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Protection against Neurobehavioral Changes Induced by Bisphenol A during Development in Rats

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ABSTRACT

Background: Bisphenol A (BPA) is an environmental estrogenic pollutant It is used in the manufacture of plastic products including beverages, dental materials and baby bottles. Exposure to BPA is unavoidable and recognized as major public health risk particularly in developing countries. Critical effects of BPA toxicity mostly occur during fetal development and postnatal development. Zinc (Zn) is an essential element for the endogenous enzymatic antioxidant processes. It is required for cell proliferation, differentiation, normal growth, immune functions and wound healing. Selenium (Se) is also nutritionally essential element with antioxidant potential. It protects brain from oxidative damage in various models of neurodegeneration.

Objective: To investigate the influence of postnatal BPA exposure during lactation on the neonates of exposed rats as well as to investigate and compare the possible protective role of Zn and/ or Se against postnatal BPA- induced developmental and neurobehavioral alterations.

Methods: Lactating dams were divided into 5 groups (8 rats/ each). Dams received daily for 21 days (from parturition until weaning) the following: Saline (1 ml/kg, P.O) for control and BPA (40 mg/kg, P.O) either alone or in combination with Zn (20 mg/kg, P.O), Se (0.1 mg/kg, P.O) or both of them. All pups were daily evaluated for physical development and for neurobehavioral development. The performance in behavioral experiments as Neonatal T-maze and Open-field test (OFT) was also examined. Brain homogenates were used to evaluate monoamines level (DA, NE and 5-HT) and oxidative stress markers (SOD, GP_X and CAT).

Results: Postnatal BPA exposure induced significant prolongation in the time of appearance of downy hair, fur development, ear opening, righting reflex, cliff avoidance, negative geotaxis 25˚, palmar grasp and auditory startle in rat pups. BPA also showed significant reduction in the number of correct choices in T-maze as well as in ambulation frequencies in OFT while showed significant elevation in rearing frequencies and latency time of rat pups. There was also significant reduction in the brain oxidative stress markers (SOD, GP_X and CAT) as well as in NE and 5-HT in addition to significant elevation in DA content. On the other hand, Zn and/ or Se co-administration with BPA improved physical and neurobehavioral development as well as performance of pups in the behavioural experiments. They also showed significant elevation in the brain oxidative stress markers (SOD, GP_X and CAT), NE and 5-HT levels while significant reduction in the brain DA content as compared to BPA alone. There was significant elevation in the number of correct choices in Zn treated group only. Co-administration of Zn and Se has no more pronounced effect than each one alone except in the effect on brain monoamines and oxidative stress markers.

Conclusion: Postnatal BPA exposure delayed some aspect of physical and neurobehavioral development as well as behavioural functions as learning, locomotors and exploratory activities, in addition to emotionality disturbance. Zn and/or Se can protect against BPA-induced alterations. The protective effect of Zn either alone or in combination was more pronounced than Se concerning learning and some reflexes. However, the protective effect of Zn and Se combination has more pronounced effect regarding brain monoamines level and oxidative stress markers only than each of them alone.

INTRODUCTION

Bisphenol A (BPA) is an industrial plasticizer with known estrogenic properties, it is widely used for manufacturing epoxy resins (for lining food and beverage cans) and dental sealants [1,2]. BPA can easily leach out from the polycarbonate plastics and epoxy resins either during heating of cans or during contact with basic and acidic substances. It was confirmed that storage, brushing and dishwashing result in polymer degradation that leading to its release [3]. The exposure to BPA has been linked to numerous health effects on the body, while the mechanism of BPA on the developing brain is still poorly understood. Hazardous of BPA leads to different heart disease, diabetes, obesity and high blood pressure as well as liver toxicity [4,5]. BPA concentrations are higher in women than in men, in children than in adults and in individuals with low household income compared with higher income [6]. BPA is lipophilic and is detected in serum as well as in breast milk; it also accumulates in human fat [7]. BPA has potent effects especially during fetal and neonatal development due to limited capacity of liver to deactivate BPA in these stages. During development of fetus, exposure to BPA is of particular concern because it can cross the placental barrier and accumulates in the fetus as well as in the placenta [8]. Prenatal exposure to BPA is associated with changes in hypothalamic pituitary and cognitive function of offspring [2,9], while continued exposure during gestation can lead to intra-uterine growth retardation [10].

Previous studies have focused on the risks of prenatal BPA exposure on the reproductive system development as well as on the nervous system development and suggested widespread negative consequences. Interestingly, exposure to small amount of BPA even below the accepted daily exposure level either during perinatal and postnatal or during adult period resulted in adverse effects on brain structure and function [11,12] through targeting synaptogenesis, memory consolidation, and dendritic development. Indeed, synaptic plasticity in the hippocampus and prefrontal cortex has been influenced by BPA, where it affects the synaptic interface encompassing the synaptic cleft and presynaptic active zone [13,14]. However, the association between BPA and the subsequent development of the brain and its functions is still unclear. Consequently, it is very important to investigate the impact of BPA exposure during postnatal development that represents the critical period of brain development in which it is highly plastic and extremely vulnerable to environmental insults [11]. Despite the fact that childhood is the greatest period of BPA exposures [15,16], exposure during postnatal period has not been completely studied.

Zinc (Zn), is a known fundamental component of the endogenous enzymatic antioxidant system. It plays an essential role in cell membrane integrity and functions in many aspects of cellular metabolism. It acts as antioxidant, or a cofactor for the antioxidant enzyme. Zn is essential for normal development of the central nervous system during fetal and postnatal life, and is required for the formation and function of a variety of proteins, enzymes, hormones, and growth factors that direct stem cell proliferation and differentiation during neurodevelopment [17-19]. Furthermore, Zn is involved also in metabolic processes outside of the CNS such as hormones transport and production of neurotransmitter precursors that will eventually affect the brain functions [18,19]. Moreover, Zn is involved in other biological processes, including the DNA synthesis, normal growth, behavioral responses, brain development, bone formation, and reproduction [20]. There is evidence that Zn deficiency results in growth retardation, testicular atrophy, skin and mucous membranes changes, poor appetite, delayed wound healing, cell-mediated immune dysfunction, and abnormal neurosensory responses [21]. Also, it was proposed that nervous system disorders, including mental disturbances and loss of sensory acuity are related to Zn deficiency [22].

