Effects of Zinc Supplementation in Neonatal Hypothyroidism and Cerebellar Distortion Induced by Maternal Carbimazole

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ABSTRACT---- ***The aim of this study is to investigate the protective role of Zn on thyroid function and cerebellum impairments induced by carbimazole (CMI) in neonats at PND 28. Animals were divided into four groups: group I acts as control; group II received carbimazole(1.35mg/kg.b.wCMI) only; group III received Zn as zinc sulphate (3mg/kg.b.w) and group IV received Zn and CMI. The administration of maternal CMI caused significant decrease in serum T4, T3 and growth hormone (GH) levels and significant increase in serum TSH levels in both dams and their newborns relative to control group. While in Zinc-hypothyroid group, a reverse pattern was noticed for all previous parameters comparing with hypothyroid group. Maternal CMI administration caused significant decrease in GSH, SOD, GPX, CAT and t-SH of cerebellum and increased prooxidant levels including MDA, H_2O_2 and NO while Zn administration to hypothyroid dams reversed this effect. CMI administration caused some degeneration and deformation innewborns cerebellum while zinc supplementation to CMI group enhanced structure, length, number and size of purkinje cells, also increase size of the granular layer.Our results indicated thatzinc has an improvement role on THs levels and histoarchiture of cerebellumdue to its antioxidant role.***

Keywords--- Carbimazole, zinc, thyroid hormones, antioxidant, prooxidant

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1-INTRODUCTION

Thyroid hormones (THs) play a curical role in the development and physiological functioning of the central nervous system (CNS) (Dipaola*et al.,* 2010; Artis*et al.,* 2012, Ahmed and Incerpi, 2013, Berbel*et al*., 2014). One of the most important functions performed by THs is the tight regulation of cellular oxgen consumption and consequent generation of reactive oxgen species (ROS) in several tissues (Mircescu, 2008; Petrulea*et al.,* 2009).

Antithyroid drugs (Hasebe*et al.,* 2008) have various effects on the thyroid function and brain development of experimental animals. CMI (methimazole (MMI) derivative) is an oral antithyroid agent used in treatment of hyperthyroidisim (Sunil *et al.*,2013). Zaidi*et al.* (2004) reported that CMI administration during pregnancy and lactation resulted into alterations in the neonatal thyroid microstructure. This thyroid dysfunction associatedwith neurological abnormalities (Pillhatsch*et al*., 2011; Cortes *et al.,* 2012).In addition, hypothyroidism during the fetal and/ or the neonatal period results in several histological changes in the neonatal rat brain.

On the other hand, normal thyroid status is dependent on the presence of many trace elements for both the synthesis and metabolism of THs (Nazifi*et al.,* 2009). THs interaction with Zn has been extensively investigated (Leblondel*et al* 1992; Freake*et al*.,2001). Some endocrine disorders and deleterious effects on brain function observed in humans, especially during the growth, are accompanied by Zn deficiency (Kwan *et al.,* 1995; Geogrieff, 2007). The fact that Zn deficiency has a suppressing effect on THs, whereas Zn supplementation has an opposite effect (Baltaci*et al.,* 2003, 2004) indicated to a relationship between Zn and THs. It has been reported that THs levels were increased in hypothyroid patient fed with Zn supplement (Kandhro*et al.,* 2008).Zn is one of the mostabundant divalent metal ions in the CNS, which is important for maintaining cognitive function (Gower-Winter and Levenson, 2012). In addition Zn plays antioxidant roles, Zn deficiency is commonly related to increase in oxidants, cellular damage and modulation of antioxidant defenses (Oteiza, 2012), whereas Zn supplementation can counteract these effects in several malignancies and diseases (Chasapis*et al.,* 2012).

The study was performed to determine potency of Zn treatment against maternal hypothyroidism induced by CMI from gestational day (GD) 13 to postnatal day (PND) 28 on thyroid functions, proxidant/ antioxidants levels and histological structure of neonatal cerebellum.

2-MATERIALS AND METHODS

2.1 Experimental animals:

The present study was carried out on 90 albino rats, 60 mature virgin female weighting 180 ± 200 gm and 30 mature males. They were obtained from the National Institute of Ophthalmology, Giza, Egypt. The adult rats were kept under observation for 2 weeks in the department animal house to exclude any intercurrent infection and to acclimatize the new conditions. The culled animals were marked, housed in metal (stainless steel) separate bottom cages with well ventilation at normal atmospheric temperature (23±2ºc) and fed on standard rodent pellet diet manufactured by the Egyptian Company for oil and soap as well as some vegetables as a source of vitamins. Tap water was used for drinking *ad libitum* and these animals were exposed to constant daily dark/light periods of 12 hour (hr) each (lights on at 06:00 hr) and 50±5% relative humidity. Generally, the protocol follows the general guidelines of animal care and the recommendation of the Canadian Council on Animal Care (CCAC; Olfert*et al.* 1993). All efforts were made to minimize the number of animals used and their suffering.

2.2 Animal grouping:

Sixteen adult female rats from GD13 to PND 28 were allocated into four groups 15 rat/ group, all groups received standard diet of97 mg Zn/kg feed (Baltaci et al., 2004)asfollows:Control group received tap water. Hypothyroid group orally administrated CMI. (1.35mg/kg.b.w)(Sakr et al.,2011).Zn group intraperitonealy injected with 3mg/kg.b.wof zinc as zinc sulphate (Baltaci et al., 2004) Zn- hypothyroid group received both Zn and CMI.

After the pregnancy, the decapitation of dams and their newborns was done at the PND 28 under mild diethyl ether anesthesia. The blood samples were taken from jugular vein centrifuged at 3000 r.p.m for 20 min and kept at – 30ºC. Cerebellum was separated from the skull and the immediatelyhomogenized in isotonic solution (0.9%Nacl). On the other hand, neonatal cerebellums were removed immediately after a rapid anaesthesia, dropped into the fixative of choice for general histological structure (haematoxylin and eosin stain; Bancroft and Stevens, 1982).

