WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 7, 892-906. Research Article ISSN 2277 – 7105

EFFECT OF *CYNODON DACTYLON* ON FREE RADICAL LOAD OF LYMPHOID ORGANS OF ASIAN CATFISH, *CLARIAS BATRACHUS*

Mishra, P.* Jurry, R., and Gupta, S.

Department of Zoology, Govt. NPG College of Science, Raipur 492010, India.

Article Received on
20 July 2014,

Revised on 12 August 2014,
Accepted on 06 Sept. 2014

***Correspondence for Author**
Dr. Preeti Mishra
Department of Zoology, Govt. NPG College of Science, Raipur 492010, India.

ABSTRACT

The aim of the present study was to asses the effect of aqueous and alcoholic extract of *Cynodon dactylon* on free radical load of lymphoid organs viz., spleen, thymus and head kidney of Asian catfish, *Clarias batrachus*. For this 36 fishes of mixed sexes were randomly divided into three group; each group consists of 12 fishes. Group one treated as control and group 2 and 3 treated as test receiving 50 and 100 ppm of aqueous or alcoholic extract of *Cynodon dactylon* every alternate day till 28 days. Test and control fishes were sacrificed after 7, 14, 21 and 28 days and lymphoid organs were collected in icecold containers for enzyme assay. The *Cynodon dactylon* treated fish showed significant increase in superoxide dismutase and catalase and decrease in lipid peroxidation. No mortality was observed at these doses. Result of ANOVA for SOD & CAT revealed a statistically highly significant main effect of treatment (T), duration (D) of experimentation and interaction effect of treatment with duration (TXD). The result of ANOVA for LPO also showed statistically significant results except alcoholic extract in thymus where duration of experimentation and interaction effect (TXD) was not significant and aqueous extract in spleen where interaction effect (TXD) was not significant.

KEY WORDS *Cynodon dactylon*, *Clarias batrachus*, aqueous & alcoholic extract, antioxidant enzyme, free radicals, lymphoid organs.

INTRODUCTION

The free radicals are the radicals with uneven number of electrons. They are very unstable molecules, search extra electron for its stability and produce another unstable molecule. Therefore, the chain of free radical is producing that cause extensive damage to cell. Super oxide anion, hydroxyl and hydrogen peroxide radicals are the example of reactive oxygen

species that can cause oxidative damage to the DNA molecules, lipids and proteins [1]. However, the cells, which are unstable by free radical attack, are destroyed by the immune system by means of phagocytes but it cannot prevent all the free radical that is produced in the cells. Antioxidant defense (AD) system which include antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), glutathioneperoxidase (GPx), glutathione-S-transferase (GST), and other lower molecular weight substances such as glutathione(GSH), vitamins and proteins located in different tissues [2] function against free radical. The ROS are generated during normal metabolism are well controlled under normal physiological conditions. However, when organisms are under stressful physiological condition the ROS overcome the body antioxidant enzyme and therefore, the organism faced health problems. Some of the medicinal plant has certain chemicals that have antioxidant properties and used as exogenous source of antioxidants in animal body.

One of the important medicinal herbs *C. dactylon* used for medicinal purposes. *Cynodon dactylon* (L) Pers. is commonly known as 'Doob' grass in India and is considered as toxic a weed which is very invasive [3] and fast growing. It is also known as "world worst weed" [4] and is found abundant along the roadsides, in lawns and uncultivable soil. It has no odor with sweet mucilaginous taste. It is one of the important medicinal weed plants of Chhattisgarh state (India). *C. dactylon* is the most sacred plant next to *Ocimum sanctum*. Hindus worship the God Ganesha with the leave of this plant religiously.

The classification of this species is as:

Kingdom	Plantae-Plants
Sub-Kingdom	Tracheobionta –Vascular Plant
Division	Magnoliophyta- Flowering plant
Class	Liliopsida-Monocotyledons
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae-Grass family
Genus	*Cynodon*
Species	*C. dactylon (L.*) Pers.

In India, this plant is used in the treatment of malaria, thirst, burning sensations in the body, anemia, asthma, bronchial problem like cough, cold, sore throat, Influenza, fever, hypertension, snake bite, kidney problem, swelling, skin diseases, leucoderma, stress and infection etc. [5]. Paul *et al*. [6] review the therapeutic uses of this plant. *Cynodon dactylon*

shows some of the pharmacological properties such as antiviral, antifungal, antiseptic, antibacterial[7-9], immunomodulatory, antihaemorrhagic, antihelminthic, analgesic, antipyretic, antioxidant[10-12] wound healing [13] and hypolipidemic activity[14].