Deficiency of Zn may be associated with increased stress and may altered emotional reactivity and depression-induced behavior as aggressiveness and anxiety along with cognitive deficits and learning-memory impairments [23,24]. It has been illustrated that Zn supplementation improved spatial learning, cognitive function, and locomotor activity of rat's offspring during the prenatal and early postnatal period. Zn is also considered an important nutrient during embryogenesis, foetal growth and functions development. It plays an important role in maintaining cellular function in the mammary gland for milk synthesis as well as for secretion [25]. Dietary Zn intake prevents alterations in the body status of lipids, so protect against some toxic effects, including oxidative damage to the cellular membranes and atherogenic action [26]. Also, the administration of Zn enhances the bone alkaline phosphatase activity and prevents bone resorption, so dietary intake of Zn may have a protective influence on the skeleton [27].

Selenium (Se), is an essential element for humans, which improves the activity of the seleno-enzyme. It is present in the active center of glutathione peroxidase (GPx), an antioxidant enzyme, which protects lipid membranes and macromolecules from oxidative damage produced by peroxides. Furthermore, it has been reported that Se has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against oxidation injury [28]. However, the most important

selenoprotein for cerebral functions is probably selenoprotein P [29]. This protein is synthesized at the cerebral level and protects the brain against free radicals damage, particularly peroxinitrite [30]. Furthermore, Se status decreases with old age [31]. Therefore, Se deficiency may be a risk factor for a decline of cognitive functions. Selenium is another important co-factor and epidemiological findings have linked a lowered Se status to neurodegenerative and cardiovascular diseases as well as to an increased risk of cancer [32]. There is an association between Se reduction and DNA damage, and oxidative stress, and some pieces of evidence that Se may affect not only cancer risk but also progression and metastasis [33]. Se intervention in subjects with a lower Se status has shown some benefits in reducing the incidence and mortality in all cancers but more specifically in liver, prostate, colo-rectal and lung cancers. Its protective effects appear to be associated with its presence in the multiform of glutathione peroxidases, which are known to protect DNA and other cellular damage from oxidative stress [34,35].

Depending on the above consideration, the aim of the present work was designed to roll out the impact of postnatal BPA exposure, exactly during lactation which is the critical period of brain development as well as the most sensitive period for brain insults. It also aimed to investigate and compare the possible protective effect of Zn and/ or Se against postnatal BPA exposure which is postulated to induce developmental and neurobehavioral impairment in the neonates.

MATERIALS AND METHODS

Animals

Adult male and female Sprague-Dawley rats (males weighing 200-220 g; females weighing 180-200 g) were used in this study. They were obtained from NILE Co. of Pharmaceutical and Chemical Industries, Egypt. Animals were kept under adequate environmental conditions. They were kept on standard diet pellets and water was given ad-libitum. Rats were housed (three to four / cage) in stainless–steel cages and kept one week prior to mating for accommodation. The work was performed according to the ethical guidelines of Al-Azhar University (Faculty of Pharmacy), Egypt.

Drugs and Chemicals

Bisphenol A, Zinc sulfate and Selenium were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Bisphenol A was freshly prepared in corn oil using tween 80 as a surfactant. Both Zinc sulfate and Selenium were freshly dissolved in distilled water.

Experimental Design

After parturition lactating dams were divided into 5 groups.

Group 1: Dams received saline (1 ml/kg) orally daily by gavage from parturition postnatal day 0 (PND 0) until weaning postnatal day 21 (PND 21) and served as control.

Group 2: Dams were received BPA solution orally daily by gavage (40 mg/kg) from parturition (PND 0) until weaning (PND 21) [36].

Group 3: Dams were received BPA solution orally daily by gavage (40 mg/kg) and after 30 minutes received Zn solution (20 mg/ kg) from parturition (PND 0) until weaning (PND 21) [37].

Group 4: Dams were received BPA solution orally daily by gavage (40 mg/kg) and after 30 minutes received Se solution (0.1 mg/ kg) from parturition (PND 0) until weaning (PND 21) [38,39].

Group 5: Dams were received BPA solution orally daily by gavage (40 mg/kg), after 30 minutes received Zn solution (20 mg/ kg) and after 30 minutes received Se solution (0.1 mg/kg) from parturition (PND 0) until weaning (PND 21).

Development of reflexes and sensory functions (neurobehavioral development) including Righting reflex, Cliff avoidance, Negative geotaxis 25°, Palmar grasp, and Auditory startle [40]. In addition rat pups were evaluated in two types of behavioral experiments: performance in the Neonatal T-maze: in which number of correct choices /10 trials was measured and performance in the Open-field test: in which latency time, ambulation frequency, rearing frequency and grooming time were measured. Additionally, the brain homogenate was used to evaluate brain monoamines levels, (DA, NE and 5-HT). Finally the brain homogenate was used to investigate oxidative stress markers (SOD, GPX and CAT) enzyme activities.

Physical Development

For each infant rat (3-4 pups/litter), time at which each of the following physical signs had been evident was recorded [40,41]. All tests were carried out on rat pups from parturition (PND 1) until weaning (PND 21).

- ***Pinna detachment:*** *Unfolding of the external ear was daily observed and recorded.*
- ***Downy hair:*** TThe appearance of primary coat of downy hair was daily observed and recorded.
- ***Fur development:*** *Complete development of fur was observed and recorded daily.*
- ***Ear opening:*** Complete opening of both ears was observed and recorded daily.