2.3 Determination of serum THs, TSH and growth hormone (GH):

Estimation of T4, T3 and TSHof both mothers and their newborns of all groups were determined in blood serum by ELISA using commercial kit (Cal biotech INC (CBI), USA Company) according to the method of Maes *et al.* (1997), Thaukr *et al.* (1997) and Burger and Patel. (1977), respectively. Neonatals GH also determined in blood serum by ELISA kit using commercial kit (DRG International, USA) according to the method of Baglia *et al.* (1992).These measurements were performed in biochemistry department at faculty of medicine, Cairo University.

2.4 Estimation of neonatal tissue zinc level:

Zinc levels of neonats cerebrum were measured by flame atomic absorption spectrophotometer with zemman background (Thermo elemental M-6 Type).

2.5 Estimation of neonatal cerebellar proxidants/ antioxidants levels:

Determination of proxidants and antioxidant defense system were estimated in our laboratory. Glutathione (GSH), glutathione-S-transferase (GST), t-SH, GPx, SOD, CAT, activities were measured according to the method of Koster *et al.* (1986), Jollow *et al.*, 1974, Pinto and Bartley (1989), Habig *et al.*, 1974,Shukla *et al.*, 1987,Aebi, 1984 respectively. Also, the lipid peroxidation LPO, nitric oxide (NO) and hydrogen peroxide (H_2O_2) concentration were estimated according to the method ofDraper and Hadley (1990), Montgomry and Dymock, 1961 and Aebi, 1984,respectively**.**

2.6 Haematoxylin and eosin stain (Bancroft and Stevens, 1982):

Cerebellum of each group were dehydrated in ascending series of ethyl alcohol then placed in xylene for clearance. The tissues were placed in paraffin wax and cut serially at 5µm thickness. These sections were de-waxed, hydrated and stained in Mayer's haemalum solution for 3 min. These sections were stained in eosin for one minute. The sections were washed in running tap water and dehydrated in ascending series of ethyl alcohol. Clearing in xylene and mounting in Canada balsam were done.

2.7Statistical analysis:

Data were analyzed using one-way ANOVA (PC-STAT) University of Georgia (1985) followed by LSD analysis to determine the main effects and compare the groups with each other. F-probability for each variable expresses the general effect between the groups. The data are presented as means ± S.E.M., and values of $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$ were considered statistically insignificant, significant highly significant and very highly significant, respectively.

3. RESULTS

3.1- Serum-hormonal levels (Table, 1&2)

In CMI group, T3 and T4 levels showed highly significant (LSD; $P<0.01$) decrease while, TSH serum levels were significantly increased in both dams and their neonats relative to control. In Zn group, maternal serum TSH and T3 levelsinsignificantly changed but T4 levels were significantly elevated (LSD;$P<0.01$), while neonats serum levels of these hormones not significantly (LSD; $P>0.05$) differ in comparing to control group.On the other hand, zinc supplementation into

maternal hypothyroid group significantly (LSD; P<0.01) increased T3 and T4 serum levels while, it significantly (LSD; P<0.01) reduced TSH serum levels of both dams and their neonats relative to hypothyroid group. Neonats GH serum levels were significantly decreased in CMI group but insignificantly differin Zn group relative to control group. In contrast, GH serum levels significantly elevated inCMI - Zn group relative to CMI group. Concerning one way ANOVA for all tested hormones, it was revealed that the general effect between groups was very highly significant (P<0.001) throughout the experimental period.

3.2. Zinc concentration in neonatal cerebellum: (Table 3)

The effect of zinc sulphate administration of hypothyroid dams on Zn levels in cerebellum illustrated in table 3. The data indicated that CMI administration significantly (LSD; p<0.01) reduced Zn level in cerebellum relative to control group also Zn administration to normal dams significantly (LSD; p<0.01) decreased newborns Zn levels in cerebellum comparing with control group. On the other hand, Zn administration to hypothyroid dams significantly (LSD; p>0.05) affected on Zn level in cerebellum relative to CMI group. Regarding one way ANOVA, the general effect between groups was very highly significant (P<0.001).

3.3.Biochemical markers in neonatal cerebellum (Table.4&5)

It is clear from table 4&5that, maternal administration of CMI caused significant (LSD; P<0.01) depletion in cerebellum antioxidant levels (GSH, t-SH,SOD GPX, and CAT) except for GST while it caused significant elevation for LPO, H_2O_2 and NO relative to control. On the other hand, Zn administration to hypothyroid dams caused significant (LSD; P<0.01) elevation in CAT, SOD, GPX, and GSH levelswhile GST and t-SH not significantly changed (LSD;P>0.05) with respect to CMI group. Also Zn exposure to hypothyroid dams caused significant (LSD; P<0.01) reduction in cerebellum content of LPO and H_2O_2 but not affected on NOrelative to CMI group. Maternal Zn group significantly (LSD; P<0.01) increased GSH, GST, LPO, H_2O_2 levels while it significant decreased levels of SOD, CAT, GPX, and t-SH in comparing to control group.Regarding one way ANOVA, the general effect between groups at PNDs 28 was very highly significant (P<0.001) for all tested during the experimental period.

3.4. Histology neonatal cerebellum as shown by H&E (Fig. 1):

In our study, the cerebellar cortex consists of four layers in normal and both treated rats; the superficial external granular layer (EGL), molecular layer (ML), Purkinje layer (PL) and deep internal granular layer (IGL). In control group, the molecular layer located before the internal granular layer (IGL). The purkinje (PC) layer comprised pear- shaped

neurons arranged in single row. A large nucleus was evident in each cell body of PCs with thick remarked cytoplasm coat. The IGL consisted of closely packed oval or round shaped neurons with large densely stained nucleus infilterated with intercellular space known as island as showing in(fig. $1A_1$, A_2). On the other hand, cerebellum of hypothyroid neonats have been shown some histopathological changes including reduction in the size and number of PCs with some degenerative changes, odematous areas between PC and IGL, and reduction in the number and size of neurons forming IGL with more intercellular space comparing with control (fig.$1B_1$-B_3). Neonats of Zn group showing normal cerebellum structure (fig. $1C_1$). Zn-hypothyroid group indicated improvement in number and size of PCs and number of neurons consisting IGL increased, more packed but still has some degenerations relative to CMI group (fig.$1D_1$).