The extract of this plant is a rich source of chemicals such as proteins, carbohydrates, mineral constituents, flavanoids, saponins, tannins, β-sitosterol, alkaloids, tri-terpenoides and glycosides [15]. Kaleeswaran *et al*. [16] reported the presence of tannins, quinines, phenol in the ethanolic extract ofthis plant. Jurry *et al*. [17] reported the presence of alkaloids, antheroquinone, flavonoids, saponins, sterols, tannins and terpenoids in aqueous and alcoholic extract of *C. dactylon*.

Fishes being aquatic inhabitants faced environmental stress due to water pollution, presence of pathogens, malnutrition, overcrowding and certain unfavorable environmental factors, and result in the production of more free radical that damage the fish tissue and decline the health. To overcome this problem, certain exogenous natural antioxidants are required. There are few papers on the antioxidant effect of plant on fish [18]. However, no work has been reported on the culturally important live fish *Clarias batrachus*. Keeping this view, the present study was planned to investigate the effect of *Cynodon dactylon* on free radical load of lymphoid organs of *Clarias batrachus*.

MATERIALS AND METHODS

Collection and preparation of plant extract

Fresh mature uninfected plants of *Cynodon dactylon* were collected from different location of Raipur district. The plants were taxonomically identified in the Department of Botany, Govt. N. P. G. College of Science, Raipur, India. The whole plant of *C. dactylon* washed with water thoroughly and shade dried at room temperature for more than 15 days. The air-dried plant were powdered in an electric blender and passed through 200 mesh sieve. The dried powder material was extracted in alcohol/distilled water. The crude extract thus obtained was filtered with Whatman filter paper no. 1 and concentrated in an incubator at 40^{o}C until the solvent completely evaporated and later stored at 4^{o}C until use.

Preparation of stock solution

The crude extracts of the plant material were used as standard stock solutions made by dissolving them in a definite amount of mother solvents and their strength were calculated. The concentration of alcoholic extract was kept high so that minimum quantity of vehicle

required for treatment. Different concentration of test solution viz., 50 and 100 ppm were prepared from stock solution by diluting with vehicle solvent. Homogeneous solution was obtained by gentle shaking or stirring.

Table1: Percentage yield of aqueous and alcoholic extracts of *Cynodon dactylon*.

Weight of plant material (g)	Plant extract			
	Aqueous extract		Alcoholic extract	
	Yield (g)	% Yield	Yield (g)	% Yield
12	1.7	14.25	01	8.33

Experimental Protocol

Clarias batrachus weighing 35- 45 gram were procured from local fish suppliers of Raipur and Jagdalpur district. Fishes were disinfected with 0.1% Potassium permanganate as describe by Joshi *et al*. [19] and acclimatized under laboratory condition for seven days. Thirty six fishes of mixed sexes were randomly selected and divided into three groups (C, T_1 and T_2), each group stocking 12 fish per aquarium with 10 litre of water. The fishes in $T_1$and T_2 groups were experimentally exposed to 50 ppm and 100 ppm dose of aqueous or alcoholic extract of *C. dactylon* respectively every alternate day upto 28 days. The third group (C) served as control. The fishes were fed with goat liver day after treatment and water was changed after 24 hours of feeding. Test and control fishes were sacrificed after 7, 14, 21 and 28 days from the start of the experiment and Lymphoid tissues viz., thymus, spleen and head kidney were collected in previously ice-cold container for antioxidant enzyme assay.

Enzyme assay

Spleen, head kidney and thymus were homogenates (10% W/v) using cold 0.1 M phosphate buffer (pH 7.4) then centrifuged at 1000 rpm for 10 minutes in cooling centrifuge [20]. The supernatant was used for the activity of Superoxide dismutase (SOD), Catalase (CAT) and Lipid peroxidation (LPO). The activity of Superoxide dismutase was determined by the method of Marklund & Marklund (1974) [21], Catalase activity was estimated according to Sinha (1972) method [22] and Lipid peroxidation was determind by the Okhawa *et al.* (1979**)** method[23].

Chemicals

Absolute ethanol, Chloroform, 2.0 mM of pyrogallol, 1.0 mM of Tris Hcl buffer, 0.2 M hydrogen peroxide, 0.01 M of phosphate buffer, 5% of dichromate acetic acid reagent

(prepared in glacial acetic acid), Sodium dodecyl sulphate, Thiobarbituric acid, n Butanol pryridin (15:1).

Statistical analysis

All data obtained were subjected to two-way Analysis of Variance (ANOVA) using SPSS version 16.0. The data were expressed as mean standard error of mean (SEM) and were analyzed by student's t- test.

RESULTS

The results of effect of aqueous & alcoholic extract of *C. dactylon* on Superoxide dismutase (SOD) and Lipid peroxidation (LPO) of lymphoid organs viz., spleen, thymus, head kidney of *C. batrachus* are shown in Table 2 & 3 and Catalase (CAT) in figure 1 to 6.