• ***Eye opening:*** *Complete opening of both eyes was daily observed and recorded.*

Neurobehavioral Development

For each infant rat (3-4 pups/litter), time at which each of the following reflexes as well as sensory functions had been evident was recorded [40,41]. All tests were carried out on rat pups from parturition (PND 1) until weaning (PND 21).

Righting reflex: The pups were placed on its back (the supine position) and monitoring the time taken to turn over and stand upright, two trials per day with a maximum time allowance of 30 seconds per trial

Cliff avoidance: The pups were placed on an edge of a cliff or a table top, and see if they are able to avoid drop, show retraction and backward movement within 60 sec.

Negative geotaxis 25°: The pups were placed on 25°an inclined plywood surface where placed in a head down position, and observed for up to 60 seconds. Each pup was given 1 trial per day.

Palmar grasp: Determine the ability of the pups to grasp a paper clip with forepaws if stroked. Each pup was given 1 trial per day.

Auditory startle: Observed until a positive startle response (the head jerk and the hind limbs extend) occurred to an auditory stimulus 15 cm over the head of the pups, each infant was given one trial per day and the presence or absence of startle response can be readily recognized.

Behavioral Experiments

They include evaluation of the behavioral development in an unfamiliar environment for infant rats.

Performance in the neonatal T-maze: *T*he test was carried out on rat pups on the PND 22.

Infant rats were alternatively tested in the neonatal T-maze which was used as index of learning and memory. The neonatal T-maze is made of black acrylic and measured 5.1 × 30.5 × 5.1 cm in the stem and 5.1 × 25.4 × 5.1 cm in the cross arm [40]. A correct choice resulted in a door being opened to a connected chamber containing the pup's home bedding and its littermates while an incorrect choice led to a dead end, followed by being picked up and placed in an empty cage for 1 min of isolation. Gates at the entrance of each choice arm prevented retracing once a decision was made. Number of correct choices out of ten trials was recorded for each neonate.

Open-Field Test (OFT): The test was carried out on rat pups on the PND 23. It is originally introduced as a measure of emotional behavior in rodents. This test provides a common measure for the assessment of motor activity, excitability, emotionality and exploratory behavior in rodents. OFT is a very important procedure in the majority of behavioral studies. It consists of a square wooden box 40 cm × 40 cm × 25 cm height [42], with red walls and white floor divided into 16 equal squares (4 × 4 cm each) using permanent paint [43,44]. The experiment was performed under white light in a quiet room between 10:00 A.M. - 4:00 P.M. All animals were taken to test situation after removing food and water from the home cage one hour before the experiment. Experimental animal were taken from their cages alternately; then gently place animal in the centre of the open field and videotaped for 3 min [45]. At the end of each trial, the open-field was thoroughly clean the arena with 10% isopropyl alcohol, and dried with paper toweling before application of a new subject in order to prevent possible effect on its behavior due to odor remained from previous rats [46].

The behavior of the experimental rat in the OPF was continuously monitored during the 3 min. observation period [44], using coded symbols for the following parameters: Latency: Time in seconds elapsed from putting the animal at the middle of the arena till it makes the first move [41]. It was measured in seconds using a stopwatch. Ambulation frequency: number of squares crossed by the animal [43,47]. It was scored as a total count during a 3 min. period. Rearing frequency: number of time the animal stood stretched on its hind limbs with and without forelimbs support [48]. It was scored during a 3 min. observation period. Grooming time: time elapsed while the animal scratching face, licking paws, fur or genitals [48].

Neurochemical Parameters (Dopamine (DA), Norepinephrine (NE) and Serotonin (5-HT) levels:

Rapidly after the last test, rats were sacrificed with minimum disturbance to avoid any changes in the concentrations of brain monoamines that may occur within few minutes [49]. Determination of dopamine was achieved using commercial ELISA kit (Dopamine Research EIA) from Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Product Number BA-10-0300 [50,51]. Determination of noradrenaline was done using commercial ELISA kit (Noradrenaline Research EIA) from Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Product Number BA-10-0200 [51,52]. Finally determination of serotonin was done using commercial ELISA kit from Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Product Number BA E-5900 [53].

Oxidative Stress Markers (Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx) and catalase (CAT) Enzymes Activities):