Table 1. Effect of treatment with zinc against maternal hypothyroidism on thyroid hormones levels of dams.

Hormones / Groups	Mothers serum THs levels					
	T3 (pg/ml)		T4 (ng/dl)		TSH (nIU/ml)	
	mean ± SE	%	mean ± SE	%	mean ± SE	%
Control	3.583±0.170[a]	-	0.845±0.020[b]	-	52.833±0.446[c]	-
CMI	1.453±0.192[c]	-59.44%	0.745±0.029[c]	-11.83%	83.700±0.495[a]	+58.42%
ZN	3.900±0.073[a]	+8.84%	0.985±0.020[a]	+16.56%	49.567±0.403[c]	-6.18%
ZN + CMI	2.737±0.094[b]	+88.36%	0.900±0.089[b]	+20.80%	67.667±0.201[b]	-19.15%
LSD at 5% Level	0.417		0.061		11.868	
LSD at 1% Level	0.569		0.084		16.186	
F-probabilty	<0.001					

-Data in all tables are expressed as mean ± SE. Number of animals in each group is six

- Values which share the same superscript symbols are not significantly different.

- F – probability expresses the effect between groups, where P<0.001 is very highly significant

Table 2.Effect of treatment with zinc against maternal hypothyroidism on thyroid hormones levels and GH of newborns.

	T3 (pg/ml)		**T4 (ng/dl)**		**TSH (nIU/ml)**		**GH(ng/ml)**	
	mean ± SE	%	mean ± SE	%	mean ± SE	%	mean ± SE	%
Control	3.337±0306[a]	-	0.890±0.040[b]	-	36.567±0.223[c]	-	3.915±0.396[a]	-
CMI	1.377±0.229[b]	-58.73%	0.720±0.081[c]	-19.10%	93.333±0.314[a]	+155.23%	1.800±0.045[b]	-54.02%
ZN	3.400±0.203[a]	+1.88%	0.930±0.036[b]	+4.49%	37.367±0.407[c]	+2.18%	4.550±0.290[a]	+16.21%
ZN + CMI	2.913±0.044[a]	+111.54	1.160±0.063[a]	+61.11%	63.700±0.333[b]	-31.74%	3.983±0.059[a]	+121.27%
LSD at 5% Level	0.641		0.125		9.628		0.732	
LSD at 1% Level	0.874		0.170		13.131		0.999	
F-probability	<0.001							

Table 3. Effect of treatment with zinc against maternal hypothyroidism on newborns zinc concentration in cerebellum.

Region / Group	Zn in Cerebellum (µg/mgptn)	
	mean ± SE	%
Control	118.950± 1.185[a]	-
CMI	102.250± 0.753[b]	-14.039
ZN	66.100± 1.601[c]	-44.430
ZN + CMI	111.150± 0.604[a-b]	+8.704
LSD at 5% Level	11.534	
LSD at 1% Level	15.731	
F-probabilty	<0.001	

-Data in tables are expressed as mean ± SE. Number of animals in each group is six

- Values which share the same superscript symbols are not significantly different.

Cerebellum	GSH(nmol/mg)		t-SH(nmol/mg)		SOD(U/100mg)		GPX (U/100mg		GST(U/100mg)		CAT(u/100mg)	
	Mean± SE	%	Mean± SE	%	Mean± SE	%	Mean± SE	%	Mean± SE	%	Mean± SE	%
Control	10.91±0.358^{b}	-	1.137±0.160^{a}	-	80.844±0.840^{a}		0.198±0.0002^{a}	-	3.087±1.099^{b}	-	0.154±0.020^{a}	-
CMI	7.876±0.249^{c}	-27.809%	0.077±0.027^{b}	-93.175%	23.34±0.343^{c}	-71.129%	0.158±0.003^{c}	-20.202%	3.326±1.11^{b}	+7.7421%	0.098±0.059^{b}	-35.86%
ZN	22.068±0.673^{a}	+102.273%	0.094±0.016^{b}	-91.653%	70.023±0.840^{b}	-13.385%	0.187±0.0009^{b}	-5.555%	3.993±0.503^{a}	+29.348%	0.098±0.037^{b}	-36.12%
ZN+CMI	10.74±0.334^{b}	+36.363%	0.200±0.021^{b}	+158.505%	76.618±0.223^{a-b}	+228.269%	0.198±0.0003^{a}	+25.316%	3.207±0.421^{b}	-3.577%	0.144±0.086^{a}	+46.51%
LSD at 5% Level	1.282		0.244		7.014		0.0005		0.250		0.0166	
LSD at 1% Level	1.748		0.333		9.566		0.0007		0.341		0.0226	
F-probability	<0.001											

- F – probability expresses the effect between groups, where P<0.001 is very highly significant

Table 4. Effect of treatment with zinc against maternal hypothyroidism on newborns antioxidant concentrations GST (U/100mg),GSH(nmol/mg),t-SH(nmol/mg),SOD(nmol/100mg), GPX (U/100mg) and catalase activity(u/100mg) in newborns cerebellum.

Table 5.Effect of treatment with zinc against maternal hypothyroidism on newborns lipid peroxidation LPO (nmol MDA/100mg/hr), H2O2 (Mm/gm) and NO (µmol/gm).

Cerebellum	LPO (nmolMDA/100mg/hr)		H_2O_2 (mM/gm)		NO content (µmol/gm)	
	Mean± SE	%	Mean± SE	%	Mean± SE	%
Control	3.860±0.686^{b}	-	0.091±0.006^{c}	-	0.058±0.097^{b}	-
CMI	5.183±0.307^{a}	+34.27%	0.230±0.023^{a}	+152.74%	0.129±0.133^{a}	+121.35%
ZN	6.196±0.242^{a}	+60.51%	0.163±0.007^{b}	+79.12%	0.043±0.075^{b}	-25.83%
ZN+CMI	2.561±0.289^{c}	-50.58%	0.071±0.008^{c}	-69.13%	0.099±0.100^{a}	-23.35%
LSD at 5% Level	1.240		0.039		0.030	
LSD at 1% Level	1.692		0.053		0.041	
F- probability	<0.001					

-Data in tables are expressed as mean ± SE. Number of animals in each group is six

- Values which share the same superscript symbols are not significantly different.

