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Maternal carbamazepine alters fetal neuroendocrine-cytokines axis

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ABSTRACT

This study detected the impact of maternal carbamazepine (CBZ) on the fetal neuroendocrine-cytokines axis. 25 or 50 mg/kg of CBZ was intraperitoneally administrated to pregnant albino rats from the gestation day (GD) 1 to 20. Both administrations of CBZ caused a hypothyroidism in dams and fetuses whereas the decreases in serum thyroxine (T4) and triiodothyronine (T3) and increases in serum thyrotropin (TSH) levels were highly significant (LSD; $P < 0.01$) at GD 20 compared to untreated control dams. Also, both administrations had undesirable impacts on the maternofetal body weight, litter weight, survival of dams and fetuses, and their food consumption in comparison to the corresponding control. These administrations also elicited a reduction in fetal serum growth hormone (GH), interferon-γ (IFNγ), interleukins (IL-2 & 4) and prostaglandin E2 (PGE2) levels. Also, the elevation in fetal serum tumor necrosis factor-alpha (TNFα), transforming growth factor-beta (TGFβ), and interleukins (IL-1β & 17) levels was observed at embryonic day (ED) 20. Moreover, there were a cellular fragmentation, distortion, hyperemia, oedema and vacuolation in the fetal cerebellar cortex due to both maternal administrations. These developmental changes were dose-dependent. These novel results suggest that CBZ may act as a developmental immunoneuroendocrine disruptor.

1. Introduction

In the last 40 years, the administration of antiepileptic drugs (AEDs) during pregnancy has adverse actions on the fetuses (Liguori and Cianfarani, 2009; Cassina et al., 2013; Thomas et al., 2017; Wen et al., 2017). Indeed, AEDs therapy has been disclosed to induce the teratogenicity (Veiby et al., 2009; British National Formulary (BNF), 2011; Kaushik et al., 2016) and endocrine disorders in both children and adults (Svalheim et al., 2015). In this context, there is intrauterine growth restriction (IUGR), and impairment in the neurocognitive behaviors (Luef, 2009). Carbamazepine (CBZ), dibenzoazepine derivative, is recognized as antiepileptic and tricyclic anticonvulsant drug (Kaushik et al., 2016; Juhel et al., 2017; Lu and Wang, 2017; Wijnen et al., 2017). However, it is considered a human teratogen and transferred the placenta to accumulate in the fetal tissues (Bath and Scharfman, 2013; Nie et al., 2016). Also, it has a moderate effect on the thyroid, hepatic and metabolic markers of children (Yılmaz et al., 2014). Importantly, CBZ initiates a central hypothyroidism (Sigurjonsdottir et al., 2014), subclinical hypothyroidism (Hamed, 2015) and growth retardation (Rättyä et al., 1999). On the contrary, one study has reported that CBZ does not initiate a hypothyroidism in patients (Post et al., 1983). Another study has postulated that CBZ can reduce the free thyroxine (FT4) level and disrupt the thyrotropin (TSH) level (Vainionpää et al., 2004). Otherwise, maternal administration of CBZ induces a neural tube defect (Morrow et al., 2006), memory loss (Uddin et al., 2016), and neuronal injury (Barkovich and Raybaud, 2004; Leventer et al., 2008; Åberg et al., 2013). Additionally, any cortical abnormalities during the first or second trimester of gestation can cause epileptic

Abbreviations: GABA, γ-aminobutyric acid; ANOVA, analysis of one way of variance; AEDs, antiepileptic drugs; BNF, British National Formulary; CBZ, carbamazepine; DC, degenerative changes; Ds, deiodinases; ED, embryonic day; EGL, external granular layer; ERK1/2, extracellular signal regulated kinase; FT4, free thyroxine; GD, gestation day; GHRH, GH-releasing hormone; GH, growth hormone; H&E, Haematoxylin and Eosin; HBV, hyperemic blood vessel; HPTA, hypothalamic-pituitary-thyroid-axis; IgA, IgG and IgM, immunoglobulins; IGF1, insulin growth factor 1; IFNγ, interferon-γ; IL-1β, 2, 4 & 17, interleukins; IGL, internal granular layer; IUGR, intrauterine growth restriction; I^-, iodide; LSD, least significant degree; ML, molecular layer; NIC, National Cancer Institute; NGF, nerve growth factor; NF-κB, nuclear factor kappa of B cells; O, oedema; PGE2, prostaglandin E2; AKT, protein kinase B; PC, Purkinje cell; PL, Purkinje layer; rT3, reverse triiodothyronine; SE, standard error; TEGL, thickened external granular layer; TRs, TH receptors; THTs, TH-transporters; TBG, thyroid binding globulin; THs, thyroid hormones; TSH, thyrotropin; T4, thyroxine; TT4, total thyroxine; TGFβ, transforming growth factor-beta; T3, triiodothyronine; TNFα, tumor necrosis factor-alpha; UDP-GTs, uridine diphosphate glucuronyl transferases; V, vacuoles; WM, white matter.