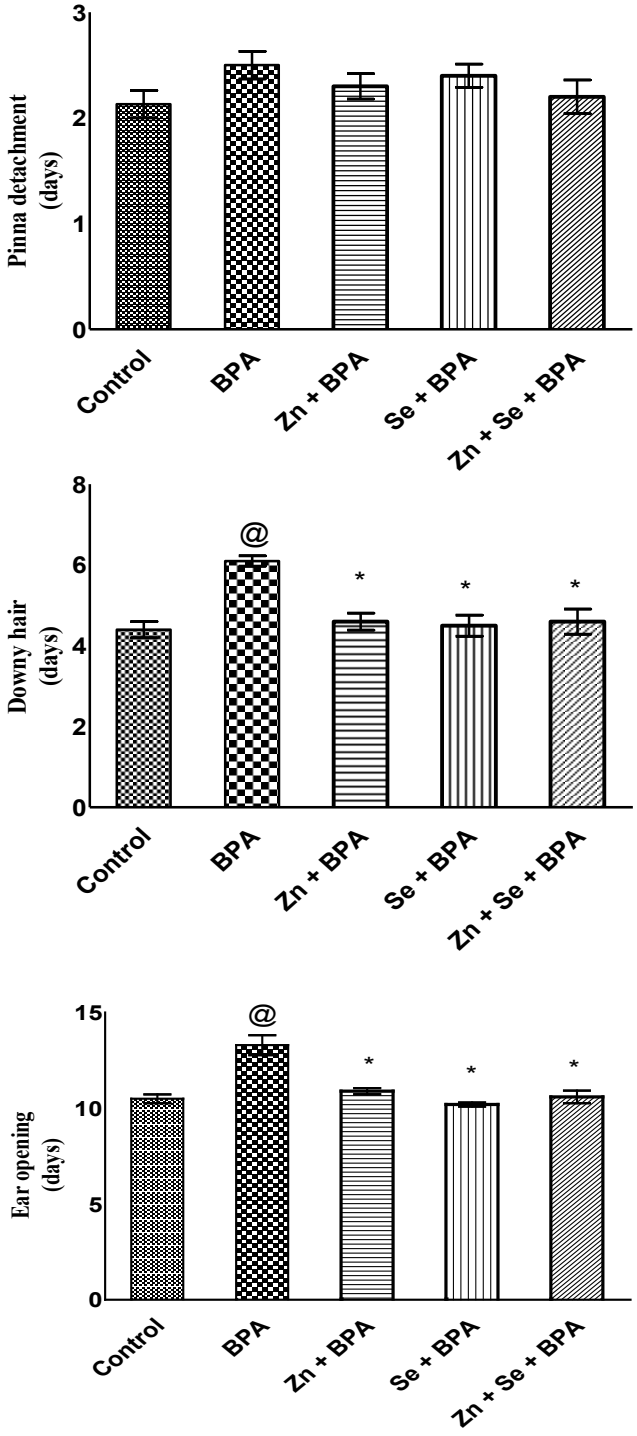
They were measured in the brain homogenate for each rat. SOD activity was assessed relying on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye [54]. The increase in absorbance at 560 nm for 5 min is measured. Determination of GP_x was achieved by the oxidation from glutathione (GSH) to oxidized glutathione (GSSG), the

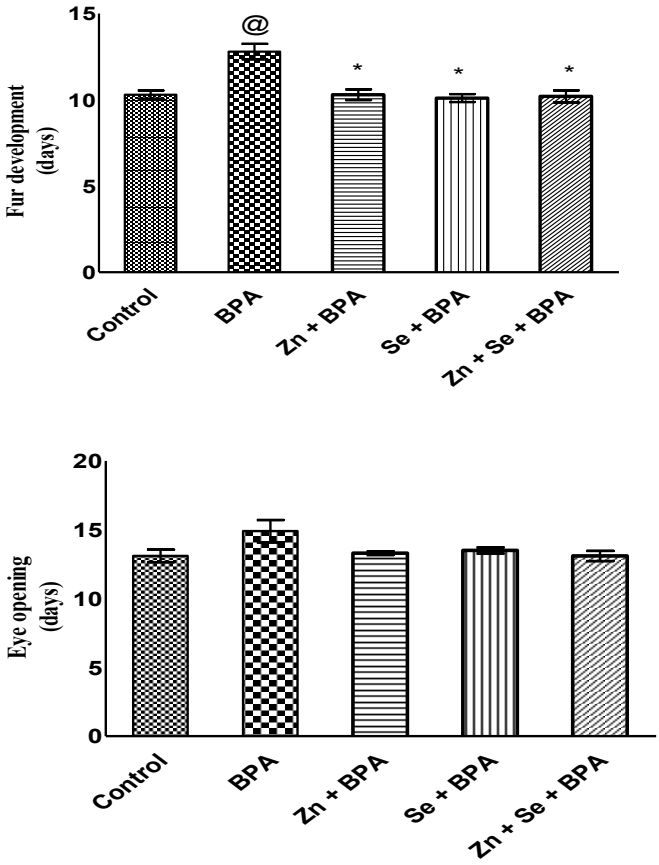
reaction was catalyzed by GPx enzyme, then coupled to the recycling of GSSG back to GSH using glutathione reductase (GSSG-R) and NADPH (β−Nicotinamide Adenine Dinucleotide Phosphate,Reduced). NADPH is oxidized to NADP +. The oxidation of NADPH to NADP + is monitored spectrophotometrically by a decrease in absorbance at 340 nm and is indicative of GPx activity, where the amount of GPx in the test sample is the rate-limiting factor of the coupled reactions [55]. On the other hand, determination of CAT is based on the decrease in light absorption at 240 nm caused by hydrogen peroxide decomposition by CAT [56].

Statistical Analysis:

Data are presented as mean ± SEM. Multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. All statistical analyses were performed using Instat (version 3) software package. Graphs were sketched using GraphPad Prism (ISI®, USA) software (version 5).

RESULTS





Note: Appearance time (days) of (i) Pinna detachment, (ii) Downy hair, (iii) Fur development, (iv) Ear open, (v) Eye open in the neonatal rats. Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,* significantly different from control or from BPA treated group at P<0.05 by using one way ANOVA followed by Tukey–Kramer multiple comparison test.

Figure 1. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on physical development of neonatal rats.

Development of Physical Signs

As shown in **Figure 1** BPA administration did not significantly prolong the time of appearance of pinna detachment in the pups. Also, Zn or Se co-administered with BPA did not significantly prolong the time of appearance as compared to BPA treated group. Additionally, co-administration of Zn and Se along with BPA did not significantly prolong the time of appearance of pinna detachment in their pups as compared to BPA, (Zn+BPA) and (Se+BPA) treated groups.

