

Full Length Research Paper

Hepatic antioxidative potential of ethyl acetate fraction of *Cynodon dactylon* in Balb/c mice

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A study was undertaken to evaluate the antioxidative potential of ethyl acetate fraction of *Cynodon dactylon* in Balb/c mice. In this present study mice were divided into the two groups and each group containing 6 mice. Group I mice were orally administered with 70 µl of DMSO and are the vehicle control for ethyl acetate fraction. Group II mice were injected with ED50 70 µl of ethyl acetate fraction of *C. dactylon*. The activity of enzymic antioxidants (U/ mg of protein) such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) were found to be significantly high in ethyl acetate fraction treated mice when compared to the control mice. The levels (g liver tissue) of nonenzymic antioxidants such as vitamin A, vitamin C, vitamin E and reduced glutathione in the ethyl acetate fraction treated mice (Vitamin A 158.224 µg, Vitamin C 7.442 µg, Vitamin E 5.383 µg and reduced GSH 489.283 nmoles) was found to be significantly higher than that found in control mice. These results suggest that ethyl acetate fraction of *C. dactylon* has very good antioxidant and hepatic protective effect of normal oxidative stress in Balb/c mice. Currently, the importance of oxidative stress in the pathophysiology of many human disorders has been highlighted, thus use of this plant as an herbal medicine is highly recommended. Hereafter, trials to establish efficacy and optimum dosage of the present herbal product for treating human chronic diseases with pathophysiology of oxidative stress are essential.

Key words: *Cynodon dactylon*, antioxidants, Balb/c mice, ethyl acetate fraction.

INTRODUCTION

Oxidative stress occurs in a cellular system when the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system. Oxidative stress plays an important role in the process of aging and pathogenesis of numerous diseases like cancer, diabetes, respiratory tract disorders, and neurodegenerative diseases (Anderson et al., 2000). ROS readily attack and induce oxidative damage to various biomolecules, including proteins, lipids, lipoproteins and DNA (Farber, 1994; Arivazhagan et al., 2001). However, the prominent levels of ROS contribute to a decline in cellular function, and have been reported

to coincide with pathologies including cancer, cardiovascular disease and neurological disorders (Muller et al., 2007). The antioxidants may mediate their effect by directly reacting with ROS, by quenching them or by chelating the catalytic metal ions (Robak and Marcinkiewicz, 1995). Several synthetic antioxidants, e.g., butylated hydroxy anisole and butylated hydroxyl toluene, are also commercially available, but these are quite unsafe and their toxicity is a serious health concern (Madhavi and Salunkhe, 1995). Plant polyphenols, is large group of natural antioxidants and polyphenols are briefly known as Vitamin P. The recent research result indicates that polyphenols may have antioxidant characteristics with potential health benefits. They may reduce the risk of cardiovascular disease and cancer (Arts and Hollman, 2005). In addition, medicinal plants

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have been a rich source of biologically active compounds and play an important role in drug discovery. Natural product researchers have sharper eye on herbal products to obtain medicinally important bioactive compounds.

The *Cynodon dactylon* commonly known as "arugum pillu" (Tamil), "doob" (Hindi), "karuka pullu" (Kanada), and "garike" (Telugu) is called creeper in India. The English name of all the East African rhizomatous species of *Cynodon* is Bermuda grass (Harlan, 1970) belonging to family of Poaceae. It is a grass native to East Africa, Asia and Australia and southern Europe. It is a weed and has been found to possess various medicinal properties. The aqueous fluid extract of *C. dactylon* leaf was used to study the anti-diabetic, antioxidant and hypolipidemic efficacy in diabetic rats (Karthik and Ravisankar, 2010), immunomodulatory activity in Swiss albino mice (Santhi and Annapoorani, 2010). Whole green plant aqueous extract of *C. dactylon* was used to study the diabetes-induced oxidative stress of diabetic rats (Prashant et al., 2010). The few literature survey only revealed that scientific investigation has been made in regard to antioxidative potential of leaf fluid extract of *C. dactylon* for antioxidative studies in Balb/c mice. Nowadays, studying antioxidative potential of *C. dactylon* leaf extracts in animal model organism is important for antioxidant researches in pharmacological sciences. Therefore, the aim of the present study was to evaluate the hepatic antioxidative potential of ethyl acetate fraction of *C. dactylon* in Balb/c mice", was accomplished to explore the antioxidant potentialities of *C. dactylon*.