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seizures (Leventer et al., 2008; Tamijani et al., 2015). Alternatively, the levels of cytokines are also impacted by CBZ treatment. Some findings have reported that the CBZ changes the levels of interleukins (IL-1β, 2, 4–6 & 10) (Basta-Kaim et al., 2008; Himmerich et al., 2013), transforming growth factor-beta (TGFβ) (Basta-Kaim et al., 2008), and tumor necrosis factor-alpha (TNFα) (Himmerich et al., 2013). Also, it causes inflammation (Björnsson, 2008), hypereosinophilia and hypersensitivity (skin involvement) (Mathieu et al., 2011). Conversely, the variation in immune markers can cause epilepsy or bipolar disorder (Himmerich et al., 2013). However, there are conflicting results regarding the impact of maternal CBZ on the fetal thyroid-brain and immune axis.

As the fetal thyroid hormones (THs) (Ahmed et al., 2008) and cytokines (Dean et al., 2012) showed a vital role in the developing brain, the goal of this study was to assess the impact of maternal administrations of CBZ (25 or 50 mg/kg) on the fetal neuroendocrine-cytokines axis. Thus, the current experiment was performed on pregnant albino rats to evaluate the following: **(1)** the alterations in the materno-fetal thyroid markers, body weight, food consumption, and survival of the dams and their fetuses; **(2)** the variations in the serum concentrations of fetal growth hormone (GH), pro-fibrotic marker (TGFβ), pro-inflammatory cytokines (TNFα, INFγ, IL-1β & 17), anti-inflammatory cytokines (IL-2 & 4) and acute-inflammatory cytokine (PGE2); and **(3)** the abnormalities in the histogenesis of the fetal cerebellum at the embryonic day (ED) 20. Indeed, this brain region is highly sensitive to any stress throughout the development (Ahmed, 2011; Ahmed et al., 2014).

2. Materials and methods

2.1. Experimental animals

Twenty-four mature virgins female Wistar rats (Rattus norvegicus) weighing 160–170 g and twelve adult males for mating only were purchased from the animal house of VACSERA in Helwan (Egypt). The rats were kept in stainless steel cages with constant light/dark cycle, temperature, and humidity throughout the experimental period. Tap water and food were provided ad libitum (Ahmed et al., 2015a,b). Before the beginning of the experiment, all animals were kept for 14 days to eliminate any intercurrent contaminations. Then, one male was coupled with two proestrous females for one or two days in a separate cage (Marcondes et al., 2002). The sperms in the vaginal smears confirmed the beginning of pregnancy and our experiment. The treatments and housing of pregnant rats were followed the overall rules of animal care in Egyptian (Zoology Department, Faculty of Science, Beni-Suef University) and Canadian Committees (Olfert et al., 1993). Notably, we did our best to decrease the animal suffering.

2.2. Experimental strategy

CBZ (Sigma Chemical Co.; dissolved in dimethylsulfoxide) was administered (i.p. injection) to pregnant rats at a dose of 25 or 50 mg/kg body weight/day during the whole pregnancy. These doses were selected according to Sitges et al. (2012) and Gómez et al. (2014), respectively. The high dose of CBZ (50 mg/kg) was equivalent to a comparable human dose of 486 mg and was given to epileptic patients per day (Reagan-Shaw et al., 2008). The control pregnant rats were injected the solvent vehicle only (i.p.). In contrast to human patients, we avoided the oral administration to prevent the stress, which could interfere with the gestation period and delay the developing brain (Manent et al., 2007).

At the end of the experiment, the dams and fetuses of all groups were sacrificed after anesthesia and tested at the gestation day (GD) 20. We followed the maternal body weight gain, the maternal mortality, the number of aborted dams, the food consumption, the litter weight, the live fetuses/litter, the number of dead fetuses/total fetuses, and the fetal body weight. We collected the blood samples for each dam and their fetuses from the jugular vein and umbilical cord, respectively. These samples were left to coagulate and centrifuged for 20 min at 3000 rpm (1006.2*g*). Their clear supernatants were directly separated into 3 Eppendorf tubes/each animal and reserved at −70 °C till utilized for various analysis. Also, the histopathological changes in the fetal cerebellum were examined at ED 20.

2.3. ELISA examination of the maternal and fetal markers

The serum concentrations of maternal and fetal TSH, T3 and T4, and fetal TNFα, GH, TGFβ, IFNγ, interleukins (IL-1β, 2, 4 & 17) and PGE2 were determined by ELISA (Spectra Max 190-Molecular Devices, USA) in Cairo University (Dep. of Biochemistry, Fac. of Medicine), Egypt. The practical kits were applied for estimation the serum concentrations of TSH, T3, T4, GH, TGFβ, and PGE2 (Millipore ELISA Kit, USA). Serum TNFα and IFNγ concentrations were examined using kits obtained from Invitrogen Corporation, USA. The IL-1β, IL-2, and IL-4 kits were purchased from R and A systems (USA) while the IL-17 kit was purchased from Cusabio (USA) according to manufacturer's instructions.