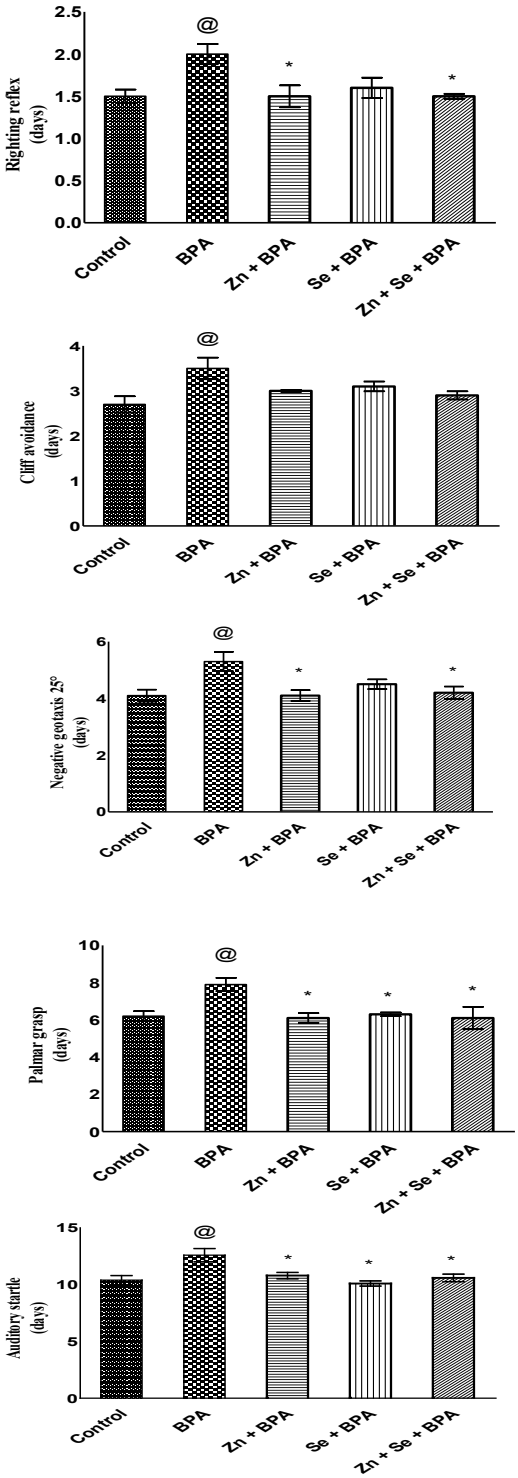
Administration of BPA induced a significant prolongation in the time of appearance of downy hair to 138.6% as compared to normal control value. However, Zn or Se co-administered with BPA significantly decreased the time of appearance of downy hair in their pups to 75.4% and 73.8% as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA significantly decreased the time of appearance of downy hair to 75.4% as compared to BPA treated group, but did not significantly change the time of appearance of downy hair as compared to (Zn+BPA) and (Se+BPA) treated groups **(Figure 1)**.

BPA administration significantly prolonged the appearance time of fur development in the pups to 124.3% compared to the value of normal control. However, Zn or Se co-administered with BPA significantly decreased appearance time of fur development in the pups to 80.5% or 78.9% as compared to BPA group. Additionally, co-administration of Zn and Se along with BPA significantly decreased the appearance time of fur development in the pups to 79.7% as compared to BPA treated group, but did not significantly change the time of appearance of downy hair in their pups as compared to (Zn+BPA) and (Se+BPA) treated groups.

Administration of BPA induced a significant prolongation in the time of appearance of ear opening in their pups to 126.7% as compared to normal control value. However, Zn or Se co-administered with BPA significantly decreased the time of appearance of ear opening in the pups to 82% and 76% as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA significantly decreased the time of appearance of ear opening to 79.6% as compared to BPA treated group, but did not significantly change the time of appearance as compared to (Zn+BPA) and (Se+BPA) treated groups.

BPA administration did not significantly change the appearance time of eye opening in the pups. Also, Zn or Se co-administered with BPA did not significantly change the appearance time of eye opening in the pups as compared to BPA treated group. Additionally, co-administration of Zn and Se along with BPA did not also change the time of eye opening in the pups as

compared to BPA, (Zn+BPA) and (Se+BPA) treated groups.



Note: Appearance time (days) of (i) Righting reflex, (ii) Cliff avoidance, (iii) Negative geotaxis 25°, (iv) Palmar grasp, (v) Auditory startle in the rats neonates. Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,* significantly different from control or from BPA treated group at P<0.05 by using one way ANOVA followed by Tukey–Kramer multiple comparison test.
Figure 2. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on development of reflexes and sensory function of neonatal rats.

Development of Reflexes and Sensory Functions (Neurobehavioral Development)

As illustrated in **Figure 2** BPA administration significantly increased the time of appearance (days) of righting reflex in their pups to 133.33% as compared to normal control value. In contrast, Zn co-administered with BPA significantly decreased the time of appearance of righting reflex in the rat pups to 75% as compared to BPA treated group, while Se co-administered with BPA

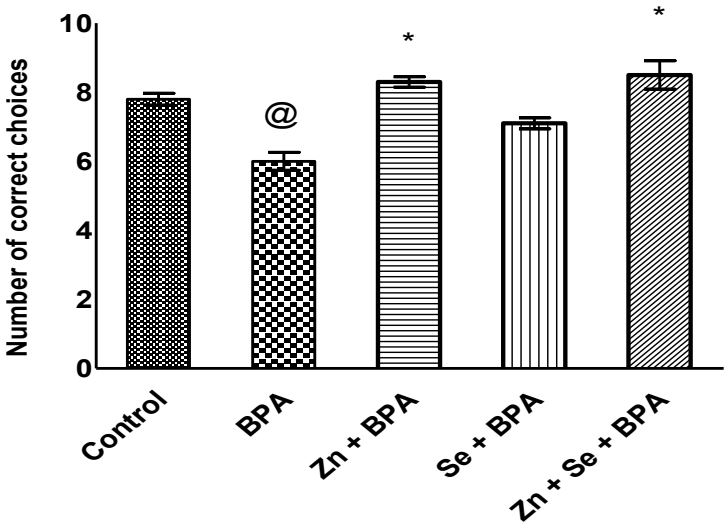
did not significantly alter the time of appearance of righting reflex in their pups as compared to BPA treated group. However, co-administration of Zn and Se along with BPA significantly decreased the time of appearance in their pups to 75% as compared to BPA treated group, but did not significantly alter the time of appearance as compared to (Zn+BPA) and (Se+BPA) treated groups **(Figure 2)**.