- F – probability expresses the effect between groups, where P<0.001 is very highly significant

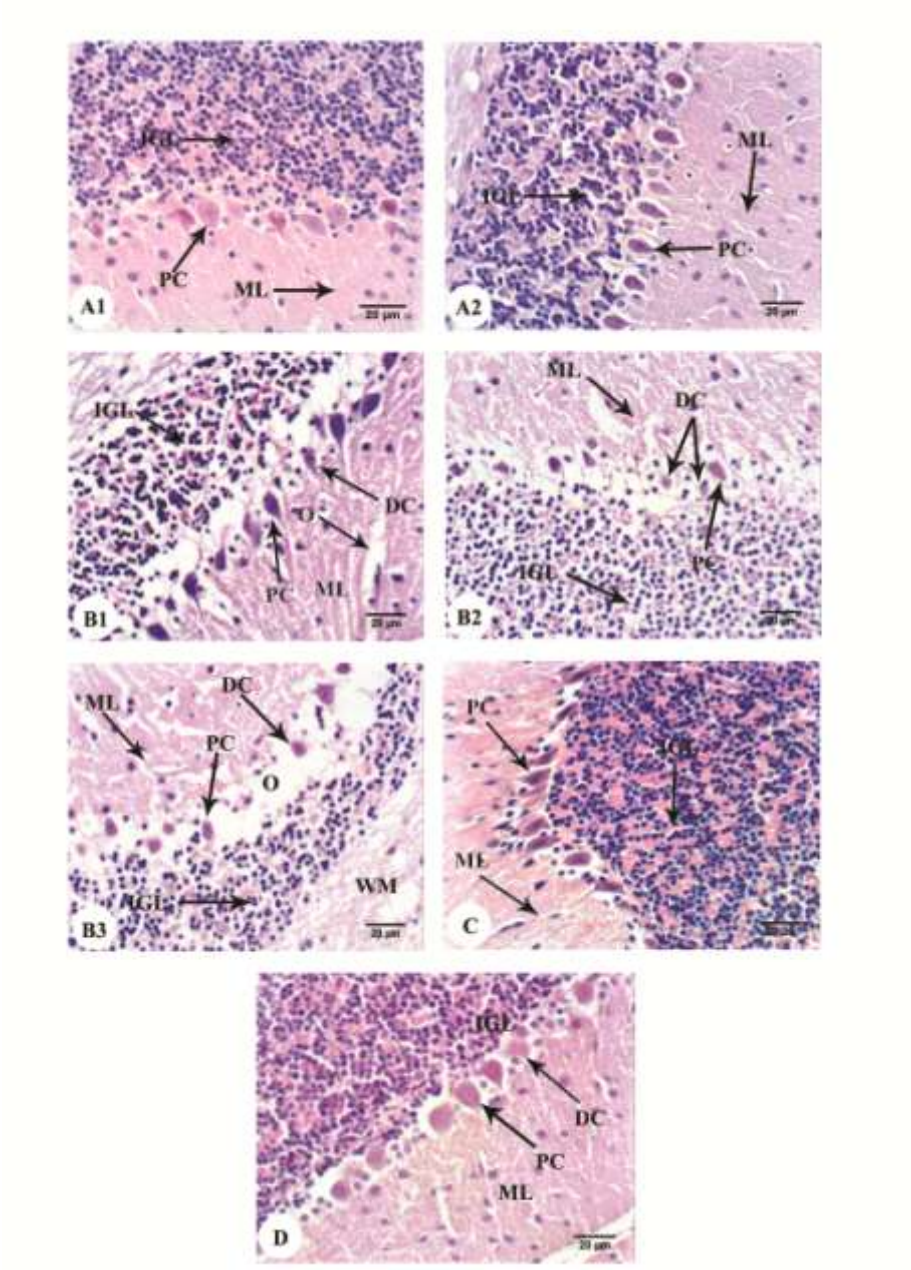

Fig.1 Sagittal sections incerebellar cortex of the newborns at PND 28 in control (A1, A2), hypothyroid (B1-B3), Zn (C) and Zn- hypothyroid groups (D).

DC: degenerative changes; IGL: internal granular layer; ML: molecular layer; O: odema; PC: purkinje cell; WM: white matter.

4. DISCUSSION

Hypothyroidism induced by antithyroid drugs such as CMI during pregnancy and postnatal periods is known to impair the maturation and development of the CNS. In the present study, the exposure of adult female rats to CMI dramatically reduced serum levels of T3 and T4 in both dams and their neonats while, it increased TSH serum levels of mothers and their newborns that emphasized the hypothyroid case. The antithyroid action of CMI is due to its conversion to MMI after absorbtion from gastrointestinal tract. MMI is transmitted through placenta or milk (Mandel and Cooper, 2001).The mechanism by which MMI exerts hypothyroidism was explained by Awad (2002) and Ahmed *et al.* (2010) who reported that MMI interferes with incorporation of iodine into tyrosyl residues of thyroglobuline (TG) and inhibits the coupling of iodotyrosyl residues to form iodothyronine, thus inhibiting the synthesis of THs.

Several trace elements are essential for normal function of thyroid status for both synthesis and metabolism of THs (Nazifi *et al*., 2009). Our results revealed that zinc administration into hypothyroid dams during and after pregnancy enhanced the T3 and T4 levels.our studies are consistent with the claim that reported that, THs levels were enhanced in hypothyroid patients fed with zinc supplement (Baltaci *et al*. 2003, 2004;Kandhro *et al*. (2008). These results may be explained as the following, (1) zinc may play a role in thyroid hormones metabolism and in the conversion of T4 to T3 (Nishiyama *et al.*, 1994).Since, Zndecrease the activity of 1, 5′- deiodinase in liver (Kralik*et al*., 1996). (2) Also, Zn is necessary for TRH synthesis (Pekary *et al*., 1991; Sciaudone *et al*., 2000) because it a major role in protein synthesis (Fabris, 1994) which included in T3 binding to its nuclear receptors (Freake *et al*., 2001). It has been reported that that zinc application increased thymic functions and in turn enhanced thyroid functions affecting the pituitary thyroid axis (Ozturk *et al*., 2004).