2.4. Histological examination of the fetal cerebellum

Cerebellar tissue samples were directly fixed in 10% neutral buffered formalin for twenty-four hours and ordered through the ethanol solutions (50%, 70%, 95% and 100%; 2 h for each change). These samples were sent to the National Cancer Institute (NIC) (Cairo, Egypt) for additional processing, clearing in xylene, embedding in paraffin wax, blocking, and serially sectioning at 6 μm and staining with Haematoxylin and Eosin (H&E) (Bancroft and Gamble, 2008).

2.5. Statistical analysis

The software of PC-STAT program was used for the statistical examination (Roa et al., 1985). Analysis of one way of variance (ANOVA) and the least significant degree (LSD) was used to distinguish the effects between the experimental groups. The results were expressed as a mean ± standard error (SE). The overall changes among the groups were established by F-probability. The means were highly significant ($P < 0.01$) and very highly significant ($P < 0.001$) different. Also, the mean values with similar superscript symbols were negligible alterations. Particularly, the number of examined samples/parameter/group was six.

3. Results

3.1. Maternal CBZ-disrupted the materno-fetal thyroid functions

Both administrations of 25 mg and 50 mg CBZ during pregnancy induced a maternofetal hypothyroidism as confirmed by an increase in the serum TSH and a decrease in the serum T4 and T3 levels at GD 20 (Table, 1). Notably, the fetal hypothyroid state was more potently in the 50 mg CBZ-treated group (−44.67% for T4, −92.44% for T3 & +228.69% for TSH) than in the 25 mg CBZ-treated group (-29.45% for T4 & −49.28% for T3 & +109.46% for TSH) (Table 1).

Table 1
Administration of CBZ changed the maternal and fetal thyroid functions at GD 20.

Day	CBZ (mg/kg)	T4	T3	TSH	T4	T3	TSH
		ng/dl					
		Dams			Fetuses		
GD 20	**0**	35.86 ± 0.114^{a}	6.84 ± 0.080^{c}	12.72 ± 0.138^{a}	17.01 ± 0.210^{b}	2.78 ± 0.109^{a}	3.38 ± 0.241^{c}
	25	26.95 ± 0.051^{b}	3.91 ± 0.098^{a}	18.90 ± 0.216^{b}	12.00 ± 0.154^{a}	1.41 ± 0.165^{c}	7.08 ± 0.158^{b}
		−24.84%	−42.83%	+48.58%	−29.45%	−49.28%	+109.46%
	50	18.67 ± 0.151^{c}	1.01 ± 0.266^{b}	24.03 ± 0.115^{c}	9.41 ± 0.259^{c}	0.21 ± 0.110^{b}	11.11 ± 0.106^{a}
		−47.93%	−85.23%	+88.91%	−44.67%	−92.44%	+228.69%
ANOVA	$P < 0.001$						
LSD 5%		0.3427	0.2912	0.4914	0.6399	0.3458	0.5324
LSD 1%		0.4739	0.4027	0.6796	0.8849	0.4782	0.7363

3.2. Maternal CBZ-decreased the maternal and fetal markers

Both maternal administrations of CBZ influenced on the maternal body weight gains (-16.49% for the low dose group & −29.19% for the high dose group) as compared to the control one (Table 2). Parallelly, mortality of dams was doubled in the 50 mg CBZ-treated group if compared to the corresponding 25 mg CBZ-treated one. However, in both treated groups, no maternal abortions were noted during pregnancy. There were marked (LSD; $P < 0.01$) changes in the maternal food consumption between the CBZ-experimental groups (−24.62% for the low dose group & −36.58% for the high dose group) and the control one. On the other hand, the weight of litters and fetuses was reduced in both 25 mg and 50 mg CBZ-treated groups in comparison to the control group. Also, both maternal administrations of CBZ-induced some fetal mortality at ED 20. In parallel, the live fetuses/litter were considerably decreased due to both maternal administrations as compared to the control. These changes became more substantial in the 50 mg CBZ-treated group than in the 25 mg CBZ-treated group (Table 2).

3.3. Maternal CBZ-altered the fetal growth markers, pro-inflammatory and acute-inflammatory cytokines

Compared to the control group, both CBZ-maternal administrations caused significantly (LSD; $P < 0.01$) decreased in fetal serum GH, IFNγ, and PGE2 concentrations at ED 20. This disturbance was noticeable in the 50 mg CBZ-treated group, where the percentage index was −85.53%, −85.43% & −75.55%, respectively in the 50 mg CBZ-treated group and −58.92%, −57.28% & −32.81%, respectively in the 25 mg CBZ-treated one (Table 3). However, these maternal administrations significantly (LSD; $P < 0.01$) increased the serum concentrations of fetal TGFβ and TNFα compared to the control values. Their averages in the 50 mg CBZ-treated group were 8.76 & 4.76 ng/dl, respectively compared to their averages in control (1.39 & 0.67 ng/dl, respectively) or in the 25 mg CBZ-treated group (4.07 & 1.63 ng/dl, respectively) (Table 3).

