.39 <sup>f</sup>	3.07 <sup>abc</sup>	4.4 <sup>bc</sup>
Vitamin E (µg / g tissue)	4.36	3.47	16.14	24.15 <sup>abc</sup>	2.04 <sup>f</sup>	6.53 <sup>abc</sup>	8.47 <sup>bc</sup>
Reduced Glutathione (nM / g tissue)	3.42	4.22	8.67	10.02 <sup>abc</sup>	4.46 <sup>f</sup>	14.01 <sup>abc</sup>	24 <sup>bc</sup>

Bc Values are mean of six mice in each group ( $p < 0.001$ ); Compared to paraffin oil group (a), PBS group (b), CCl<sub>4</sub> group (c), silymarin plus CCl<sub>4</sub> group (d), protein fraction plus CCl<sub>4</sub> (e), CCl<sub>4</sub> group compared to all other groups (f). 1- unit defined as the amount of enzyme required to decrease the absorbance by 0.05 units at 240 nm; 2- unit defined as the amount of enzyme that gives 50% inhibition of the extent of NBT reduction 1 min; 3- unit defined as the nano moles of GSH oxidized / min

**Liver Marker Enzymes:**

To evaluate the normal functioning of the liver, the hepatocellular marker enzymes such as serum glutamic Oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were assessed in the serum of all the experimental mice. The activity of SGOT and SGPT was found to be significantly decreased in Cynodon dactylon administered mice when compared to PBS control. The activity of SGOT and SGPT was found to be significantly increased in CCl<sub>4</sub> the hepatotoxin induced animals (Table 3). In the present study, MDA content of the liver of Cynodon dactylon plus CCl<sub>4</sub> treated mice was found to be significantly ( $p < 0.001$ ) decreased when compared to the control groups and Silymarin plus CCl<sub>4</sub> treated groups (Table 2).

**Table 2. Liver marker enzymes level of lipid peroxides in the experimental animals treated with protein fraction of Cynodon dactylon**

Treatment group	SGOT p (U/L)	SGPT q (U/L)	Lipid peroxides (nM MDA / mg protein)
Pbs	53.49	48.69	5.03
Paraffin oil	52.35	52.64	4.17
Silymarin	55.82	55.08	0.65
Protein fraction	33.59	34.12	0.64
CCl <sub>4</sub>	118.25	108.19	11.06 <sup>d</sup>
Silymarin + CCl <sub>4</sub>	46.49*	69.79*	5.01 <sup>ac</sup>
Protein fraction + CCl <sub>4</sub>	78.56*	74.72*	4.10 <sup>ab</sup>
CD ( = 0.05)	2.01	0.041	0.545

**Discussion**

Catalase is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity is found in the red cells and liver. It decomposes H<sub>2</sub>O<sub>2</sub> and protects the tissue from highly reactive hydroxyl and is thought to be the first line of defense against oxidative damage caused by H<sub>2</sub>O<sub>2</sub> and other radicals induced by carcinogen. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. 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. The catalase

activity of CCl<sub>4</sub> group was found to be significantly lower than that found in control groups. Superoxide radicals are one of the most important reactive O<sub>2</sub> free radicals constantly produced in living cells. Superoxide anion is the first reaction product of O<sub>2</sub> which is measured in terms of inhibition of generation of O. 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 dismutates superoxide radicals O<sub>2</sub><sup>-</sup> into H<sub>2</sub>O<sub>2</sub> plus O<sub>2</sub>, thus participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. Glutathione peroxidase is also considered to be an important H<sub>2</sub>O<sub>2</sub> removing enzyme in mammalian cells and is more important than catalase for removing H<sub>2</sub>O<sub>2</sub>.

GPx is involved in the defense mechanism against oxidative damage. The induction of GPx, which is the central importance in the detoxification of peroxides and hydro peroxides, was measured in the hepatic cytosol where these processes have fundamental importance. In the present study, an increase in hepatic GPx activity was noticed in mice treated with the protein fraction of Cynodon dactylon when compared to that of control groups and Silymarin administered groups. Administration of extracts of P. aculeate and Silymarin were found to enhance the hepatic activities of GPx, GST, SOD and CAT in CCl<sub>4</sub> intoxicated rats. In accordance with these reports the enzymic antioxidants of the selected plant constitute the foremost defense system that limit the toxicity associated with free radicals and are intimately involved in the preventions of cellular damage.

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. 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 (Vit A, Vit C and Vit E) by the supplement. 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. The level of vitamin C in the liver of mice administered with protein fraction of Cynodon dactylon showed significant increase when compared to that of the standard antioxidant Silymarin as well as the control groups and also reported that administration of Cleome gynandra L. Leaf extract significantly increased the levels of vitamin C in arthritis induced rats.

The administration of protein fraction of Cynodon dactylon afforded a significant increase in the levels of vitamin E in the liver of mice when compared to that of the control groups and Silymarin administered groups. A flavonoid rich fraction from *Spermacoce hispida* significantly increased the hepatic levels of non enzymic. A similar kind of increase in hepatic Antioxidative vitamins was shown by methanolic extract of *Careya arborea* Roxb and silymarin in rats with CCl<sub>4</sub> induced liver damage in a study by Sambath kumar et al. The hepatic levels of GSH in mice administered with protein fraction of Cynodon dactylon plus CCl<sub>4</sub> showed a significant increase in comparison with the control groups and the Silymarin group. The non-enzymatic antioxidant Glutathione is one of the most abundant tripeptides present in the liver. Glutathione is the most important non-protein compound containing thiol group, which acts as a substrate for GST and GPx involved in preventing the deleterious effect of oxygen radicals. Das et al. reported a decreased activity of GPx in mice after ethanol exposure which might be due to the inactivation of enzyme and ROS. In consistence with this report, the protein fraction of Cynodon dactylon enhances the levels of GSH liver of mice.

When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. In the present study, the activity of SGOT and SGPT was found to be significantly decreased in Cynodon dactylon administered mice when compared to PBS control. The activity of SGOT and SGPT was found to be significantly increased in CCl<sub>4</sub> the hepatotoxin induced animals. In aqueous and ethanolic extracts of *A. marmelos* and Thirupathi et al. in methanolic extract of leaves of *Balanites roxburghii* (BLR) showed significant decrease in the level of biochemical markers such as SGOT and SGPT. Lipid peroxidation (LPO) refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semi stable peroxides which in turn damage the enzymes, nucleic acids membranes and proteins. The extent of lipid peroxidation is measured through malondialdehyde content (MDA), a pro-oxidant factor that determines the oxidative damage. In the present study, MDA content of the liver of protein fraction plus CCl<sub>4</sub> treated mice was found to be significantly ( $p < 0.001$ ) decreased when compared to the control groups and Silymarin plus CCl<sub>4</sub> treated groups. This decrease in MDA level indicated the inhibition of lipid peroxidation and enhancement of Antioxidative defense mechanisms to prevent formation of excessive free radicals.

#### 4. Conclusion

The results obtained clearly showed that the protein fraction of Cynodon dactylon had significant hepatoprotective potential by enhancing the activities of enzymic antioxidants, increasing the levels of non enzymic antioxidants, marker enzymes and antilipid peroxidative role by decreasing the lipid peroxide levels. These effects were found to

be more significant than that of Silymarin, the standard antioxidant and the CCl<sub>4</sub> the hepatotoxin. Administration of Cynodon dactylon plus CCl<sub>4</sub> significantly decreases the levels of liver marker enzymes, hepatic enzymic and non enzymic antioxidants. The results also imply that the hepatoprotective effects of Cynodon dactylon may be due to its antioxidant property.

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