THs may regulate the growth and development, in part, affecting GH function (Ahmed *et al*., 2010). In the present study, GH concentration decreased in the hypothyroid newborns. GH is a key factor controlling postnatal growth and development (Wong *et al.*, 2006). Also, THs influence growth in part by altering the secretion and effects of pituitary GH via mRNA or growth factors (Saranac *et al*., 2013), while GH in turn mediates the growth and function of the thyroid as well as TH metabolism (Ahmed and Incerpi, 2013; Saranac *et al.*, 2013). Our result indicated that Zn supplementation increased GH levels in the hypothyroid newborns in comparing with control, where zinc participate in GH synthesis, secretion and action on somatomedin-c production. Zinc administration was found to be able to elevate basal plasma GH in normal individuals (Brando-Neto *et al.*, 1987Moreover, IGF-1 synthesis in liver is dependent on zinc, which mediates the growth promoting effect of GH (Hambigde *et al*., 1976;Cossack,1984).
Our presented results in table 2 indicated that maternal hypothyroid group showed significant decrease in Zn concentration in neonats cerebellum relative to control group while, Zn supplementation into hypothyroid group significantly increased Zn concentration in neonats cerebellum comparing to CMI group. Pimenta et al. (1992) reported a delay in intestinal absorbtion and zinc assimilation by tissues in hypothyroid patients after Zn ingestion. It is well known that changes in trace mineral absorption andexcretion in the gastrointestinal tract are primary mechanisms for maintaining trace mineral homeostasis (King et al., 2000).

Our result revealed that CMI caused significant decrease in the antioxidant markers (SOD, CAT, GPX, GSH, t-SH) and increasing LPO, H_2O_2 and NO levels in cerebellum in comparing to control group, while Zn administration of hypothyroid dams reversed these patterns effect relative to CMI group. Hypothyroidism induced by antithyroid drugs disturbed the balance between ROS generation and the antioxidant defense systems (Bhanja and Jena, 2013) causing an increase of oxidant species.Thus it is suggested that CMI induced oxidative stress which results in alterations that observed cerebellum structure.Generally, SOD and CAT are closely related to the direct elimination of reactive oxgen where, SOD catalyzes the reduction of superoxide anion into hydrogen peroxide which subsequently detoxified by CAT and GPX at both intra and extra cellular levels. Therefore, the reduction in the activity of these enzymes may result in accumulation of superoxide radical and hydrogen peroxide.Therefore, we can infer that high levels of H_2O_2 indicate suppression in the activity of SOD, CAT and GPX and induced lipid peroxidation.

TH is important in maintaining the GSH homeostasis in the developing rat brain (Ahmed *et al*., 2010) since, hypothyroidism reduced cellular thiol reserve and changed glutathione/GSH-px content in the brain of suckling rats (Dasgupta *et al.*, 2005).In addition Progressive hypothyroidism during the postnatal rat brain development led to a decline in GSH level (Mogulkoc *et al*., 2005), GPX activity (Ahmed, 2012) associated with an increase in H_2O_2 (Bhanja and Chainy, 2010), LPO (Bhanja and Chainy, 2010). Hypothyroid rats, exhibit high activity of nitric oxide synthetase (NOS) in organs such as heart, muscle, kidney and liver (Carreras *et al*., 2001; Quesada *et al.*, 2002) which explain high levels of NO. The reduction in antioxidant level of hypothyroid newborns may be explained by decreasing in Zn level in cerebellum.

On the other hand, our data postulated that Zn administration of hypothyroid mothers causing significant increase in the antioxidant levels of newborns cerebellum and decreasing the oxidants levels in comparing to hypothyroid group. Zn is an essential trace metal that acts as antioxidant by neutralizing free radical generation (Powell, 2000). Hypothyroid rats showed significant decrease in Cu and Zn levels compared with the controls (Alturfan *et al.*, 2007). The reduction in LPO levels following Zn supplementation to hypothyroid rats referring to its antiperoxidative effect which enhanced cell membrane stabilization against oxidative damage (Bettger and O'Dell, 1981).In addition Zn caused increasing in GSH level which consumed in detoxification of peroxide result from lipid peroxidation and to cope with increasing levels of H_2O_2 resulted due

to CMI supplementation. Regarding plasma GPX, an increased activity was shown in elderly subjects supplemented with Zn and Se (Girodon *et al*., 1997). The reduction in SOD, CAT and GPX activities after Zn supplementation into normal rat may be explained by decreasing in Zn concentration in cerebellum since Zn is an essential component of Zn-Cu SOD (Prasad et al., 1993), which in turn causing increasing in LPO and H_2O_2. Zn administration to normal rats increased GSH and GST levels could possibly be because Zn enhanced metallothionine which is very rich in cysteine, and is an excellent scavenger of OH or during its action in reducing active oxygen formation (Ranjbar et al., 2002) in addition Zn plays an important role in regulation of cellular glutathione that is vital to cellular antioxidant defense (Parat et al., 1997). Zinc acts as an inhibitor for NADPH oxidases enzymes that stimulate formation of O^{-2} from oxgen(Prasad, 2008). Zinc is an important structural element of NOS (nitric oxide synthatase) enzymes and is known to inhibit its catalytic activity (Miriam *et al.,* 2014). These results may illustrate the protective role of these trace elements as cofactors of antioxidant enzymes in limiting oxidative stress.