3.4. Maternal CBZ-perturbed the fetal interleukins markers

Data presented in Table 4 proved that both maternal administrations of CBZ-induced a substantial (LSD; $P < 0.01$) increase in serum concentrations of fetal IL-1β & 17, and considerable (LSD; $P < 0.01$) diminution in the serum concentrations of fetal IL-2 & 4 with respect to the control group. These inconsistencies became noteworthy in the 50 mg CBZ-treated group (+329.59% for IL-1β, +346.41% for IL-17, −73.46% for IL-2 & −82.96% for IL-4) when compared to relevant 25 mg CBZ-treated group (+115.69% for IL-1β, +216.38% for IL-17, −39.48% for IL-2 & −38.38% for IL-4) (Table 4).

In general, the disturbance in all maternofetal markers was more apparent in the 50 mg CBZ-treated group than in the 25 mg CBZ-treated group. One way ANOVA for the studied markers exhibited a very highly profound ($P < 0.001$) effect among the experimental groups (Tables 1–4).

3.5. Maternal CBZ-caused some histopathological changes in the developing cerebellum

The cerebellum of the control fetuses showed a normal distribution in their layers at ED 20 (Fig. 1A_{1-4}). On the other hand, both administrations of the maternal CBZ-induced a materno-fetal hypothyroidism led to a severe impairment in the normal cerebellar structure (Fig. 1B & C). The lesions in the 25 mg CBZ-treated group appeared in the form of hyperemia in the internal granular layer (IGL) and external granular layer (EGL), thickening in the EGL, and cellular distortions in the IGL and Purkinje cells (PCs) at ED 20 (Fig. 1B_{1-3}). Fig. 1B_4 showed that the PCs were disorganized, oedema in the IGL and Purkinje layer (PL), and vacuolar degenerative changes in the molecular layer (ML). In the 50 mg CBZ-treated group, a vacuolation in ML (spongiosis),

Table 2
Maternal administrations of CBZ changed the maternal and fetal markers at GD 20.

Day	CBZ (mg/kg)	Maternal body weight gain (g)	Mean of food consumption (g)	No. of dead dams/ pregnant rats	No. of aborted dams	Litter weight (g)	Mean of live fetuses/litter	No. of dead fetuses/ total fetuses	Fetal body weight (g)/litter
GD 20	**0**	69.05 ± 0.634^{a}	14.05 ± 0.087^{a}	0/8	0	63.92 ± 0.400^{a}	9.50 ± 0.223^{a}	0/99	6.87 ± 0.327^{a}
	25	57.66 ± 0.937^{b}	10.59 ± 0.216^{b}	1/8	0	41.10 ± 0.451^{b}	7.50 ± 0.221^{b}	4/81	4.28 ± 0.295^{b}
		−16.49%	−24.62%			−35.70%	−21.05%		−37.70%
	50	48.89 ± 0.897^{c}	8.91 ± 0.266^{c}	2/8	0	33.17 ± 0.710^{c}	5.50 ± 0.220^{c}	6/72	3.43 ± 0.377^{c}
		−29.19%	−36.58%			−48.10%	−42.10%		−50.07%
ANOVA		$P < 0.001$				$P < 0.001$			$P < 0.001$
LSD 5%		1.6227	0.6163			1.6228	0.6738		0.3416
LSD 1%		2.2441	0.8523			2.2442	0.9319		0.4725

Table 3
Maternal administrations of CBZ changed the growth, pro-fibrotic, pro-inflammatory, and acute-inflammatory markers at ED 20.

Day	CBZ (mg/kg)	GH	TGFβ	TNFα	IFNγ	PGE2
		ng/dl				
ED 20	**0**	5.60 ± 0.203^{a}	1.39 ± 0.134^{b}	0.67 ± 0.178^{a}	2.06 ± 0.113^{c}	7.65 ± 0.147^{c}
	25	2.30 ± 0.138^{c}	4.07 ± 0.129^{c}	1.63 ± 0.146^{b}	0.88 ± 0.167^{a}	5.14 ± 0.183^{b}
		−58.92%	+192.80%	+143.28%	−57.28%	−32.81%
	50	0.81 ± 0.089^{b}	8.76 ± 0.102^{a}	4.76 ± 0.145^{c}	0.30 ± 0.156^{b}	1.87 ± 0.093^{a}
		−85.53%	+530.21%	+610.44%	−85.43%	−75.55%
ANOVA	$P < 0.001$					
LSD 5%		0.4286	0.3888	0.3992	0.2026	0.4696
LSD 1%		0.5928	0.5377	0.5521	0.2803	0.6494

Table 4
Maternal administrations of CBZ changed the fetal interleukins (IL-1β, 2, 4 & 17) at ED 20.