MATERIALS AND METHODS

Animals

In this experiment, a total of 12 Balb/c male mice were used. The current work was carried out after approval by our Institutional animal ethical committee (Registered no. 623/02/b/CPCSEA). The experimental procedure was followed accordance with guidelines on Committee for the Purpose of Control and Supervision of Experiments on Animal Facility (CPCSEA), Government of India. The 5 to 7 weeks old mice were procured from the animal breeding station, Kerala Agricultural University, Thrissur, in India. The mice were maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$) with dark/light cycle (14/10 h). They were housed in polypropylene neat cages, bottomed with husk and fed standard pellet diet and water and *libitum*. The mice were acclimatized to laboratory conditions for 15 days before the commencement of the experiments. In this experiment the mice were divided into the two groups and each group containing 6 mice. Group I mice were orally administered with 70 μl of DMSO and are the vehicle control for ethyl acetate fraction. Group II mice were injected with ED50 70 μl of ethyl acetate fraction of *C. dactylon*.

Chemicals and extraction methods

In this experimental study chemicals and enzymes were procured from Sigma Chemical Co. (St. Louis, MO, USA), and SISCO Research Laboratories (Maharashtra, India). Fresh leaves of *C. dactylon* was taken for in this experimental study. The leaves were

washed with distilled water and blotted to dry using filter paper. The dried leaves were cut into small pieces. Organic extracts were prepared using 80% ethyl acetate and 80% methanol from the leaves of *C. dactylon*. 20% aqueous extract was also prepared and lyophilized. The total polyphenolic content in ethyl acetate, methanol and water extracts were assayed calorimetrically by means of Folins-Ciocalteu method as described by Malick and Singh, (1980).

Enzyme assays

The activity of enzymic antioxidants likes catalase, superoxide dismutase and glutathione peroxidase was analyzed with the excised liver samples. Catalase, (EC 1.11.1.6) activity was estimated by the method of Luck, (1974), Superoxide dismutase, (EC 1.15.1.1) was estimated by the method of Kakkar et al. (1984) and the activity of Glutathione peroxidase, (EC 1.11.1.9), in the liver was assessed by the method of Rotruck et al. (1973). In order to find out the specific activity of protein present in the liver homogenates was estimated by the method of Shakir et al. (1994).

Non enzyme assays

The levels of the nonenzymic antioxidants such as vitamin A, C, E and reduced glutathione were assessed from the excised liver samples. vitamin A, B, C and reduced glutathione (GSH) were assayed by following methods of Bayfield and Cole (1980); Roe and Keuther (1953); Rosenberg (1992) and Moron et al. (1979) respectively.

Statistical analysis

The experiment results are expressed as means \pm SE. Statistical data were analyzed for significant difference by one-way analysis of variance followed by Duncan's multiple range tests at a $p < 0.05$ was considered statistically significant using SAS software (SAS Version 6, 4th Edition).

RESULTS

The results of the total polyphenolic content of ethyl acetate, methanol and aqueous extracts of *C. dactylon* were presented (Figure 1 and Table 1). The results were revealed significant variation in ethyl acetate extract of *C. dactylon* was possessed highest polyphenolic content (49 mg/g of leaves), followed by methanolic (23 mg/g of leaves) and aqueous extracts (9.7 mg/g of leaves).