THs in rats mainly affect cerebellar development during the first 2-3 weeks of postnatal life (Wang *et al.*, 2012) and is required for normal maturation of this region (Ahmed,2011; Ahmed and Incerpi, 2013).Whereas, they increase the rate of neuronal proliferation in the cerebellum (Bhanja and Chainy, 2010).Our result indicated that, CMI induced hypothyroidism caused reduction in number and size of PCs. Altered in antioxidant defense system in hypothyroid neonats status disturbed histology of cerebellum so that perinatal hypothyroidism dramatically affects the morphogenesis of this region.Our study indicated that Zn supplementation to hypothyroid dams improved neonats cerebellum structure. Neurons have been shown to be highly sensitive to a deficit of Zn; in rats postnatal Zn deficiency impairs the normal development of the cerebellum, affecting the number and differentiation of purkinje, basket, stellate and granule cells (Sandstead, 2003). Zn supplementation into hypothyroid dams improved cerebellum histological structure during its effect on thyroid hormone levels and its antioxidant role since, Zn administration increased THs levels of hypothyroid dams.

5. CONCLUSION

We concluded that Zn has a protective role on newborns against hypothyroidism induced by carbimazole during pregnancy, where Zn play an important role in THs production and function and play an important role in brain development. Zinc increase growth of the newborns through its effect on GH. CMI induced hypothyroidism is associated with a marked alteration in enzymatic and non enzymatic antioxidant components. Our result showed that the nutritional antioxidant Zn is an important neuroprotective element that may be used as dietary supplement against oxidative damaged induced by hypothyroidism.

6. CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

7. ACKNOWLEDGMENTS

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8.REFERENCES

- Aebi, H. 1984. Methods enzymol. 105, 121-126.
- Ahmed, R.G., 2012.Maternal-newborn thyroid dysfunction.In the Developmental Neuroendocrinology, pp. 1-369. Ed R.G. Ahmed. Germany: LAP LAMBERT Academic Publishing GmbH & Co KG.
- Ahmed, R.G., Incerpi, S., 2013. Gestational doxorubicin alters fetal thyroid–brain axis. Int. J. Devl. Neuroscience .31, 96–104.
- Ahmed, O.M., Abd El-Tawab, S.M., Ahmed, R.G., 2010. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones-neurotransmitters and adenosinergic system interactions. International Journal of Developmental Neuroscience. 28, 437–454.
- Alturfani, A. A., Zengin, E., Dariyerli, N., Alturfan, E. E., Gumustas, M. K., Aytac, E., Aslan, M., Balkis, N., Aksu, A., Yigit, G., Uslu, E., Kokoglu, E., 2007. Investigation of zinc and copper levelsin methimazole-induced hypothyroidism:relation with the oxidant-antioxidant status, Folia Biologica (Praha). 53, 183-188.
- Artis, A.S., Bitiktas, S., Taskin, E., Dolu, N., Liman, N., Suer, C., 2012. Experimental hypothyroidism delays field excitatory postsynaptic potentials and disrupts hippocampal long-term potentiation in the dentate grus of hippocambal formation and Y-maz performance in adult rats. Journal of Neuroendocrinology. 24 (3), 422-433.
- Awad, M.F.I., 2002. Increased risk of fetal anomalies following maternally induced hypothyroidism in female albino rats. Ph.D. Thesis, Fac. of Sci., Cairo Univ., Egypt.
- Baglia, L.A., Cruz, D., Shaw, J .E., 1992. Production of immunoreactive forms of growth hormone by the Burkitt tumor serum-free cell line of Ramos.Endocrinology. 130, 2446-2454.
- Baltaci, A.K., Mogulkoc, R., Bediz, C.S., Ugur A., 2003. Pinealectomy and zinc deficiency have opposite effects on thyroid hormones in rats. Endocr Res.29, 473- 81.