Day	CBZ (mg/kg)	IL-1β	IL-2	IL-4	IL-17
		ng/dl			
ED 20	**0**	2.23 ± 0.116^{c}	7.80 ± 0.184^{a}	11.80 ± 0.172^{b}	2.93 ± 0.136^{a}
	25	4.81 ± 0.165^{a}	4.72 ± 0.127^{b}	7.27 ± 0.205^{c}	9.27 ± 0.317^{c}
		+115.69%	−39.48%	−38.38%	+216.38%
	50	9.58 ± 0.234^{b}	2.07 ± 0.118^{c}	2.01 ± 0.096^{a}	13.08 ± 0.201^{b}
		+329.59%	−73.46%	−82.96%	+346.41%
ANOVA	$P < 0.001$				
LSD 5%		**0.5391**	0.5416	0.4958	0.7894
LSD 1%		**0.7456**	0.7490	0.6856	1.1041

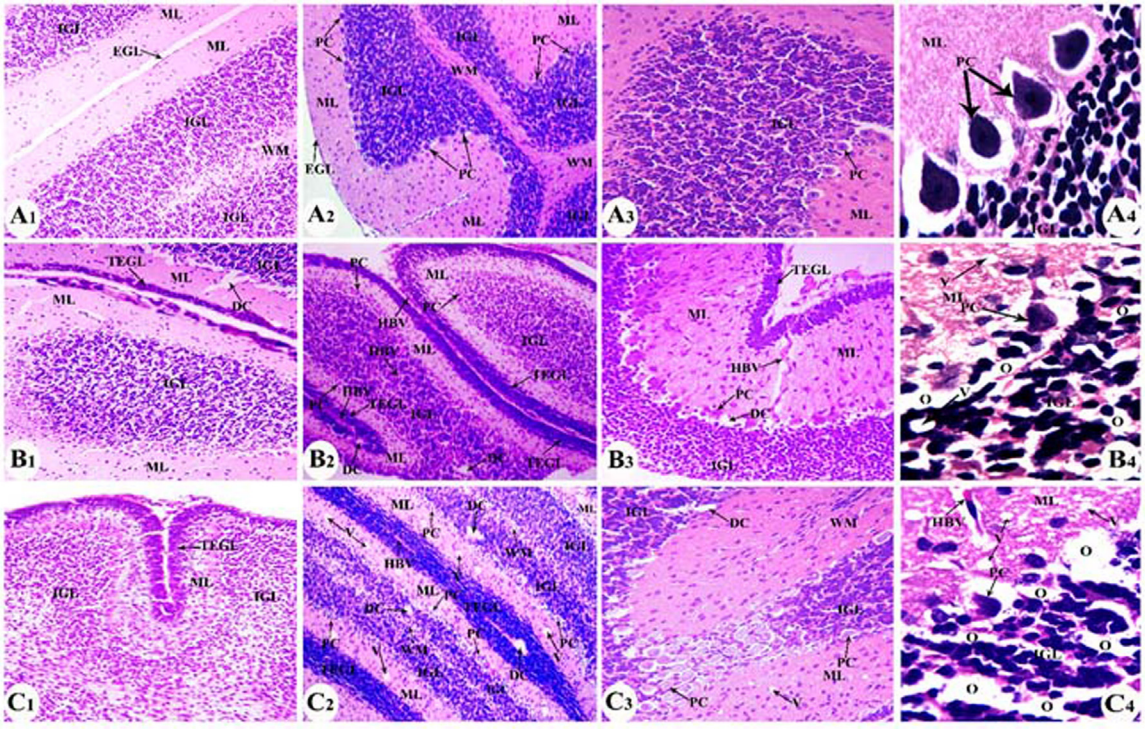

Fig. 1. Sagittal sections in the fetal cerebellum at the embryonic day 20 in control (A_1, X200; A_2, X400; A_3, X600 and A4, X1000), 25 mg/kg CBZ (B_1, X200; B_2, X400; B_3, X600 and B4, X1000), and 50 mg/kg CBZ (C_1, X200; C_2, X400; C_3, X600 and C4, X1000), (H&E stain). Where, DC, degenerative changes; EGL, external granular layer; HBV, hyperemic blood vessel; IGL, internal granular layer; ML, molecular layer; O, oedema; PC, Purkinje cell; TEGL, thickened external granular layer; V, vacuoles; WM, white matter.

severe degenerative changes (oedema, pyknotic nuclei, and cell death) in the IGL and PL, and reduction in the size of the PCs were noted at ED 20 (Fig. $1C_{1\text{-}4}$). Further, disperse depositions of damaged PCs were observed in the IGL (Fig. $1C_{2,3}$). Notably, reduction in the cellularity populations of the IGL, patches cell damage and a loss of the standard association were noticed in both CBZ-treated groups (Fig. $1B_4$ & C_4).

