ISSN No:-2456-2165

Total Protein, Protein Carbonyl, Glutathione and Enzymatic Antioxidants in Brain Sub Regions of Male Wistar Rat after Dietary Zinc Deficiency Antioxidant Study in Brain Sub Regions after Dietary Zinc Deficiency

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Abstract:- Micronutrient zinc is an essential trace element being crucial for maintaining the normal biological functions. Pre-pubertal male Wistar rats (35-45 gm) were divided into 4 groups: (1) Negative Control (NC) - fed with standard feed (Ashirwad Industries, Chandigarh) and tap water was provided ad libitum. (2) Zinc Control (ZC) - Fed with diet containing 100 µg/gm zinc. Tap water was provided ad libitum. (3) Pair fed (PF) - Fed with 100 µg/gm Zn diet but the amount of feed given was equal to the feed consumed by Zinc deficient group. Tap water was provided ad libitum. (4) Zinc Deficient (ZD) - fed with 1.00 µg/gm zinc diet and demineralized water was provided ad libitum. Experiments were conducted for 2-, 4- and 6- weeks. Total protein, protein carbonyl, glutathione, superoxide dismutase (Total SOD, Cu-Zn SOD and MnSOD) and catalase were assessed. Results revealed significant (P<0.05) decrease in total protein, glutathione and Cu-ZnSOD activity after dietary zinc deficiency while concentration of protein carbonyl and activities of total SOD, MnSOD and catalase increased. Study indicates that insufficient dietary zinc results in alteration of total protein, glutathione, SOD and catalase with consequent formation of protein oxidation (carbonyl) indicating the generation of reactive oxygen species leading to oxidative stress which would impede the functional aspects of brain sub-regions.

Keywords:- *Zinc deficiency, Brain sub regions, total protein, protein carbonyl, glutathione, superoxide dismutase and catalase.*

I. INTRODUCTION

Nutritional zinc deficiency of zinc has affected nearly 2 billion people in developing countries [1] [2] with prevalence in low and middle income countries [3] and reported to be four times more common in malnourished children [4]. According to Food and Health Organization one third of the population resides in countries having high zinc deficiency with India being one of them. Myriads of biological functions involve zinc which has been widely reported.2800 human proteins are potentially zinc-binding in vivo, corresponding to 10% of all human proteome [5].

Authors [6] [7] [8] [9] [10] [11] [12] [13] reported that normal functioning of brain requires zinc for neuronal proliferation, neurotransmission, neuromodulator, synaptic plasticity, learning and memory. In various regions of brain neurons contain free zinc ion with high concentration in neurons of cortex, amygdala, olfactory bulb, hippocampus and neocortex region [14] [15] [16]. Zinc in brain as well as its circulation maintains the blood brain barrier and it is the integrity of this barrier which is responsible for maintaining zinc homeostasis especially during pathological state [17]. Various aspects of zinc transporters have been discussed [18] [19] [20] [21] [22] with ZnT2 transporter localized in choroidal epithelia and freshly isolated plexus tissues in choroidal Z310 cells [23]. ZnT3 loads zinc into brain synaptic vesicles of a subset of glutamate neurons [24].

Under normal conditions antioxidant decreases the perturbations caused due to generation of ROS/RNS either by functioning as a catalytic agent, reducing, quenching/scavenging reactive species or by protecting the molecules [25]. Carbonylation – protein modification usually affecting lysine, arginine and proline is an indicator of oxidative protein damage and has also been associated with neurodegenerative disease [26] [27] [28] [29].Glutathione(GSH) – a tripeptide thiol and an antioxidant has been reported in neurons although its concentration is less than that of astrocytes [30] with Cys being an important substrate for synthesis of GSH in neurons [31] while astrocytes utilizes both Cys or cystine for synthesis [32]. It antagonizes the concentration of reactive oxygen species while efficiently triggering the antioxidant defense mechanisms [33] .Superoxide dismutase (EC