In this experiment were carried out to find out the effect of ethyl acetate fraction of *C. dactylon* on the activities of enzymic antioxidants in the liver of control and experimental mice. The results of the prominent three selected enzymic activities viz., catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) are presented (Table 2). The results revealed that significantly increased the activity of enzymic antioxidants such as CAT, SOD and GPX in ethyl acetate fraction of *C. dactylon* (CAT 87.6926 U/mg, SOD 55.1606 U/mg and GPX-5.5032 U/mg), treated mice when compared to DMSO control (CAT 26.8681 U/mg, SOD 26.8605 U/mg



Figure 1. Noticeable visible variability of total polyphenolic substance from ethyl acetate (1), methanolic (2) and aqueous (3) extracts of *C. dactylon*.

Table 1. Total polyphenolic content of ethyl acetate, methanolic and aqueous extracts of *C. dactylon*.

S. No.	Name of the extracts	*Polyphenolic content in mg/g of leaves
1.	Ethyl acetate extract	49
2.	Methanolic extract	23
3.	Aqueous extract	9.7

*As catechol equivalent.

Table 2. Activities of enzymatic antioxidants in the liver of mice treated with ethyl acetate fraction of *C. dactylon* and DMSO control.

S. No.	Groups	CAT (U/mg protein)*	SOD (U/mg protein) [@]	GPX (U/mg protein) [#]
1.	DMSO	26.868 ± 1.824 ^b	26.861 ± 1.130 ^b	1.597 ± 0.131 ^b
2.	Ethyl acetate extract	87.693 ± 5.287 ^a	55.161 ± 0.612 ^a	5.503 ± 0.118 ^a

The values are means ± SD of six replicates; values are different within the column are significantly different superscripts at $p < 0.05$ by Dungan's multiple range test (DMRT). *1 Unit - Amount of enzyme required to decrease the absorbance by 0.5 units at 240 nm/mg protein, @1 Unit - Amt. of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 min/mg protein, #1 Unit - n moles of GSH oxidized / min/ mg protein.

and GPX 1.5965 U/mg).

In this experimental study was besides carried out to explore the effect of ethyl acetate fraction of *C. dactylon* on the levels of nonenzymic antioxidants such as the levels of vitamin A, C, E and reduced glutathione in the control and experimental mice were assessed.

The results of nonenzymic antioxidant levels in the liver of mice induced with ethyl acetate fraction of *C. dactylon* are presented (Table 3). The results have significant variable activity of nonenzymic antioxidants such as vitamin A, C, E and reduced glutathione in the liver of mice administered with ethyl acetate fraction (Vitamin A 158.224 ± 1.764 µg/g liver tissue, Vitamin C 7.442 ± 0.153 µg/g liver tissue, Vitamin E 5.383 ± 0.535 µg/g liver tissue and reduced GSH 489.283 ± 2.516 nmoles/g liver tissue) were found to be significantly high

when compared to treatment with DMSO (Vitamin A 39.370 ± 0.558 µg/g liver tissue, Vitamin C 2.430 ± 0.362 µg/g liver tissue, Vitamin E 0.762 ± 0.019 µg/g liver tissue and reduced GSH 281.635 ± 1.798 nmoles /g liver tissue). Consequently, nonenzymic activity results were reflected high antioxidant potential of ethyl acetate fraction of *C. dactylon* than the control DMSO.

DISCUSSION

Extract of *C. dactylon* is endowed with phytochemicals; these phytochemicals have been known to be biologically active, aiding these activities through different mechanisms. The antioxidative effect is mainly due to phenolic components, such as phenolic acids and

Table 3. Levels of nonenzymic antioxidants in the Liver of mice treated with ethyl acetate fraction of *Cynodon dactylon* and DMSO.