Result of ANOVA for SOD & CAT revealed a statistically highly significant main effect of treatment (T), duration (D) of experimentation and interaction effect of treatment with duration (TXD). The result of ANOVA for LPO also showed statistically significant results except alcoholic extract in thymus where duration of experimentation and interaction effect (TXD) was not significant and aqueous extract in spleen where interaction effect (TXD) was not significant.

On an average both, the extract treated group showed statistical significant change as compare to control in all the three lymphoid organs of *C. batrachus*. The aqueous extract showed higher activity of SOD in Spleen and thymus as compared to head kidney. The high dose (100ppm) showed higher elevation in SOD activity as compare to low dose (50ppm). The result of catalase activity clearly indicated that low dose is more effective as compare to high dose for enzyme elevation. In general, there is a decline in lipid peroxidation in all lymphoid organs as compared to control.

Table-2: Effect of aqueous and alcoholic extract of *C. dactylon* on SOD activity in lymphoid organs of *C. batrachus*.

Lymphoid organ	Days	SOD activity unit/min/mg protein					
		Aqueous Extract of *C. dactylon*			Alcoholic extract of *C. dactylon*		
Spleen		Control	Low dose	High dose	Control	Low dose	High dose
	7	12.68±0.40	11.50±0.45	57 .35±2.57**	36.52±0.29	21.36±0.22***	126.54±0.28***
	14	20.62±0.80	16.59±0.37*	202.63±0.73***	12.19±0.12	51.37±0.28***	134.45±0.26***
	21	16.67±0.37	5.96±0.40**	343.71±0.43***	16.11±0.06	81.41±0.31***	67.31±0.24***
	28	12.52±0.29	6.48±0.29**	15.91±0.52*	9.63±0.14	38.53±0.26***	30.76±22.91
	ANOVA Summary T* D*** T×D***				**ANOVA Summary T*** D*** T×D***		
Thymus	7	10.88±0.37	14.82±0.33 *	36.74±0.41***	82.45±0.12	30.28±0.25***	9.6 ± 0.11***
	14	12.69±0.25	10.36±0.44 *	93.05±0.43***	48.16±0.16	47.88± .26	35.8 ± 0.29 ***
	21	11.77±0.29	6.21±0.51 *	149.18±0.41***	48.72±0.21	65.59±0.19***	98.23 ± 0.16 ***
	28	11.27±0.22	7.99±0.47 *	277.31±0.39***	13.26±0.25	143.59±0.39***	47.78 ± 0.14 ***
	ANOVA Summary T* D*** T×D***				**ANOVA Summary T*** D*** T×D***		
Head kidney	7	123.96±2.38	6.0±0.42***	20.88± 0.33***	14.23±0.15	48.77±0.13***	53.39±0.24***
	14	2.53± 0.20	15.17±0.47**	171.22±0.53***	52.26±1.23	82.89±0.15**	60.32±0.25*
	21	12.6±0.23	24.11±0.41**	320.74±0.41***	79.66±0.33	24.72±0.20***	6.90±0.08***
	28	45.46±1.76	11.72±0.62**	128.38±0.41***	65.7±0.25	36.73±0.25***	6.56±0.23***
	ANOVA Summary T*** D*** T×D***				ANOVA Summary T*** D*** T×D***		

The values are expressed as Mean ± SEM. ***p<0.001., ** p<0.01., * p<0.05 Statistical test was done by ANOVA, where T= Treatment, D= Duration of treatment.

Table-3: Effect of Aqueous and alcoholic extract of *Cynodon dactylon* on Lipid per-oxidation in lymphoid organs of *C. batrachus*