1.15.1.1) an antioxidant with three distinct class (E-SOD; Cu-Zn SOD and MnSOD) each having distinct sub cellular localization catalyzing the conversion of superoxide radical into molecular oxygen and hydrogen peroxide functioning as a major cellular defense against O2• - and peroxynitrite [34] [35]. The decomposition of hydrogen peroxide to molecular oxygen and water is catalyzed by catalase, thus bringing to an end the detoxifying reaction started by SOD [36]. The study evaluates total protein, protein carbonyl, glutathione and enzymatic antioxidants (SOD and catalase) in Wistar rat brain sub-regions after dietary zinc deficiency.

II. MATERIALS AND METHODS

A. Synthetic diet

The diets were formulated by using ICN Research Diet Protocol (1999). The ingredients of the diet (per kg diet) were- Egg white/ albumin- 180 gm, Corn oil- 100 gm, Corn starch- 443 gm, Sucrose- 200 gm, Cellulose -30 gm, Choline chloride- 2 gm, DL- methionine- 7 gm, AIN- 76 salt mixture- 35 gm, AIN- 76C vitamin-antibiotic mixture- 10 gm.

B. Basal diet

Zinc contents of basal diet from each lot was estimated at 213.9 nm in air-acetylene flame n GBC 902 Atomic Absorption Spectrophotometer and zinc concentration adjusted to 1.0 µg/gm and 100 µg/gm by addition of appropriate amounts of zinc sulphate.

C. Experimental Protocol

Male *Rattus norvegicus* (Wistar rats) (30-40 days of age,pre-pubertal; 35-45 gm) were divided into four groups with 10 animals in each group: Group 1: Negative control – fed with standard feed (Ashirwad Industries, Chandigarh) and tap water was provided ad libitum .Group 2 : Zinc control / ZC group- Fed with diet containing 100 µg/gm zinc. Tap water was provided ad libitum. (c) Group 3: Pair-fed group / PF group- Fed with 100 µg/gm Zn diet but the amount of feed given was equal to the feed consumed by Zinc deficient group the previous day to account for stress and starvation effect caused due to low diet intake. Tap water was provided ad libitum (d) Group 4: Zinc deficient / ZD group- animals were fed with 1.00 µg/gm zinc diet and demineralized water was provided ad libitum. Male Wistar rats were housed in isolation in polypropylene cages with stainless steel grills. Cages and water bottles were washed with detergent solution, demineralized water and finally rinsed in 1% EDTA solution prepared in demineralized water for removal of zinc traces. Experiments were conducted for 2-, 4- and 6-weeks and approved by University Department Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA (1678/GO/Re/S/12)].

After completion of experiments, animals were anesthetized; brain excised, cleaned off of extraneous tissue, weighed on Sartorius BS 124 S electronic balance and processed for biochemical studies.

D. Biochemical Studies

Brain sub –regions were homogenized in Remi tissue homogenizer and centrifuged at 2000 rpm (Remi –R8C laboratory centrifuge) or 10,000 rpm (Sigma refrigerated high –speed centrifuge 4 K15 using 18015 rotar) to remove cell debris. The absorbance was read on Systronics spectrophotometer 169 (Serial No. 827). Total protein [37], Protein carbonyl [38], Glutathione (GSH) [39] [40], Superoxide Dismutase (SOD)- (i) Total SOD [41] (ii) Cu-Zn SOD [42] and (iii) Mn-SOD (Total SOD – Cu-Zn SOD) and Catalase [43] were analyzed.

E. Statistical Analysis

Data expressed as mean ± SEM. One way Analysis of Variance (ANOVA) was carried out separately for 2-, 4- and 6- week experimental groups and post- hoc test (Tukey's Multiple Comparison test) was applied if the difference was found to be significant. Data were analyzed using Graph Pad Prism Version 7.0e. $P < 0.05$ was examined to be significant.