Administration of BPA significantly increased time of appearance of cliff avoidance in their pups to 129.63% as compared to normal control value. However, Zn or Se co-administered with BPA did not significantly alter the time of appearance of cliff avoidance in their pups as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA did not significantly alter the time of cliff avoidance appearance in rat pups as compared to BPA, (Zn+BPA) and (Se+BPA) treated groups.

BPA administration significantly increased appearance time (days) of negative geotaxis 25° of the rat pups to 129.3% as compared to control. In contrast, co-administration of Zn with BPA significantly decreased the appearance time to 77.36% as compared to BPA group. However, Se co-administration did not significantly alter time of negative geotaxis 25° appearance. On the other hand, co-administration of both Zn and Se along with BPA significantly decreased the time of appearance of negative geotaxis 25° in the rat pups to 79.24% as compared to BPA group, but did not alter it as compared to either Zn or Se alone.

BPA administration significantly increased the time of appearance (days) of palmar grasp in their pups to 127.42% as compared to normal control value. In contrast, Zn or Se co-administered with BPA significantly decreased the time of appearance of palmar grasp in their pups to 77.22% and 79.74% respectively as compared to BPA treated group. Additionally, co-administration of Zn and Se along with BPA significantly decreased the time of palmar grasp appearance in the pups to 77.22% as compared to BPA treated group, but did not significantly affect the time of appearance as compared to (Zn+BPA) and (Se+BPA) treated groups.

Administration of BPA significantly increased time of appearance (days) of auditory startle in rat pups to 121.15% as compared to control value. In contrast, Zn or Se co-administered with BPA significantly decreased time in their pups to 85.71% and 80.16% as compared to BPA treated group respectively. Additionally, co-administration of Zn and Se along with BPA significantly decreased the time of appearance of auditory startle in the pups to 84.13% as compared to BPA treated group, but did not significantly alter the time of appearance as compared to (Zn+BPA) and (Se+BPA) treated groups.

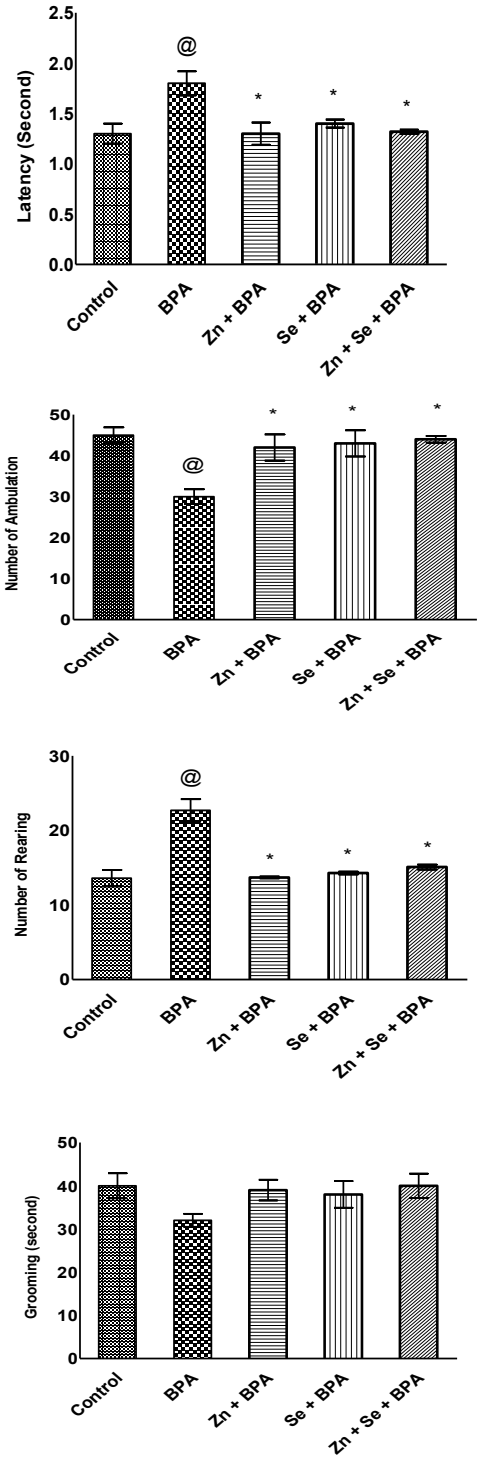


Note: Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,* significantly different from control or from BPA treated group at P<0.05 by using one way ANOVA followed by Tukey–Kramer multiple comparison test.

Figure 3. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on performance in the neonatal T-maze.

Performance in the Neonatal T-Maze

As shown in **Figure 3**, BPA administration induced a significant reduction in the number of correct choice in T- maze in their pups to 76.92% as compared to normal control value. In contrast, Zn co-administered with BPA induced a significant elevation in the number of correct choices in T- maze in their pups to 138.33% as compared to BPA treated group. However, Se co-administered with BPA did not significantly alter the number of correct choices in T maze in the pups as compared to BPA treated group **(Figure 3)**. Additionally, co-administration of both Zn and Se along with BPA resulted in significant elevation in correct choices number of the pups in T- maze to 141.7% as compared to BPA group, but did not significantly alter them as compared to either Zn or Se alone.



Note: Latency time (i), Ambulation frequency (ii), Rearing frequency (iii), Grooming time (iv) of the neonates.
Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,* significantly different from control or from BPA treated group at $P<0.05$ by using one way ANOVA followed by Tukey–Kramer multiple comparison test.