Lymphoid organ	Days	LPO µmo of MDA/min/mg protein					
		Aqueous Extract of *C. dactylon*			Alcoholic extract of *C. dactylon*		
		Control	Low dose	High dose	Control	Low dose	High dose
Spleen	7	2.03 ± 0.22	0.77±0.069*	0.5 ± 0.03*	2.54 ± 0.26	1.08 ± 0.05*	0.16 ± 0.01*
	14	1.53 ± 0.20	0.4 ±0.06*	0.37 ± 0.04*	1.70 ± 0.19	0.5 ± 0.06*	0.07 ± 0.01*
	21	1.63 ± 0.29	0.03±0.01*	0.26 ± 0.04*	2.02 ± 0.26	0.09 ± 0.01	0.17 ± 0.01*
	28	1.6 ± 0.23	0.34 ±0.05*	0.3 ± 0.06*	1.57 ± 0.21	0.2 ± 0.06*	0.12 ± 0.01*
	ANOVA Summary T*** D*** T×D ns				ANOVA Summary T*** D*** T×D**		
Thymus	7	1.23 ± 0.17	1.35 ± 0.05	1.98 ± 0.10	1.17 ± 0.17	0.33 ± 0.01	0.12 ± 0.01*
	14	.01 0.27	0.69 ± 0.01	1.00 ± 0.05	1.83 ± 0.23	0.24 ± 0.03*	0.34 ± 0.25*
	21	1.33 ± 0.18	0.03 ± 0.01*	0.04 ± 0.01	1.23 ± 0.20	0.17 ± 0.01*	0.44 ± 0.01
	28	1.16 ± 0.12	0.52 ± 0.01*	0.8 ± 0.01	1.5 ± 0.23	0.18 ± 0.01*	0.05 ± 0.01*
	ANOVA Summary T*** D*** T×D***				ANOVA Summary T*** D ns T×D ns		
Head kidney	7	1.7 ± 0.17	1.56 ± 0.11	1.40 ± 0.07	1.47 ± 0.25	1.82 ± 0.07	0.65 ± 0.06
	14	1.23 ± 0.20	0.85 ± 0.05	0.67 ± 0.02	1.60 ± 0.17	1.00 ± 0.13	0.54 ±0.06*
	21	1.43 ± 0.20	0.08 ± 0.01*	0.18 ± 0.02*	1.74 ± 0.27	0.4 ± 0.06*	0.50 ± 0.04*
	28	0.96 ± 0.23	0.67 ± 0.02	0.48 ±0.02	1.58 ±0.23	0.92 ± 0.01	0.45 ± 0.02*
	ANOVA Summary T*** D*** T×D**				ANOVA Summary T*** D** T×D***		

The values are expressed as Mean ± SEM. ***p<0.001., ** p<0.01., * p<0.05, Statistical test was done by ANOVA, where T= Treatment, D= Duration of treatment.

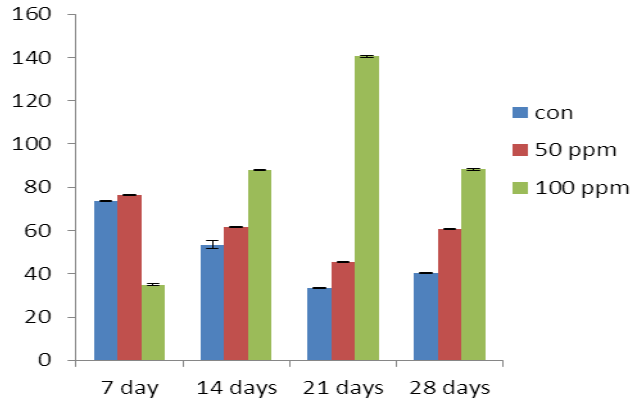

Figure- 1: Effect of aqueous extract of *Cynodon dactylon* on slpeen catalase

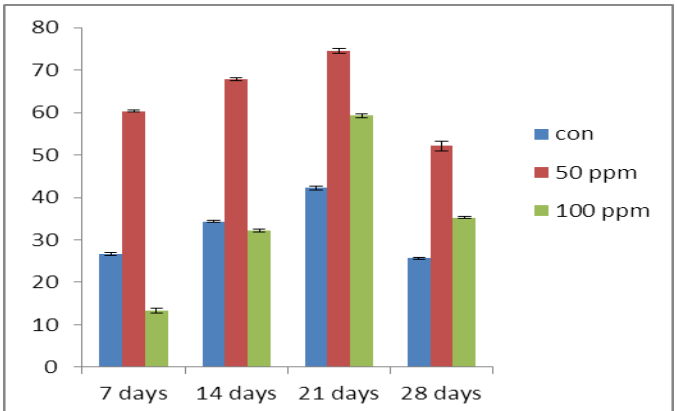

Figure-2: Effect of alcoholic extract of *Cynodon dactylon* on spleen catalase

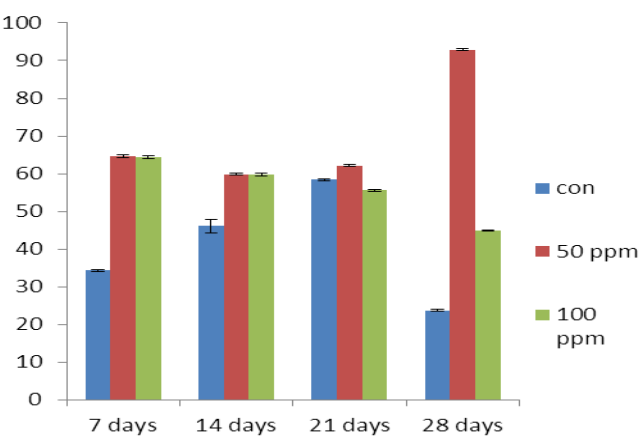

Figure-3: Effect of aqueous extract of *Cynodon dactylon* on head kidney catalase