III. RESULTS

There was a significant ($P<0.05$) decrease in total protein concentration after 2-, 4- and 6- weeks of experiment when zinc deficient (ZD) groups were compared with their respective controls(NC and ZC) as well as pair fed (PF) groups. Decrease was also significant when pair fed group of 2-, 4- and 6 week experiment were compared with respective NC and ZC groups (Table 1).

Protein carbonyl concentration increased significantly ($P<0.05$) after 2-, 4- and 6- weeks of dietary zinc deficiency when compared with their respective controls (NC and ZC) and paired groups. Significant increase was evident when pairfed groups were compared with respective controls (NC and ZC) (Table 2).

Glutathione concentration decreased significantly ($P<0.05$) after 2-, 4- and 6- weeks of experiment when zinc deficient groups were compared with their respective controls(NC and ZC) and pair fed groups. Decrease was also significant when pair fed group of 2- , 4- and 6 week experiment were compared with respective NC and ZC groups (Table 3).

After 2-, 4- and 6- weeks of dietary zinc deficiency total SOD activity increased significantly ($P<0.05$) when zinc deficient groups were compared with their respective NC, ZC and PF groups. Comparison of three experimental PF groups with respective NC and ZC groups revealed significant increase ($P<0.05$) (Table 4). Cu-Zn SOD activity decreased significantly($P<0.05$) after 2-, 4- and 6- weeks of dietary zinc deficiency when zinc deficient groups were compared with their respective NC,ZC and PF groups. Decrease was also significant ($P<0.05$) when PF groups(2-, 4- and 6- weeks experiment) were compared with their respective NC and ZC groups(Table 5). Mn SOD activity increased significantly ($P<0.05$) when zinc deficient groups (2-,4- and 6- weeks experiment) were compared with their respective NC,ZC and PF groups. Similar pattern of increase was evident when pair fed groups of the three experiments

was compared with their respective NC and ZC groups (Table 6).

Catalase activity increased significantly ($P<0.05$) when zinc deficient groups of 2-,4- and 6- weeks experiment were compared with their respective NC,ZC and PF groups. Similar pattern of increase was observed when pair fed groups of 2-, 4- and 6 weeks experiment was compared with their respective NC and ZC groups (Table 7).

IV. DISCUSSION

Nutritional zinc deficiency causes oxidative stress which implies critical imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms in the body [44]. It is a disturbance in the prooxidant-antioxidant balance causing possible cellular damage [45]. Animal studies have revealed that zinc is essential for the synthesis of nucleic acids [46] and proteins [47] and is crucial in protein metabolism [48]. Oxidative stress generated apoptosis cascade which causes ROS increases and/or antioxidant decreases, disruption of intracellular redox homeostasis and irreversible oxidative modifications of lipid and protein [49]. Reference [50] reported disruption in protein metabolism in male rats after dietary zinc deficiency. Further, reference [51] suggested that the effects of zinc deficiency on protein metabolism is not due to (i) inability to form functional mRNA's/monosomes or (ii) overall decline in protein synthesis but it appears to selectively to decrease synthesis of some proteins(repression of genes) and increasing others(activation of genes). Decreased serum hormonal (FSH,LH and testosterone) profile was reported [52]. Decline in total protein in brain sub–regions after dietary zinc deficiency suggests deterioration of enzymes, proteins and hormones. The evident decrement in total protein in pairfed groups could be the outcome of stress and starvation.

Carbonylation causes major changes in protein structure as well as function leading to formation of protease resistant protein aggregates which would affect the cells making it susceptible to oxidative stress [53], Carbonyl stress increases chemical modification of proteins leading to oxidative stress and tissue damage [54]. Accumulation of protein carbonyl which increased progressively with duration of zinc deficiency reflects loss of structural /catalytic function of proteins disrupting the cellular functions.