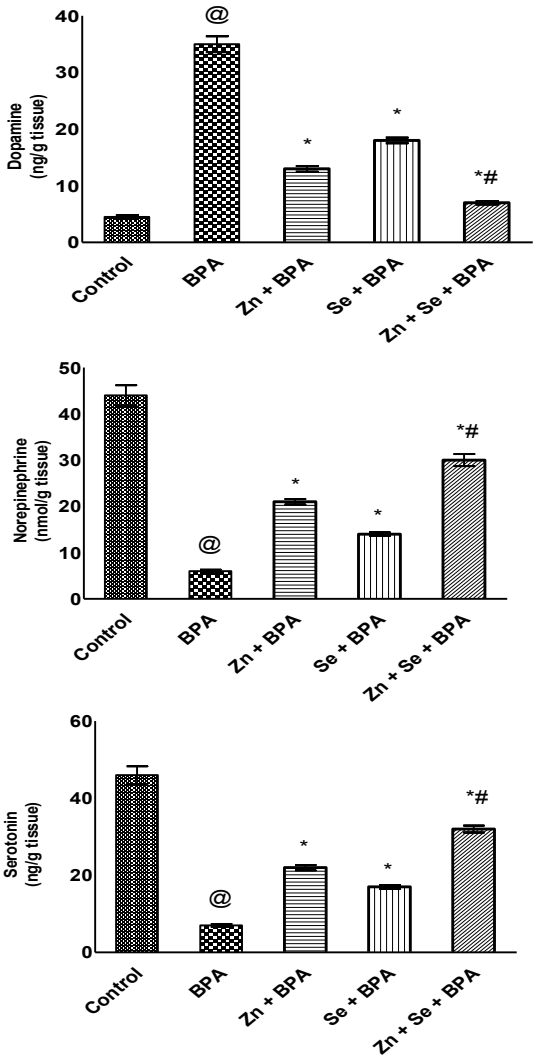
Figure 4. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on the performance of neonatal rats in the open-field test.

Performance in the Open-Field Test (OFT)

As illustrated in **Figure 4** BPA administration induced a significant elevation in the latency time in their pups to 138.5% as compared to normal control value. In contrast, Zn or Se co-administered with BPA induced significant reduction in the latency time to 72.2% and 77.8% respectively as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA induced significant reduction in the latency time to 73.3% as compared to BPA treated group, but did not significantly change the latency as compared to (Zn+BPA) and (Se+BPA) treated groups. BPA administration induced significant reduction

in ambulation frequency in rat pups to 66.7% as compared to normal control value. In contrast, Zn or Se co-administered with BPA induced a significant elevation in the ambulation frequency of pups to 140% and 143.33% respectively as compared to BPA treated group **(Figure 4)**. Additionally, co-administration of both Zn and Se along with BPA induced significant elevation in the ambulation frequency in their pups to 146.7% as compared to BPA treated group, but did not significantly change ambulation frequency as compared to (Zn+BPA) and (Se+BPA) treated groups respectively.

Administration of BPA induced significant elevation in the rearing frequency to 167% as compared to control value. In contrast, Zn or Se co-administered with BPA induced a significant reduction in rearing frequency in their pups to 60.4% and 63% respectively as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA induced a significant reduction in the rearing frequency in to 66.5% as compared to BPA treated group, but did not significantly change rearing frequency as compared to (Zn+BPA) and (Se+BPA) treated groups. BPA administration did not significantly change the grooming time as compared to control. Also, Zn or Se co-administered with BPA did not significantly change the grooming time in their pups as compared to BPA treated group. Co-administration of both Zn and Se along with BPA did not also significantly change the grooming time in their pups as compared to BPA, (Zn+BPA) and (Se+BPA) treated groups.



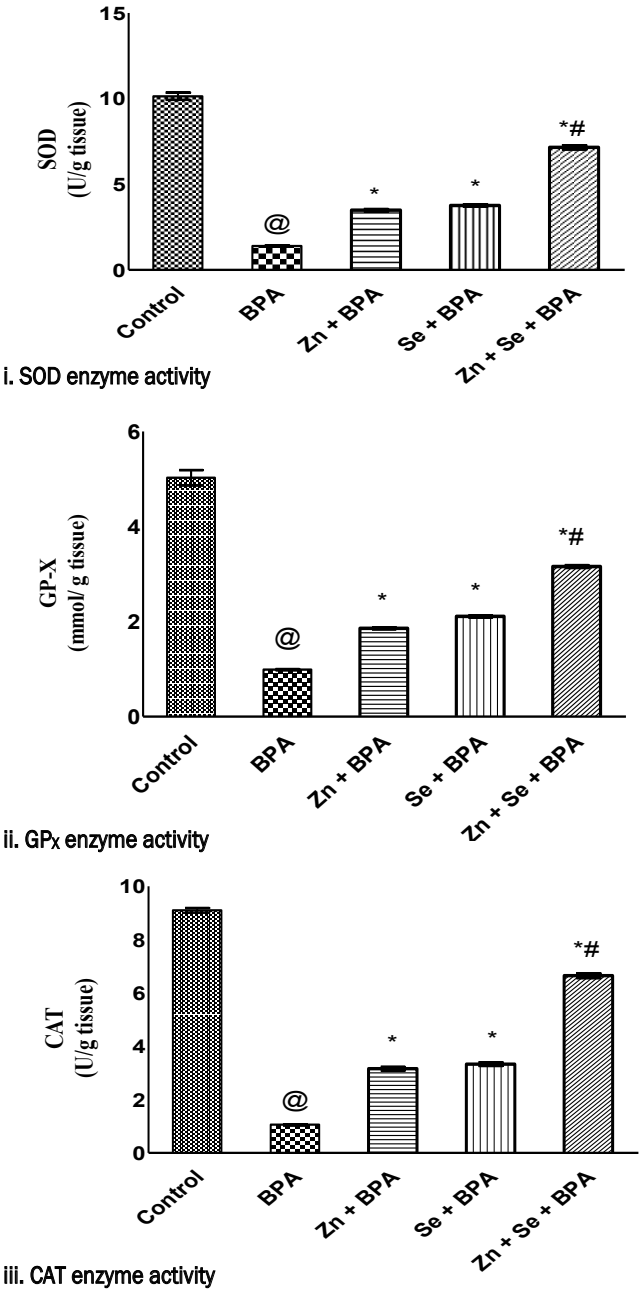
Note: Dopamine (i), Norepinephrine (ii), Serotonin (iii) levels in brain of the neonates.
Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,*, # significantly different from control, BPA and Zn or Se treated groups respectively at $P<0.05$ using one way ANOVA followed by Tukey – Kramer multiple comparison test.