S. No	Groups	Vit. A $\mu\text{g/g}$ liver tissue	Vit. C $\mu\text{g/g}$ liver tissue	Vit. E $\mu\text{g/g}$ liver tissue	Reduced GSH nmoles/g liver tissue
1.	DMSO	39.370 \pm 0.558 ^b	2.430 \pm 0.362 ^b	0.762 \pm 0.019 ^b	281.635 \pm 1.798 ^b
2..	Ethyl acetate fraction	158.224 \pm 1.764 ^a	7.442 \pm 0.153 ^a	5.383 \pm 0.535 ^a	489.283 \pm 2.516 ^a

The values are means \pm SD of six replicates; values are different within the column are significantly different superscripts at $p < 0.05$ by Dungan's multiple range test (DMRT).

phenolic diterpenes (Pourmorad et al., 2006). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Polyphenols are mainly responsible for the antioxidant activity; the obtained amount of total polyphenolic content of ethyl acetate, methanol and aqueous extracts of *C. dactylon* was found significant variation in ethyl acetate extract of *C. dactylon* possessed highest polyphenolic content followed by methanolic and aqueous extracts (Table 1). The leaf extract of *C. dactylon* contain anti-diabetic and antioxidant activities in diabetic rats (Karthik and Ravisankar, 2010) also supported our results findings.

Glutathione, SOD and CAT are protecting the cell constituents from oxidative damage. Despite these extensive defense systems, biomolecule damage may still occur and persist within the cell. The significant increase in the activities of SOD and CAT suggests a greater level of endogenous antioxidant associated with the leaf extract treatment resulting in an enhanced free radical scavenging activity (Karuna et al., 2009).

The increase in SOD activity also may indirectly play an important protective role in preserving the activity of CAT. CAT is a hemeprotein, which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals (Chance et al., 1952). Whole green plant aqueous extract of *C. dactylon* have significant antioxidant effect in diabetic rats (Prashant et al., 2010). Effect of ethyl acetate fraction of *C. dactylon* on the activities of enzymic antioxidants in the liver of control and experimental mice results were showed that significant increase in the activity of enzymic antioxidants such as CAT, SOD and GPX in ethyl acetate fraction of *C. dactylon* treated mice; when compared to DMSO control. This enzymatic activity results was reflected the superior high antioxidant potential of ethyl acetate fraction of *C. dactylon* than DMSO control. These similar results were obtained earlier researchers Veena et al. (2002) in dry stem crude extraction of *Tinospora cordifolia*, and polyphenols extracts in tea (Frei and Higdon, 2003).

The nonenzymatic antioxidant levels in the liver of mice induced with ethyl acetate fraction of *C. dactylon* have been possess significant variable activity of

nonenzymatic antioxidants such as vitamin A, C, E and reduced glutathione in the liver of mice administered with ethyl acetate fraction were found to be significantly high when compared to treatment with DMSO. This nonenzymatic activity results were reflected the high antioxidant potential of ethyl acetate fraction of *C. dactylon* than the control DMSO. Herein finding is related with the findings of water and ethanol extracts of *Foeniculum vulgare* seeds, (Münir et al., 2003) and ethyl acetate, methanol and water extract of the fruit of *Dillenia indica* (Abdille et al., 2005) results also supported our findings.

The present studies are soundly indicated that ethyl acetate fraction of *C. dactylon* has very good potential to improve human antioxidant status and prevent normal oxidative stress that happens daily due to normal exposure to many causal chemicals and conditions. This potential of ethyl acetate fraction of *C. dactylon* seems to be due to its bioactive antioxidant components, especially polyphenols and flavonoids. In recent years the importance of oxidative stress in the pathophysiology of many human disorders has been highlighted, thus use of this plant as an herbal medicine is highly recommended. Trials to establish efficacy and optimum dosage of the present herbal product for treating human chronic diseases with pathophysiology of oxidative stress are essential.

Conclusion

The present study revealed that the ethyl acetate fraction of *C. dactylon* leaves is a potent source of natural antioxidants, containing a unique mixture of polyphenols, flavonoids and aliphatic compounds. Therefore, the ethyl acetate fraction of *C. dactylon* can be recommended as a natural, low cost, locally available antioxidative agent for alleviating the chronic degenerative disorders such as immunosuppressive, arthritis, cardiovascular diseases, aging and diabetes mellitus suggesting its use in naturaceuticals.

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