Glutathione, an antioxidant is the most powerful weapon against oxidative and nitrosative stress that result due to excess of free radical generation [55]. A significant decrease in the reducing capacity of glutathione has been used as a marker of oxidative stress in rats and other animal models [56]. Stress caused decrement in GSH and free sulfhydryl groups [57] [58]. In present study glutathione concentration in brain sub-regions decreased significantly which can be contributed to oxidative stress after dietary zinc deficiency and due to stress in pair-fed groups.Several authors [59] [60] [61] in their studies observed enhanced oxidative damage –membrane lipid peroxidation, protein carbonylation as well as DNA breakage due to loss of superoxide dismutase activity. Variable degree of oxidative damage in the cytoplasm, nucleus and mitochondria using Sod1−/− mice [60] and Sod2+/− mutants [62] [59] were observed. Using *nestin – Cre-loxp* system brain specific SOD2-deficient mice (B-SOD2$^{-/-}$) was generated which reveled decreased mitochondrial complex II activity ,growth retardation, spongiform neurodegeneration in motor cortex, hippocampus as well as brain stem accompanied by gliosis [63]. Neuronal nitration on account of endogenous production of superoxide radical/ peroxynitrite can affect the expression of Cu-Zn SOD [64]. Increased total SOD and Mn SOD activity was observed after deficiency indicative of altered expression /catalytic activity. Overexpression of total SOD and MnSOD probably in response to oxidative stress to protect the brain cells from degeneration on account of accumulation of superoxide anion radical and peroxynitrite formation which in turn would increase H_2O_2 concentration promoting production of hydroxyl radical. Moreover, Cu-Zn SOD activity in brain sub regions decreased in the present study which could probably be due to (i) low availability of copper to maintain its expression (ii) oxidative stress as well as (ii) neuronal nitration. Stress and starvation (due to limited food provided) in pair fed groups also exhibited altered activity.Catalase mRNA was demonstrated in brain neurons and glia cells [65]. H_2O_2 is also generated by protonated form of superoxide (hydroxyperoxyl radical) which reacts with iron /copper to generate HO • (Fenton reaction / Haber Weiss reaction) which is detrimental to the cells. High activity of catalase in brain sub regions was observed in the present study to lower the noxious H_2O_2 which can be due to oxidative stress caused by dietary zinc deficiency.

ISSN No:-2456-2165

V. TABLES

Groups	Total Protein (mg /g)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	43.98± 0.003	46.52± 0.002	42.45± 0.003	45.42± 0.004
2ZC	43.97± 0.004	46.51± 0.003	42.44± 0.005	45.42± 0.005
2PF	42.36± 0.002^{b*d*}	45.65± 0.004^{b*d*}	40.36± 0.002^{b*d*}	44.04± 0.003^{b*d*}
2ZD	39.67± 0.005^{c*e*f*}	42.32± 0.002^{c*e*f*}	38.25± 0.003^{c*e*f*}	41.37± 0.004^{c*e*f*}
4NC	48.56± 0.002	45.65± 0.001	61.56± 0.004	63.91± 0.005
4ZC	48.55± 0.004	45.66± 0.002	61.56± 0.003	63.91± 0.006
4PF	46.69± 0.003 ^{b*d*}	43.25± 0.003 ^{b*d*}	59.41± 0.003 ^{b*d*}	61.41± 0.004 ^{b*d*}
4ZD	42.56± 0.001^{c*e*f*}	39.35± 0.004^{c*e*f*}	56.36± 0.005^{c*e*f*}	58.76± 0.004^{c*e*f*}
6NC	65.27± 0.003	68.96± 0.005	70.96± 0.002	69.93± 0.003
6ZC	65.28± 0.004	68.95± 0.003	70.95± 0.004	69.93± 0.007
6PF	64.69± 0.003 ^{b*d*}	65.63± 0.002 ^{b*d*}	68.05± 0.002 ^{b*d*}	65.58± 0.004^{b*d*}
6ZD	60.48± 0.005^{c*e*f*}	61.52± 0.004^{c*e*f*}	64.56± 0.003^{c*e*f*}	61.02± 0.002^{c*e*f*}