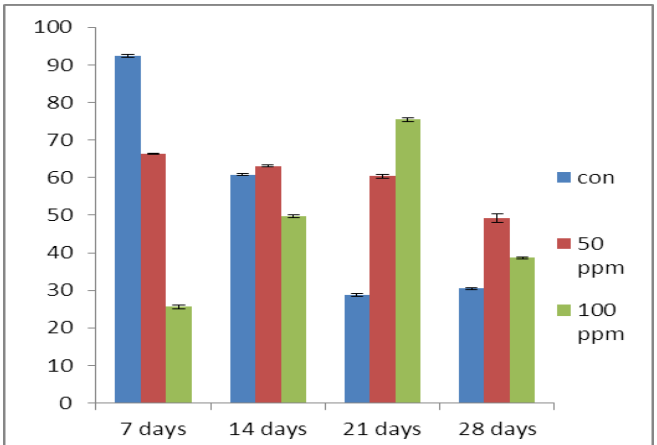

Figure-4: Effect of alcoholic extract of Cynodon dactylon on head kidney catalase

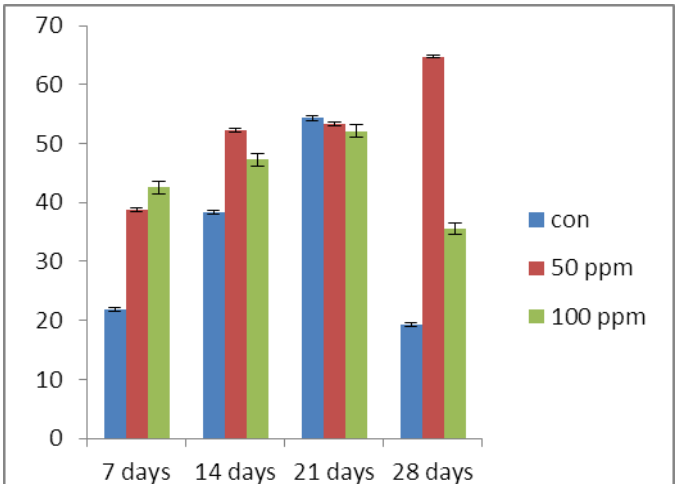

Figure-5: Effect of aqueous extract of *Cynodon dactylon* on thymus catalase

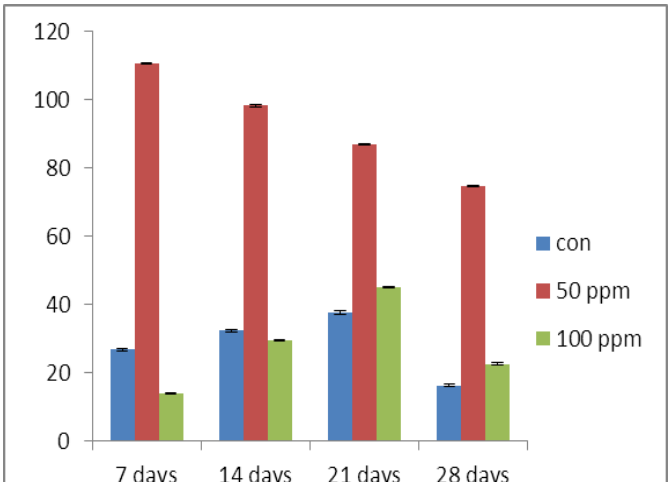

Figure-6: Effect of alcoholic extract of *Cynodon dactylon* on thymus catalase

DISCUSSION

In the present study, the effect of varied concentration of aqueous and alcoholic extract of *Cynodon dactylon* on free radical load of lymphatic organ of air breathing catfish, *Clarias batrachus* at different time points have been assessed. Spleen, head kidney and thymus which are the main lymphoid organs in fish, play an important role in the formation of Red blood cells, cells of immune system, synthesis of immunoglobulins, filteration of blood and recycling of iron. Free radicals are produced by endogenous cellular sources during normal cell metabolism. Mitochondrial respiration is the main endogenous source of ROS. Increase production of ROS can cause oxidation of proteins, lipid peroxidation and DNA oxidation, alternation in gene expression and change in cell redox status [24-25]. Oxygen free radicals are reportedly involved in the toxicity of numerous chemicals and also in pathogenesis of many diseases [26]. The antioxidant enzymes break the chain of free radical formation and regulate the tissue damage.

In *C. batrachus* the treatment of aqueous and alcoholic extract of *C. dactylon* significantly increase the activity of superoxide dismutase and catalase. The result of our study was supported by the study of Thiruvengada *et al* [27] who found that *C.dactylon* enhanced free redical scavenging activity in rat. A number of other investigators reported similar results in mammals with *C.dactylon* [28, 5, 29].