4. Discussion

There are synergistic actions between the materno-fetal thyroid axis and cytokines to maintain the normal fetal progress and cerebellar development during the gestation period. Several studies reported that the maturation of the GH, growth factors

and cytokines controlled by the THs (Ahmed, 2015, 2016a,b; Candelotti et al., 2015). Otherwise, immune cytokines (interleukins, TGFβ, IFNγ or TNFα) might have a dynamic role in the development, and in the programming of neuroendocrine axes (Dahlgren et al., 2006; Ahmed et al., 2015a; Ahmed, 2016b). Further, PGE2 regulates the cellular proliferation (Ota et al., 2012), and the developing cerebellum (Dean et al., 2012). Thus, any disturbance in the hypothalamic-pituitary-thyroid axis (HPTA) and cytokines axis may probably influence the neurodevelopmental mechanism, particularly the developing cerebellum (Ahmed et al., 2008, 2014).

The current data displayed that the maternal administrations of CBZ (25 or 50 mg/kg) during the gestation period appear to induce hypothyroidism at GD 20 via thyroid dyshormonogenesis in both dams and their fetuses. This hypothyroidism was dose dependent. Previously, the administration of CBZ caused a reduction in the concentrations of T4, FT4, T3 & reverse triiodothyronine (rT3) (Verrotti et al., 2009; Yılmaz et al., 2014; Svalheim et al., 2015; Zevenbergen et al., 2016) associating with an elevation in the concentration of TSH (Thomas et al., 1998). It competes with THs on the thyroid binding globulin (TBG) (Surks and DeFesi, 1996; Simko and Horacek, 2007). The mechanism of this disruption could be attributed to: **(1)** elevation of the FT4/total thyroxine (TT4) ratio (Surks and DeFesi, 1996); **(2)** inhibition of the iodide (I^-) utilization, the Na^+/I^- symporter, and the THs synthesis (Isojärvi et al., 2001); **(3)** initiation of the TH-metabolism by the cytochrome P-450 iso-enzymes (Isojärvi et al., 1995); **(4)** stimulation of TH-glucuronoconjugation by uridine diphosphate glucuronyl transferases (UDP-GTs) (Isojärvi et al., 2001; Simko and Horacek, 2007); and **(5)** alteration of the peripheral metabolism of TH by deiodinases (Ds) activity (Simko and Horacek, 2007) or by the transmembrane TH-transporters (THTs) in the several biological tissues (including hypothalamus and pituitary) (Verrotti et al., 2009). Thus, CBZ may increase the breakdown and elimination of THs (increase the metabolic clearance rate). These data imply that CBZ may distort the materno-fetal THs and HPTA homeostasis producing hypothyroidism.

Alternatively, our findings proposed that both maternal administrations of CBZ also had unwanted effects on the maternal/fetal body weight, the litter weight, the survival of dams/fetuses, and the maternal food consumption. Exposure to CBZ reduces the pregnant body weight gain (Diav-Citrin et al., 2001), the fetal body weight (Gerenutti et al., 2008), and the number of litters (Åberg et al., 2013), and increases the fetal resorption (Vorhees et al., 1990). These defects may disrupt the mating process and parturition (Åberg et al., 2013). Also, the reduction in maternal food consumption in our study could be one of these abnormalities. In general, these abnormalities might reflect the teratological effect of CBZ during the development. Consistent with these data, the serum concentration of fetal GH was profoundly declined in both maternal CBZ-treated groups at studied embryonic day compared to the corresponding control one. Various mechanisms were discussed to clarify these defects. AEDs stimulate or inhibit the synthesis and metabolism of the GH-releasing hormone (GHRH) by cytochrome P-450 iso-enzymes (Leśkiewicz et al., 2008). These results are reinforced by Artama et al. (2005) who reported that the prenatal exposure to CBZ disrupts the GH-insulin growth factor 1 (IGF1) axis, and Hamed (2015) who reported that the CBZ disturbs the hormonal system, the binding protein, and the HPTA function. The effect extended to reduce the metabolism during pregnancy (Gedzelman and Meador, 2012), and the development of the fetuses (Kilic et al., 2014; Farmen et al., 2015). Pérez et al., in 2008, elucidated that the growth retardation is probably due to the antiproliferative effects of CBZ (increasing the mitotic index and blocking the anaphase and metaphase). In line with these observations, THs distortion and metabolic disorders can cause this retardation (Rättyä et al., 1999; Ahmed, 2013). Thus, it may infer that the maternal and fetal hypothyroidism can deplete the uteroplacental transfer and prenatal development.