Table 1: Total protein in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where, * P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

Groups	Protein Carbonyl (nmoles/mg protein)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	1.61±0.003	1.66±0.001	1.44±0.008	1.66±0.004
2ZC	1.61±0.004	1.66±0.009	1.44±0.007	1.66±0.005
2PF	1.69±0.003 ^{b*d*}	1.76±0.003 ^{b*d*}	1.69±0.001 ^{b*d*}	1.85±0.003^{b*d*}
2ZD	1.81±0.003 ^{c*e*f*}	1.96±0.004 ^{c*e*f*}	1.75±0.001 ^{c*e*f*}	1.99±0.003 ^{c*e*f*}
4NC	1.72±0.001	1.82±0.001	1.57±0.005	1.85±0.005
4ZC	1.72±0.004	1.82±0.003	1.57±0.007	1.86±0.005
4PF	1.80±0.002 ^{b*d*}	1.90±0.009 ^{b*d*}	1.82±0.001 ^{b*d*}	1.95±0.003 ^{b*d*}
4ZD	2.05±0.009 ^{c*e*f*}	2.04±0.009 ^{c*e*f*}	1.92±0.004 ^{c*e*f*}	2.12±0.003 ^{c*e*f*}
6NC	1.94±0.009	1.92±0.009	1.65±0.004	1.91±0.004
6ZC	1.93±0.001	1.92±0.007	1.65±0.009	1.91±0.009
6PF	1.99±0.009 ^{b*d*}	2.04±0.009 ^{b*d*}	1.92±0.001 ^{b*d*}	2.04±0.003 ^{b*d*}
6ZD	2.24±0.008 ^{c*e*f*}	2.14±0.008 ^{c*e*f*}	2.08±0.002 ^{c*e*f*}	2.21±0.003 ^{c*e*f*}

Table 2: Protein carbonyl in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where, * P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

ISSN No:-2456-2165

Groups	Glutathione (GSH) (µM GSH /mg)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	28.75± 0.003	30.66± 0.001	25.49± 0.002	26.92± 0.004
2ZC	28.75± 0.002	30.31± 0.002	25.92± 0.001	26.92± 0.004
2PF	27.31± 0.005 b*d*	29.89± 0.004 b*d*	24.67± 0.002 b*d*	24.25± 0.005 b*d*
2ZD	25.95± 0.004 c*e*f*	28.11± 0.002 c*e*f*	22.89± 0.005 c*e*f*	23.75± 0.003 c*e*f*
4NC	36.21± 0.001	39.92± 0.001	33.44± 0.003	34.73± 0.003
4ZC	36.38± 0.002	39.23± 0.004	33.96± 0.004	34.73± 0.003
4PF	34.98± 0.003 b*d*	37.51± 0.005 b*d*	31.62± 0.003 b*d*	32.11± 0.004 b*d*
4ZD	32.62± 0.001 c*e*f*	35.87± 0.003 c*e*f*	28.01± 0.005 c*e*f*	27.96± 0.003 c*e*f*
6NC	53.25± 0.003	56.62± 0.004	49.34± 0.003	50.18± 0.003
6ZC	53.47± 0.004	56.46± 0.002	49.85± 0.004	50.18± 0.004
6PF	50.51± 0.004 b*d*	53.33± 0.004 b*d*	46.21± 0.002 b*d*	47.23± 0.005 b*d*
6ZD	46.32± 0.005 c*e*f*	47.15± 0.002 c*e*f*	40.63± 0.003 c*e*f*	40.01± 0.004 c*e*f*

Table 3: Glutathione in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where, * P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