Our results were in agreement with some other investigation using different plant in mammalian models viz *Tinospora cordifolia* [30], *Phyllanthus amarus* ([31], *Mucuna pruriens* [32], *Bauhinia racemosa* [33], *Cissus quadrangularis* [34]., *Ocimum sanctum* [35], *Achyranthes aspera* [36], *Barringtonia acutangulata* [37], *Gmelina asiatica* [20], *Leonotis leonurus* [38], *Euphorbia thymifolia* [39]. A significant increase in super oxide dismutase and catalase activity was reported in brain and kidney tissue after the treatment of propolis, which is a hive product collected by honey bees from plants and Ginseng as compare to control in rats [40]. Alagammal *et al.* [41]reported similar results with *Polygala rosmarinifolia* against CCl4 induced hepatotoxicity in rats. SOD and CAT constitutes a mutually supportive team of defense against reactive oxygen species (ROS) [42].

SOD is a metallic protein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of super oxide anion. It is responsible for the dismutation of highly reactive and potentially toxic radicals to less toxic hydrogen peroxide [43] which is finally detoxified by catalase to nontoxic end products [44]. Inability to remove these toxic

radicals can initiate a damaging effect on polyunsaturated fatty acids and structural proteins of plasma membranes. Catalase, an enzyme that occurs in almost all aerobically respiring organisms, serves to protect cells from the toxic effect of hydrogen peroxides, by catalyzing the hydrogen peroxides to water and [45]. Catalase activity becomes important at high concentration of hydrogen peroxide at which the enzyme decomposes most of this compound [46].

The result of present study clearly indicated the significant lowering of lipid peroxidation after the treatment of aqueous and alcoholic extract of *Cynodon dactylon* in spleen, head kidney and thymus of *Clarias batrachus* fish. Similar comparable results were reported by Metwally *et al.* [18] in *Oreochromis niloticus treated with Allium sativum*. Dash *et al.*[47]. reported the significantly decrease lipid peroxidation in rats treated with *Ichnocarpus frutescens*. Similarly Hyunghee *et al.* [48] reported the antioxidant potential of ginseng by decreasing MDA level and increasing SOD & CAT activity in human. MDA is a major oxidation product of polysaturated fattyacids. It is a significant indicator of membrane lipid peroxidation arising from interacting reactive oxygen types with cellular membrane [49]. Plant derived natural products viz., tannins, flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including anti-oxidant. Flavonoid content of the plant is reported to be responsible for the antioxidant effect [40]. Flavonoids inhibit lipid peroxidation at the initiation stage by acting as scavengers of superoxide anions and hydroxylradicals [50]. It has been proposed that flavonoids terminate chain radical reactions by donating hydrogen atoms to the peroxy radical forming a flavonoid radical which in turn reacts with free radicals thus terminating the propagating chain [51-52]. Our previous work clearly reported the presence of flavonoids in the aqueous and alcoholic extract of *C. dactylon*. Higher activity of SOD and catalse and decrease lipid peroxidation after the exposure of *C. dactylon* clearly indicates the antioxidant potential of *Cynodon dactylon* in *Clarias batrachus*. Thus its use may improve the fish health.

CONCLUSION

The present study has shown that *C.dactylon* increased the antioxidant activity in *C.batrachus* by increasing SOD and CAT activity and decreasing lipid peroxidation. Thus, it can be used as prophylactic measure in fish. Further work is necessary to isolate active principles and elucidate the actual mechanism involved in the antioxidant activity of this plant in fish**.**

ACKNOWLEDGEMENT

We are grateful to Prof S.V.K. Prasad, Principal, Govt. N.P.G. College of Science, Raipur, India for encouragement and providing necessary facilities. One of us (JR) is thankful to UGC, New Delhi, India for the award of Rajeev Gandhi National fellowship.

REFERENCES

1. Farber JL. Mechanism of cell injury by activated oxygen species. Environ. Health Perspect, 1994; 102: 17-8.
2. Frei B. Molecular and biological mechanisms of antioxidant action. FASEBJ, 1999; *13*: 963-2.
3. Fernandez ON. Establishment of *Cynodon dactylon* from stolen and rhizome fragments. Weed Research**,** 2003; 53: 130-9.
4. Holm LG, Plucknett DL, Pancho JV, Herberger JP. The world's worst weeds. University press, 1977; ISBN 0-8248-0295-0 pp. 609.
5. Karthik D, Ravikumar S. A study on the protective effect of *Cynodon dactylon* leaves extract in diabetic rats. Biomed Environ Sci, 2011; 24(2): 190-10.
6. Paul R, Mandal A, Dutta KA. An updated overview on *Cynodon dactylon (*L.) Pers. *I J R A*, 2012; 3(1): 11-4.
7. Devi G K, Thirumaran RA and Anantharaman P. Antibacterial activity of selected medicinal plants from Parangipettai coastal regions; Southeast Coast of India. *J. Applied Sciences*, 2009; 7 (9): 1212-4.
8. Chaudhari Y, Mody HR, Acharya VB. Antibacterial activity of *Cynodon dactylon* on different bacterial pathogens isolated from clinical samples. Int J of Pharmaceutical Studies and Research, 2011; 11(1): 16-5.
9. Rao AS, Nayanatara AK, Kaup RS, Sharma A, Kumar AB, Vaghasiya BD, Kishan K, Pai SR. Potential antibacterial and antifungal activity of aqueous extract of *Cynodon dactylon*. Int J of Pharmaceutical Sciences and Research, 2011; 2 (11): 2889-5.
10. Garg V, Khosa R. Analgesic and antipyretic activity of aqueous extract of *Cynodon dactylon.* Pharmacology Online, 2008; 3:12-7.
11. Kumar, A, Sawarkar HA, Deshmukh VS, Mishra KK, Singh M, Verma T, Kashyap P. *Cynodon dactylon* (L.) Pers. pharmacological and medicinal application. Int J of Herbal Drug Research, 2011; 1(1): 1-7.