The results of the present investigation revealed that both maternal administrations of CBZ perturbed the fetal pro-inflammatory (TNFα, INFγ, IL-1β & 17), pro-fibrotic (TGFβ), acute-inflammatory (PGE2), and anti-inflammatory (IL-2 & 4) markers. Both CBZ administrations induced a reduction in fetal serum IFNγ, interleukins (IL-2 & 4) and PGE2 levels and an increase in fetal serum TNFα, TGFβ, and interleukins (IL-1β & 17) at ED 20 compared to the control one. These alterations could reflect the anticonvulsive/antiepileptic effects of CBZ. Interestingly, the CBZ can exert these drastic actions through three mechanisms. **(1)** reducing the production of PGE2 (Matoth et al., 2000), IL-2 & 4 (Marmurowska-Michałowska et al., 2004; Basta-Kaim et al., 2008; Himmerich et al., 2014), and TNFα & IL-1β (Himmerich et al., 2013; Gómez et al., 2014). This immunosuppressive action resulted in the reduction of the levels of immunoglobulins (IgA, IgG, and IgM), the protein synthesis in the lymphocytes, and the expression of CD4+ or CD8+ by T cells (Marmurowska-Michałowska et al., 2004; Basta-Kaim et al., 2008). **(2)** elevating the production of TGFβ (Basta-Kaim et al., 2008), TNFα (Fiszer, 2001; Himmerich et al., 2014), IL-1β, 2, 5 & 6 (Andrzejczak, 2011), and INFγ (Aihara et al., 2003). This immunostimulation action resulted in **(A)** the activation of macrophages in adipose tissues (Himmerich et al., 2009), and Kupffer cells in liver tissue (Himmerich et al., 2005); and **(B)** the presence of hematological leukocytosis or atypical lymphocytosis (Degirmenci et al., 2016). Alternatively, this behavior reflected the nutritional overload (increased appetite) effect of CBZ (Himmerich et al., 2005). **(3)** modifying the γ-aminobutyric acid (GABA) (antagonist effect) and ion channels receptors of immune cells (Himmerich et al., 2013). The variations in these results depending on the threshold dosages of CBZ, developmental period, experimental models, and nutritional status. Collectively, it is legitimate to suggest that the maternal CBZ might change the synthesis, transport, and actions of the present cytokines, thereby varying the fetal immune system. An alternative elucidation for the present results is that the maternofetal hypothyroidism-induced by the maternal CBZ seems to impact the programming of fetal immune responses, and the reverse may be true. These communications are expected to be very complicated. In this regard, thyroid disorders (hypothyroidism) might distort the immune markers (De Vito et al., 2011; Ahmed et al., 2015b), and the productions of the cytokines/chemokines during the fetoplacental development (Silva et al., 2014). Concurrent with these observations, the disturbances in the immune system can initiate several endocrine diseases (De Vito et al., 2011).

Concomitantly with the current materno-fetal hypothyroidism, some histopathological alterations were noticed in the fetal cerebellar cortex of both maternal CBZ groups at ED 20. These changes characterized by a hyperemia in the EGL and IGL, thickening in the EGL, vacuoles in ML, and fragmentation of the cellular element (oedema, pyknotic nuclei, and cell death) in the IGL and PL, and reduction in the size of the PCs. These destructive effects became more noticeable in the 50 mg CBZ-treated group than in the 25 mg CBZ-treated one. Previously, the administration of CBZ induces neuronal apoptosis (Lekera and Neufeld, 2003). One interpretation of those observations is that the CBZ blocks the voltage-dependent Na^+ & K^+ channels (McLean and Macdonald, 1986), and decreases their density or permeability (Sitges et al., 2011; Gómez et al., 2014) throughout the early developmental period (Manent et al., 2007). With those interpretations, AEDs change the neuronal nuclear factor kappa of B cells (NF-κB) (Young et al., 2016), the glutamate-gated ion channel, and Ca^{2+}