- Baltaci,A.K., Mogulkoc, R., Bediz, C.S.,Ugur,A., 2004. Opposite effects of zinc and melatonin on thyroid hormones in rats. Toxicology.195, 69-75.
- Bancroft, J.D., Stevens, A., 1982. In Theory and practice of histological techniques, pp 121.2nd Edn. Edinburgh, UK: Churchill-Livingstone.
- Berbel, P., Navarro, D., Román, G.C., 2014. An evo-devo approach to thyroid hormones in cerebral and cerebellar cortical development: etiological implications for autism. J Frontiers in endocrinology. 5, 146 28.
- Bettger, W.J., O'Dell, B.L., 1981. A critical physiological role of zinc inthe structure and function of biomembranes, Life Sci. 28, 1425–1438.
- Bhanja, S., Chainy, G.B.N., 2010. PTU-induced hypothyroidism modulates antioxidant defence status in the developing cerebellum. Int. J. Dev. Neurosci. 28, 251-262.
- Bhanja, S., Jena, S., 2013.Modulation of antioxidant enzyme expression by PTU-induced hypothyroidism in cerebral cortex of postnatal rat brain.Neurochem. Res. 38(1), 42-49.
- Brandao-Neto, J., Kimachi, T., Verissimo, J.M., Shuhama, T., Marchini, J.S., Antunes-Rodrigues, J., 1987.Acute effects of human growth hormone on zinc, copper, calcium and magnesium metabolism in normal subjects. Brazilian journal of medical and biological research= Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica. 21(1), 43-47.
- Burger, H. G., Patel, Y. C., 1977. Thyrotropin releasing hormone –TSH. Clinic.Endocrinol.and Metab. 6, 831-00.
- Carreras, M.C., Peralta, J.G., Converso, D.P., Finocchietto, P.V., Rebagliati, I., Zaninovich, A.A., Poderoso, J.J., 2001. Modulation of liver mitochondrial NOS is implicated in thyroid-dependent regulation of O2 uptake.Am J Physiol Heart Circ Physiol. 281,H2282–H2288.
- Chasapis, C.T., Loutsidou, A.C., Spiliopoulou, C.A., Stefanidou, M.E., 2012. Arch. Toxicol. Zinc and human health: an update. J Archives of toxicology. 86(4),521–534.
- Cortes,C., Eugenin, E., Aliaga, E., Carreno, LJ., Bueno, S.M., Gonzalez, P.A., Gayol, S., Naranjo, D., Noches, V., Marassi, M.P., Rosenthal, D., Jadue, C., Ibarra, P., Keitel, C., Wohlk, N., Court, F., Kalergis, A.M., Riedel, C.A., 2012. Hypothyroidism in the adult rat causes incremental changes in the brain- derived neurotrophic factor, neuronal and astrocyte apoptosis, gliosis, and deterioration of postsynaptic density. Thyroid 22 (9), 951-963.
- Cossack, Z. T., 1984. Somatomedin-C in zinc deficiency, Experentia. 40, 489–499.
- Dasgupta, A., Das, S., Sarkar, P.K., 2005. Thyroid hormone stimulates gammaglutamyl transpeptidase in the developing rat cerebra and in astroglial cultures.J. Neurosci. Res. 82, 851–857.
- Dipaola, M., Luders, E., Dilulio, F., Cheruubini, A., Passafiume, D., Thompson, P.M., Caltagirone, C., Toga, A.W., Spalletta,G., 2010. Callosal atrophy in mild cognirive impairment and Alzheimer's disease: different effects in different stages. NeuroImage 49, 141-149.
- Draper, H.H., Hadley, M., 1990.Malondialdehyde determination as index of lipid peroxidation.Methods Enzymol.186, 421-31.
- Fabris, N., 1994. Neuroendocrine-immune aging: an integrative view on the role of zinc. Ann N Y Acad Sci.719, 353–68.
- Freake, H.C., Govoni, K.E., Guda, K., Huang, C., Zinn, S.A., 2001.Actions and interactions of thyroid hormone and zinc status in growing rats. J. Nutr. 131, 1135–1141.
- Gardner, D.L., Dodds, T.C., Royd, J.D., 1976. Human Histology, third ed. Churchill Livingstone, New York.
- Georgieff, M.K., 2007. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr.85, 614S–20SS.
- Girodon, F., Blache, D., Monget, A.L., Lombart, M., Brunet-Lecompte, P., Arnaud, J., Richard, M.J., Galan, P., 1997.Effect of a two-year supplementation with low doses of antioxidant vitamins and/or minerals in elderly subjects on levels of nutrients and antioxidant defense parameters. J. Am. Coll. Nutr. 16, 357-365.
- Gower-Winter, S.D., Levenson, C.W., 2012. Zinc in the central nervous system: from molecules to behavior. Biofactors.38,186–93.
- Habig, W and Pabst, M Jakoby, W.J., 1974. BIOL. Chem. 249, 7130-7139
- Hambigde, K. M., Walravens, P. A., Brown, R. M.,Webster, J.,White, S.,Anthony, M.,Roth, M.L., 1976. Growth limiting zinc deficiency in children from Denver Colorado, Am. Clin.Nutr.29, 734.
- Hasebe, M., Matsumoto, I., Imagawa, T., Uehara, M., 2008.Effects of an anti-thyroid drug, methimazole, administration to rat dams on the cerebellar cortex development in their pups. Int. J. Dev. Neurosci. 26, 409–414.Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R., 1974.Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzeneoxide as the hepatotoxic intermediate. Pharmacol. 11(3), 151-169.
- Kandhro, G.A., Kazi, T.G., Afridi, H.I., Naveed Kazi, N., Arian, M.B., Sarfraz, R.A., Syed, N., Baig, J.A., Shah, A.Q., 2008. Evaluation of iron in serum and urine and their relation with thyroid function in female goitrous patients. Biol Trace Elem Res. 125,203-102.
- King, J. C., D. M. Shames, and L. R. Woodhouse. 2000. Zinc homeostasis in humans. J. Nutr. 130,1360S–1366S.