Groups	Total SOD (Units mg protein /hr)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	10.36±0.018	9.76±0.027	6.63±0.015	10.37±0.015
2ZC	7.79±0.026	9.78±0.014	6.63±0.012	10.36±0.017
2PF	8.55±0.018 b*d*	10.165±0.015 b*d*	7.55±0.019 b*d*	11.93±0.011 b*d*
2ZD	10.12±0.015 c*e*f*	12.11±0.018 c*e*f*	8.95±0.020 c*e*f*	14.10±0.034 c*e*f*
4NC	8.78±0.014	10.47±0.019	7.02±0.011	15.13±0.014
4ZC	8.78±0.013	10.48±0.013	7.02±0.014	15.13±0.011
4PF	9.78±0.015 b*d*	11.34±0.019 b*d*	9.32±0.010 b*d*	18.11±0.018 b*d*
4ZD	14.18±0.017 c*e*f*	15.19±0.014 c*e*f*	12.18±0.020 c*e*f*	22.30±0.022 c*e*f*
6NC	9.46±0.016	14.46±0.021	9.27±0.019	29.12±0.014
6ZC	9.47±0.016	14.46±0.015	9.27±0.021	29.14±0.017
6PF	11.14±0.018 b*d*	16.00±0.020 b*d*	10.77±0.023 b*d*	34.99±0.333 b*d*
6ZD	18.92±0.040 c*e*f*	23.34±0.021 c*e*f*	16.13±0.020 c*e*f*	40.11±0.015 c*e*f*

Table 4: total Superoxide dismutase (SOD) in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where,
* P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

ISSN No:-2456-2165

Groups	Cu-Zn SOD (Units mg protein /hr)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	4.73±0.016	5.22±0.014	4.53±0.011	6.928±0.013
2ZC	4.75±0.018	5.22±0.021	4.51±0.012	6.937±0.012
2PF	4.23±0.014 ^{b*d*}	4.63±0.018 ^{b*d*}	3.65±0.017 ^{b*d*}	5.414±0.013^{b*d*}
2ZD	3.36±0.020 ^{c*e*f*}	3.26±0.022 ^{c*e*f*}	2.89±0.013 ^{c*e*f*}	5.041±0.017^{c*e*f*}
4NC	5.95±0.019	6.89±0.013	5.51±0.012	7.234±0.012
4ZC	5.94±0.021	6.88±0.018	5.51±0.014	7.251±0.017
4PF	3.87±0.012 ^{b*d*}	3.75±0.011 ^{b*d*}	2.92±0.013 ^{b*d*}	4.928±0.012^{b*d*}
4ZD	2.99±0.019 ^{c*e*f*}	2.85±0.014 ^{c*e*f*}	1.98±0.015 ^{c*e*f*}	3.456±0.017^{c*e*f*}
6NC	7.88±0.013	10.72±0.022	7.62±0.019	14.09±0.014
6ZC	7.87±0.010	10.71±0.023	7.62±0.010	14.0±9±0.020
6PF	3.02±0.020 ^{b*d*}	2.98±0.022 ^{b*d*}	2.12±0.014 ^{b*d*}	3.528±0.011^{b*d*}
6ZD	1.98±0.016 ^{c*e*f*}	1.63±0.019 ^{c*e*f*}	0.58±0.012 ^{c*e*f*}	2.020±0.022^{c*e*f*}

Table 5: cu-zn Superoxide dismutase (SOD) in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where, * P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