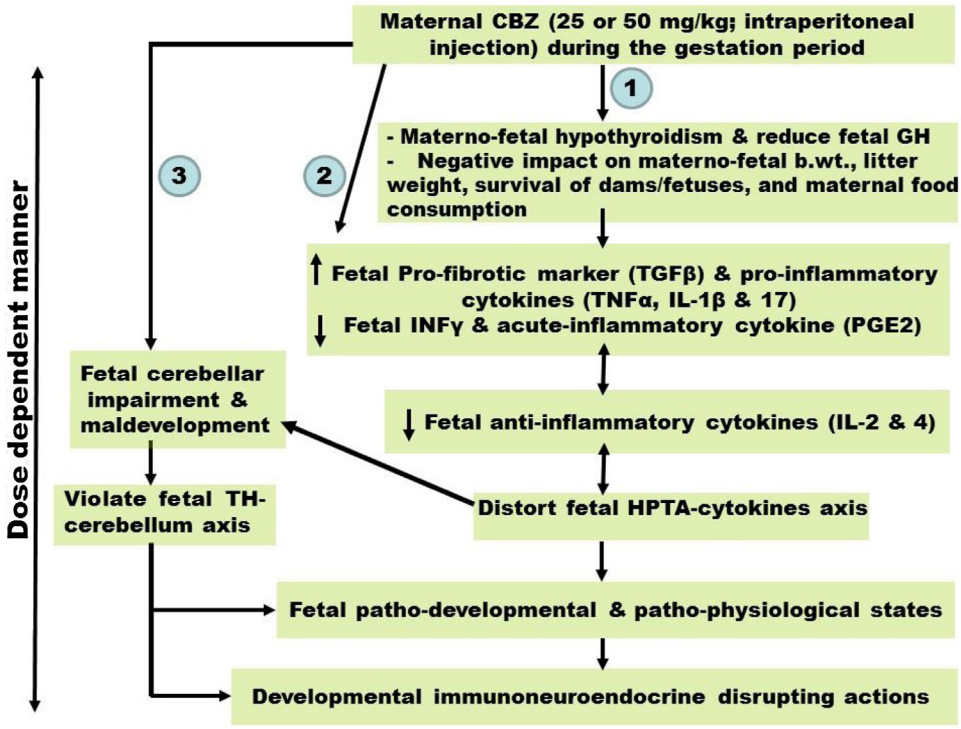

Fig. 2. Schematic diagram of the harmful action of the maternal CBZ on the developmental neuroendocrine-cytokines homeostasis. The maternal CBZ might act as a fetal immunoneuroendocrine disruptor. This hypothesis could illustrate by two pathways. **(A) Direct pathway:(1)** Maternal CBZ-induced the materno-fetal hypothyroidism, and disrupted the fetal HPTA, the maternal/fetal body weight, the litter weight, the survival of dams/fetuses, and the maternal food consumption; **(2)** It altered the fetal cytokines; and **(3)** It impaired the histogenesis of the fetal cerebellum. **(B) Indirect pathway:** The materno-fetal hypothyroidism by the maternal CBZ could interrupt the maturation of the fetal THs/GH-cytokines axes (the reverse may be true), and elicit the patho-developmental and patho-physiological states on the developing cerebellum. This, in turn, might destruct the developing immunoneuroendocrine axes. These alterations were dose-dependent.

ion channels permeability (Sitges et al., 2011). Another interpretation is that the AEDs disturb the vitamin K or folate metabolism (Ramsay and Slater, 1991), and reduce the neurotrophins 3 & 4, protein kinase B (AKT) and extracellular signal-regulated kinase (ERK1/2) (Ikonomidou and Turski, 2010). This disruption can induce the neurodegeneration (neuroapoptosis) and cortical deformation during the development (Bittigau et al., 2003; Kim et al., 2007). Further to those proapoptotic actions, AEDs can delay the cellular differentiation, neurogenesis, synaptogenesis, myelination, and axonal arborization (Ikonomidou and Turski, 2010; Bath and Scharfman, 2013). This disturbance may impair the normal brain development.

Herein, a novel observation is that the maldevelopment in the fetal cerebellar cortex due to the maternal administrations of CBZ seems to be due to the materno-fetal hypothyroidism and fetal GH-cytokine dysfunctions. Accordingly, the hypothyroidism can distort the nuclear TH receptors (TRs) (Ahmed, 2015), cerebellar nerve growth factor (NGF) (Singh et al., 2003), and developing cerebellum (Ahmed et al., 2012, 2014; Ahmed and Incerpi, 2013). These results are supported by Lasoń et al. (2011) who emphasized that AEDs disrupt the neuropeptides, neurosteroids, adenosine, antioxidant system, and genetic/epigenetic factors. This distortion leads to the impairment of the neurobehaviors (Tamijani et al., 2015), the production of oxidative stress (Aycicek and Iscan, 2007), and the neurotoxicity of developing cerebellum (Ikonomidou and Turski, 2010). Similar observations are reported by Ravizza and Vezzani (2006) who postulated that the dysregulations in the cytokines stimulate the inflammation, cell death, neuronal degeneration, and seizures. Thus, it can conclude that the CBZ has adverse actions in the endocrine and immune systems, that therefore alters the developing cerebellum. The severity of the maternofetal hypothyroidism and the dysfunctions of the fetal TH/GH-cytokines might be detrimental to the health of the fetuses.

5. Conclusion & future direction

Maternal administrations of CBZ caused a maternofetal hypothyroidism representing a good biomarker for the alterations of the fetal HPTA-cytokines-cerebellum axes. A new insight provided by this examination is that the maternal CBZ might act as a fetal immunoneuroendocrine disruptor producing complex and mosaic actions during the prenatal period. These disordered became substantial in the 50 mg CBZ-treated group (Fig. 2). Thus, we suggest that CBZ might be unsafe during pregnancy. However, these effects might rely on the concentration of CBZ, route of administration, and animal species (age, developmental period, and sex type). Additional examinations are required to corroborate these results with the human health to maximize the maternal health and minimize the fetal risk. Future examinations are vital to exploring the impact of the maternal CBZ on the gene expression during the development.

Conflict of interest

None stated.

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