12. Hema TA, Arya AS, Suseelan S, John Celestinal RK, Divya P V. Antimicrobial activity of five South Indian medicinal plants against clinical pathogens. Int J Pharm Bio Sci, 2013; 4(1): 70-11.
13. Dande P, Khan A. Evaluation of wound healing potential of *Cynodon dactylon*. Asian J. of Pharmaceutical and Clinical Research, 2012; 5(3): 161-4.
14. Visvanathan S, Janathul Firdous S, Bharathi V. Protective effect of *Cynodon dactylon* extract on high diet induced hyperlipidemia in experimental animals. World J of Pharmaceutical Research, 2014; 3(5): 513-5.
15. Thakare VM, Chaudhari RY, Patil VR. Potential medicinal plant *Cynodon dactylon* (L.) Pers. Asian J Pharmaceutical Sciences and Research, 2011; 1(6):1-9.
16. Kaleeswaran B, Ilavenil S, Ravikumar S. Growth response, feed conversion ratio and antiprotease activity of *Cynodon dactylon* (L.) mixed diet in *Catla catla* (Ham.). J of Animal and Veterinary Advances, 2011; 10: 511-7.
17. Jurry R, Gupta S**,** Mishra P. Phytochemical screening of *Cynodon dactylon.* Asian J of Chem and Environ Res, 2013; 6 (1-2): 17-5.
18. Metwally M A A. Effects of garlic (*Allium sativum*) on some antioxidant activities in *Tilapia nilotica* (*Oreochromis niloticus*). *World Journal of Fish and Marine Sciences*, 2009; 1: 56-9.
19. Joshi PK, Harish D, Bose M. Effect of lindane and malathione exposure to certain blood parameters in a fresh water teleost fish, *Clarias batrachus*. Pollution Resources, 2002; 21:55-3.
20. Merlin NJ, Parthasarthy V. Antioxidant and hepato-protective activity of chloroform and ethanol extract of *Gmelina asiatica* aerial parts. J Med Plant Res, 2011; 5(4): 533-6.
21. Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay of superoxide dismutase. Eur J Biochem, 1974; 47: 469-5.
22. Sinha AK. Coloimetric assay of catalase. Anal Biochem, 1972; 47:389-6.
23. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 1979; 95: 351-8.
24. Blake DR, Allen RE, Lunec J. Free radicals in biological system: a review oriented to inflammatory processes. Br Med Bull, 1987; 43: 371-15.
25. Livingstone DR. Oxidative stress in aquatic organism in relation to pollution and agriculture. Revue de Medecine Veterinaire, 2003; 154: 427-4.