Groups	Mn SOD (Units mg protein /hr)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	3.06±0.014	4.55±0.014	2.10±0.014	3.45±0.017
2ZC	3.09±0.023	4.57±0.014	2.12±0.016	3.44±0.021
2PF	4.34±0.018 ^{b*d*}	5.54±0.013 ^{b*d*}	3.90±0.014 ^{b*d*}	6.54±0.014^{b*d*}
2ZD	6.77±0.014 ^{c*e*f*}	8.84±0.017 ^{c*e*f*}	6.07±0.017 ^{c*e*f*}	9.07±0.013^{c*e*f*}
4NC	2.85±0.015	3.59±0.016	1.51±0.019	7.90±0.012
4ZC	2.85±0.016	3.59±0.021	1.51±0.020	7.89±0.015
4PF	5.91±0.017 ^{b*d*}	7.59±0.016 ^{b*d*}	6.40±0.020 ^{b*d*}	13.20±0.015^{b*d*}
4ZD	11.20±0.018 ^{c*e*f*}	12.35±0.015 ^{c*e*f*}	10.20±0.015 ^{c*e*f*}	18.86±0.012^{c*e*f*}
6NC	1.60±0.014	3.75±0.014	1.66±0.015	15.06±0.018
6ZC	1.61±0.012	3.76±0.017	1.65±0.018	15.04±0.017
6PF	8.13±0.017 ^{b*d*}	13.00±0.029 ^{b*d*}	8.65±0.018 ^{b*d*}	31.80±0.013^{b*d*}
6ZD	16.95±0.031 ^{c*e*f*}	21.71±0.021 ^{c*e*f*}	15.56±0.021 ^{c*e*f*}	38.10±0.013^{c*e*f*}

Table 6: Manganese Superoxide dismutase (SOD) in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where, * P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

Groups	Catalase (Kat.f)			
	Frontal	***Parietal***	***Occipital***	***Cerebellum***
2NC	0.112±0.006	0.131±0.001	0.102±0.001	0.177±0.002
2ZC	0.112±0.002	0.131±0.001	0.102±0.001	0.177±0.004
2PF	0.113±0.001 ^{b*d*}	0.132±0.004 ^{b*d*}	0.102±0.001 ^{b*d*}	0.178±0.001 ^{b*d*}
2ZD	0.131±0.002 ^{c*e*f*}	0.145±0.002 ^{c*e*f*}	0.109±0.002 ^{c*e*f*}	0.196±0.007^{c*e*f*}
4NC	0.133±0.001	0.133±0.001	0.102±0.005	0.182±0.008
4ZC	0.132±0.001	0.132±0.001	0.102±0.005	0.182±0.003
4PF	0.135±0.008 ^{b*d*}	0.135±0.008 ^{b*d*}	0.103±0.007 ^{b*d*}	0.188±0.006^{b*d*}
4ZD	0.171±0.005 ^{c*e*f*}	0.171±0.005 ^{c*e*f*}	0.112±0.006 ^{c*e*f*}	0.272±0.005^{c*e*f*}
6NC	0.117±0.001	0.134±0.001	0.103±0.004	0.183±0.006
6ZC	0.1117±0.001	0.134±0.001	0.103±0.001	0.1836±0.002
6PF	0.119±0.001 ^{b*d*}	0.141±0.005 ^{b*d*}	0.105±0.009 ^{b*d*}	0.1911±0.001^{b*d*}
6ZD	0.231±0.009 ^{c*e*f*}	0.271±0.001 ^{c*e*f*}	0.121±0.005 ^{c*e*f*}	0.5117±0.003^{c*e*f*}

Table 7: catalase in brain sub-regions of Wistar rat after 2-,4- and 6- weeks of dietary zinc deficieny (MEAN ± SEM)

Where,
* P<0.05 Significant
F= Frontal lobe of the brain, P= Parietal lobe, O= Occipital lobe & C= Cerebellum,
a= NC Vs ZC, b = NC Vs PF, c= NC Vs ZD, d=ZC Vs PF, e =ZC Vs ZD, f =PF Vs ZD
Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups

ACKNOWLEDGMENT

Ms. Yukti Kanwar thanks University Grants Commission, New Delhi, for the award of the Non NET fellowship. The authors gratefully acknowledge Department of Zoology, Centre for Advanced Studies, University of Rajasthan, Jaipur, India, for providing necessary facilities.

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