- Koster, J.F., Biermond, P., Swaak, A.J.G., 1986. Intracellular and extracellular sulph-hydryl levels in rheumatoid arthritis. Ann. Rheum. Dis. 45, 44-46.
- Kralik, A., Eder, K., Kirchgessner, M., 1996. Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. Horm Metab Res. 28(5), 223–6.
- Kwan, E.Y., Lee, A.C., Li, A.M., Tam, S.C., Chan, C.F., Lau, Y.L., Low, L.C., 1995.A cross-sectional study of growth, puberty and endocrine function in patients with thalassaemia major in Hong Kong. J. Paediatr. Child Health. 31, 83–87.
- Leblondel, G., Le Bouil, A., Allain, P., 1992. Influence of thyroparathyroidectomy and thyroxine replacement on Cu and Zn cellular distribution and on the metallothionein level and induction in rats. Biol. Trace Elem. Res. 32, 281–288.
- Maes, M., Mommen, K., Hendrickx, D., Peeters, D., D'Hondt, P., Ranjan, R., De Meyer, F., Scharpe, S., 1997.Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers.Clin.Endocrinol. (Oxford) 46 (5), 587–598.
- Mandel, S.J., Cooper, D.S., 2001.The use of antithyroid drugs in pregnancy and lactation. J. Clin. Endocrinol.Metab. 86, 2354-2359.
- Mano, T., Sinohara, R., Sawai, Y., Oda, N., Nishida, Y., Mokumo, T., Asano, K., Ito, Y., Kotake, M., Hamada, M., 1995.Changes in lipid peroxidation and free radical scavengers in the brain of hyper- and hypothyroid aged rats.J Endocrinol. 147,361–36
- Mircescu, G., 2008. Oxidative stress of chronic kidney disease. Acta Endocrinologica (Buc) 4, 433-446.
- Miriam M.Cortese-Krott ., LarissaKulakov ., ChristianOpländer., VictoriaKolb-Bachofen., Klaus-D.Kröncke., ChristophV..Suschek., 2014. Zinc regulates iNOS-derived nitric oxide formation in endothelial cells. RedoxBiology.2, 945–954.
- Mogulkoc, R., Baltaci, A.K., Aydin, L., Oztekin, E., Sivrikaya, A., 2005. The effect of thyroxine administration on lipid peroxidation in different tissues of rats with hypothyroidism.Acta Physiologica Hungarica. 92 (1), 39–46.
- Montgomer, H.a.c., Dymock, J.F., 1961. Analyst, 86, 414.
- Nazifi, S., Mansourian, M., Nikahval, B., Razavi, S.M., 2009.The relationship between serum level of thyroid hormones, trace elements and antioxidant enzymes in dromedary camel (Camelus dromedarius).Trop Anim Health Prod.41,129–34.
- Nishiyama, S., Futagoishi-Suginohara, Y., Matsukura, M., Nakamura, T., Higashi, A., Shinohara, M., Matsuda, I., 1994. Zinc supplementation alters thyroid hormone metabolism in disabled patients with zinc deficiency. J Am Coll Nutr. 13(1), 62–7.
- Olfert, E.D., Cross, B.M., McWilliam, A.A. (Eds), 1993. In Guide to the care and use of experimental animals.vol 1. Ottawa, Ontario, Canada: CCAC.
- Oteiza, P.I., 2012. Zinc and the modulation of redox homeostasis. Free Radic. Biol.Med. 53, 1748–1759.
- OzturkGuler, Akbulut, K.G., Afrasyap, L., Sevinc, D., 2004. Effect of melatonin treatment on liver and thymus zinc levels in young and middle-aged rats. J Trace Elem Exp Med.17, 75–80.
- Parat, M.O., Richard, M.J., Beani, J.C., Favier, A., 1997. Involvement of zinc in intracellular oxidant/antioxidant balance, Biol. Trace Elem. Res. 60,187–204.
- PC- STAT, 1985.One way analysis of variance.Version 1A(c) copyright. Programs coded by Roa, M., Blane, K., Zonneberg, M. Univ. of Georgia, USA.
- Pekary, A.E., Lukaski, H.C., Mena, I., Hershman, J.M., 1991. Processing of TRH precursor peptides in rat brain and pituitary is zinc dependent. Peptides.12, 1025- 32.
- Petrulea, M.S., Duncea I., Muresan, A., 2009. Thyroid hormones in excess induce oxidative stress in rats. Acta Endocrinologica (Buc), V (2), 155-164.
- Pilhatsch, M., Marxen, M., Winter, C., Smlka, M.N., Bauer, M., 2011. Hypothyroidism and mood disorders: integrating novel in sights from brain imaging techniques. Thyroid Research 4 (August(Suppi.1)),S3.
- Pimenta, W.P., Brandão-Neto, J., Curi, P.R., 1992.The assessment of zinc statusby the zinc tolerance test in thyroid disease.J Trace Elmen Med Biol. 9, 34-7.
- Pinto, R.E., Bartley, W., 1989.The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenates.Biochem. J. 112, 109-115.
- Powell, S.R., 2000. The antioxidant properties of zinc. J. Nutr. 130(5S Suppl), 1447S-1454S.
- Prasad, A.S., 1993. Zinc and enzymes, in: A.S. Prasad (Ed.), Biochemistry of Zinc, Plenum Press, New York, pp. 17–53.
- Prasad, A.S., 2008. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc, Exp. Gerontol. 43, 370–377.
- Quesada, A., Sainz, J., Wangensteen, R., Rodríguez-Gomez, I., Vargas, F., Osuna, A., 2002.Nitric oxide synthase activity in hyperthyroid and hypothyroid rats. Eur J Endocrinol. 147,117–122.

- Ranjbar, A., Pasalar, P., Abdollahi, M., 2002.Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticidemanufacturing workers, Hum. Exp. Toxicol. 21, 179–182.
- Sakr, S.A.R., Hoda Abdel- hafez Mahran, Amany Ebrahem Nofal., 2011. Effect of selenium on carbimazole induced testicular damage and oxidative stress in albino rats. J, Trace Elements in Medicine and Biology. 25, 59-66.
- Sandstead, H. H., 2003. Zinc is essential for brain development and function. J. Trace Elem. Exp. Med. 16,165–173.
- Saranac, L., Stamenkovic, H., Stankovic, T., Markovic, I., Zivanovic, S., Djuric, Z., 2013. Growth in children with thyroid dysfunction.The Current Topics in Hypothyroidism with Focus on Development.119-134.
- Sciaudone, M.P., Chattopadhyay, S., Freake, H.C., 2000. Chelation of zinc amplifies induction of growth hormone mRNA levels in cultured rat pituitary tumor cells. J Nutr.130, 158–63.
- Shukla, G.S., Hussain, T., Chandra, S.V., 1987.Possible role of superoxide dismutase activity and lipid peroxide levels in cadmium neurotoxicity: in vivo and in vitro studies in growing rats. Life Sci. 14, 2215-2225.
- Sunil, K., Kota., Lalit, K., Meher., Siva, K., Kota., Sruti Jammula., Kirtikunmar, D.,
- Modi., 2013. carbimazole induced cholestatic hepatitis in Graves disease; Indian journal of Endocrinology and metabolism. 17(2), 326-328.
- Thakur, C., Saikia, T.C., Yadav, R.N., 1997. Total serum levels of triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH) in school going children of Dibrugarh district: an endemic goiter region of Assam. Indian J Physiol pharmacol.41 (2), 167-70.
- Wang, Y., Zhong, J., Xu, H., Wei, W., Dong, J., Yu, F., Wang, Y., Gong, J., Shan, Z., Teng, Y., Chen, J., 2012. Perinatal iodine deficiency and hypothyroidism increase cell apoptosis and alter doublecortin and reelin protein expressions in rat cerebellum. Arch. of Med. Res. 43, 255-264.
- Wong, A.O., Zhou, H., Jiang, Y., KO, W.K., 2006. Feedback regulation of growth hormone synthesis and secretion in fish and the emerging concept of intrapituitary feedback loop. Comp. Biochem. Physiol. Part A: Mol. Integr. Phsiol. 144(3), 284-305.
- Zaidi, T.M., Khan, A.A., Hasan, B.M., Faruqi, A.N., 2004. Carbimazole induced thyroid histopathy in albino rats during development. J Anat Soc Ind, 53(2),14–7.
- Zhang, L., Cooper-Kuhn, C.M., Nannmark, U., Blomgren, K., Kuhn, H.G., 2010.Stimulatory effects of thyroid hormone on brain angiogenesis in vivo and in vitro.J.Cereb. Blood Flow Metab. 30(2), 323–335.