26. Rey A, Banerjee BD. Stress free radicals and the immune response, modulation by drug. Arch Phamacol, 1998; 358: 739.
27. Thiruvengada Rajan VS, Ellaiah P, Madhusudhana chetty C. Invitro anti-oxidant and alpha-amylase inhibitory activity of isolated compound from ethyl acetate extract of *Cynodon dactylon* and piper betle. Int J Pharm and Indian Research, 2011; 1(4): 261-5.
28. Santhi R, Kalaiselvi K, Annapoorani S. Antioxidant efficacy of *Cynodon dactylon* leaf protein against ELA implanted swiss albino mice. J of Pharmacy Research, 2010; 3(2): 228-3.
29. Saradha Devi KM, Annapoorani S, Ashokkumar K. Hepatic antioxidative potential of ethyl acetate fraction of *Cynodon dactylon* in balb/c mice. J of medicinal Plant Research, 2011; 5(6): 992-5.
30. Desai VR, Kamat JP, Sainis KB. An immunomodulator from *Tinospora cordifolia* with antioxidant activity in cell free systems. Proc Indian Acad Sci (Chem Sci), 2002; 114(6):713-7.
31. Karuna R, Sreenivasa Reddy SS, Saralakumari D. Antioxidant potential of aqueous extract of *Phyllanthus amarus* in rats. Indian J of Pharmacology, 2009; 41(2): 64-4.
32. Sivaraman D, Ratheesh Kumar KS, Muralidaran P. Effect of ethanolic seed extract of *Mucuna pruriens* (L.) DC. Var. Utilis on haloperidol induced tardive dyskinesia in rats. Int J. of Pharmaceutical Sciences Review and Research, 2010; 3(2): 106-18.
33. Gupta M, Mazumder U. Antitumor activity and antioxidant role of *Bauhinia racemosa* against Ehrlich ascites carcinoma in Swiss albino mice. Acta Pharmaco Sin, 2004; 25: 1070-6.
34. Jainu M, Shyamala Devi. In vitro and in vivo evaluation of free radical scavenging potential of *Cissus quadrangularis*. African J. of Biomedical Research, 2005; 8: 95-5.
35. Goel A, Kumar S, Singh DK, Bhatia AK. Wound healing potential of *Ocimum sanctum* Linn. with induction of tumor necrosis factor (TNF-α). Indian J of Experimental Biology, 2010; 48: 402-5.
36. Malarvili T, Gomathi N. Effect of *Achyranthes aspera* (L.) seeds on redox and oxidative status in plasma and selected tissues of rats fed with high doses of fructose. Biosci Biotech Res Asia, 2009; 6(2): 659-5.
37. Babre N, Debnath S, Manjunath YS, Parameshwas P, Wankhed SV, Hariprasath K. Antioxidant potential of hydroalcoholic extract of *Barringtonia acutangula* Linn. roots on stretozotocin-induced diabetic rats. Int J of Pharmaceutical Sciences, 2010; 2(4): 201-3.

38. Oyedemi SO, Afolayan AJ. In vitro and in vivo antioxidant activity of aqueous leaves extract of *Leonotis leonurus* (L.) R. Br. Int J of Pharmacology, 2011; 7(2): 248-9.
39. Mamatha GC, Prabhakar T, Pandya N, Maduri V, Neelima K, Raju Erumalla VN. Antitumor activity and anti-oxidant status of *Euphorbia thymifolia* Linn. against ehrlich ascites carcinoma in swiss albino mice. World J of Pharmaceutical Research, 2014; 3(5): 853-12.
40. Diab AA, EI-Sayed A, EI-Aziz A, Hendaw, AA, Zahara MH, Hamza R. Antioxidant role of both Propolis and Ginseng against neurotoxicity of Chloropyrifos and Profenofos in male rats. Life Science J, 2012; 9(3): 987-22.
41. Alagammal M, Lincy MP, Mohan, VR. Hepatoprotective and antioxidant effect of *Polygala rosmarinifolia* Wight & Arn against CCl4 induced hepatotoxicity in rats. Journal of Pharmacognosy and Phytochemistry, 2013; 2(1):118.
42. Bandyopadhyay U, Das, Banerjee RK. Reactive oxygen species: Oxidative damage and pathogenesis. Current sci. 1999; 77: 658.
43. Fridovich I. Superoxide radical as an endogenous toxicants. Ann Rev Pharmacol Toxicol, 1983; 23: 239-18.
44. Niwa Y, Iizawa O, Ishimoto K, Akamatsu CH, Kanoh T. Age dependent basal level and induction capacity of copper, zinc and manganese superoxide dismutase and other scavenging enzyme activities in leucocytes from young and elderly adults. Am J Pathol, 1993; 143(1): 312-9.
45. Moosavi-Movahedi, Wilkinson AE, Jones MN. Interaction of *Aspergillus niger* catalase with Sodium n dodocyl sulphate. Pure and Applied Chem, 1987; 66(1): 71-5.
46. Liczmanski AE. Toksyeznosctlenu II Mechanizmy obronne. Post Biochem, 1988; 34: 293-8.
47. Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupta P, Maiti BC, Maity TK. Evaluation of hepato-protective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R. Br. on peracetamol induced hepato-toxicity in rats. Tropical J. of Pharmaceutical Research, 2007; 6 (3): 755-11.
48. Hyunghee LA, Frank JGB, Michung YA. Ginsenoside Rf, a component of ginseng, regulates lipoprotein metabolism through peroxisomes proliferate or activated receptors. Biochemical and Biophysical Research Communication, 2006; 339: 196-8.
49. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human diseases: an overview. Methods Enzymol, 1990; 186: 1-85.

50. Torel J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochemistry, 1986; 25: 383– 3.
51. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercitin in lipid peroxidation. Biochemical Pharmacology, 1989; 38: 1763– 7.
52. Robak J, Grygleuski RJ. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology, 1988; 37: 837–5.