Figure 5. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on brain monoamines level of neonatal rats.

Neurochemical Parameters

As illustrated in **Figure 5**, BPA administration induced a significant elevation in the brain DA content to 777.8% as compared to normal control value. In contrast, Zn or Se co-administered with BPA induced a significant reduction in the brain DA content to 37.14% and 51.43% as compared to BPA treated group. Co-administration of both Zn and Se along with BPA induced significant reduction in brain DA content to 20%, 53.85% and 38.97% as compared to BPA, (Zn + BPA) and (Se + BPA) treated groups

respectively. Administration of BPA induced significant reduction in brain NE level to 13.64% as compared to control. In contrast, Zn or Se co-administered with BPA induced a significant elevation in the brain NE level to 350% and 233.3% respectively as compared to BPA treated group. Additionally, co-administration of both Zn and Se along with BPA induced a significant elevation in the brain NE level to 500%, 142.86% and 214.3% as compared to BPA, (Zn + BPA) and (Se + BPA) treated groups respectively. BPA administration also induced significant reduction in brain 5-HT level to 15.2% as compared to control. In contrast, Zn or Se co-administered with BPA induced significant elevation in brain 5-HT level to 314.3% and 242.9% respectively as compared to BPA. Co-administration of both Zn and Se along with BPA induced a significant elevation in brain 5-HT level to 457.14%, 145.5% and 188.23% as compared to BPA, (Zn + BPA) and (Se + BPA) treated groups respectively **(Figure 5)**.



Note: SOD (i), GPX (ii), CAT (iii) in brain of the neonates.
Values are expressed as (Means ± S.E.M).
Neonates number in each group (n)=28-32.
@,*, # significantly different from control, BPA and Zn or Se treated groups respectively at P<0.05 using one way ANOVA followed by Tukey – Kramer multiple comparison test.

Figure 6. Effect of Zinc (Zn), Selenium (Se) and their combination with Bisphenol A (BPA) on brain antioxidant enzymes of neonatal rats.

Oxidative Stress Markers (SOD, GP_x and CAT)

As illustrated in **Figure 6**, BPA administration induced a significant reduction in the brain SOD activity to 13.8% as compared to control value. In contrast, Zn or Se co-administered with BPA induced a significant elevation in the brain SOD activity to 249.3% and 268.6% respectively as compared to BPA treated group. Co-administration of Zn and Se along with BPA induced a significant

elevation in brain SOD activity to 511.4%, 205.2% and 190.4% as compared to BPA, (Zn + BPA) and (Se + BPA) treated groups respectively. Administration of BPA induced a significant reduction in brain GPX activity to 19.7% as compared control. In contrast, Zn or Se co-administered with BPA induced a significant elevation in the brain GPX activity to 187.9% and 213.13% respectively as compared to BPA group. Additionally, co-administration of both Zn and Se along with BPA induced a significant elevation in brain GPX activity to 319.2%, 170% and 149.8% respectively as compared to BPA, (Zn + BPA) and (Se + BPA) groups. BPA administration induced a significant reduction in the brain CAT activity to11.6% as compared to control. In contrast, Zn or Se co-administered with BPA induced a significant elevation in the brain CAT activity to (307.55% and 314%) respectively as compared to BPA treated group **(Figure 6)**. Additionally, co-administration of both Zn and Se along with BPA induced a significant elevation in brain CAT activity to 627.4%, 199.7% and 210.4% as compared to BPA, (Zn + BPA) and (Se + BPA) treated groups respectively.

DISCUSSION

Bisphenol-A (BPA) is widely used for manufacturing of polycarbonate plastics, epoxies and resins and is found in numerous household objects such as water bottles, can linings, baby bottles, and dental resins. The exposure to BPA has been associated with many negative health effects on the body, but the mechanism of BPA on the brain during development is poorly understood until now. In this work, we want to investigate whether BPA exposure during a developmental phase when brain connectivity is being organized can cause long-term deleterious effects on brain function and plasticity that outlast the BPA exposure [2,57].

In the current study, the postnatal BPA exposure was carried out during lactation period. It is well known that, BPA has been transferred from mother to fetus during lactation [58]. A pervious study has shown that the BPA has been detected in adult serum, maternal circulation [59], amniotic and placental fluids [60], breast milk [58,61], and the urine of infants [6]. Results of the current study showed that, postnatal administration of BPA to lactating dams induced a decline in the postnatal physical and neurobehavioral development in rat pups as indicated by prolongation in the time of appearance of some landmarks of physical growth (including downy hair, fur development, ear opening) and by prolongation in the time of appearance of some reflexes and sensory function (Righting reflex, cliff avoidance, negative geotaxis 25°, palmar grasp, and auditory startle) in rats pups, with respect to corresponding control group. BPA exposure weren't altering most of the physical sign which have been evident early in the postnatal life as pinna detachment, eye opening. These findings are in agreement with Ema et al. [62], who found that developmental landmarks (e.g., age at eye opening) were reported to be unaffected by oral BPA treatment at doses ranging 0.2–200 μg/kg/day to rats.

The present study disagrees with the work of Mendl and Paul [63]; Cameron [64] and Palanza et al. [65], which showed that two reflexive responses, the righting reflex and cliff-drop aversion, which can provide information concerning physical and motor development as well as sensory function and/or processing. The two reflexive behaviors and the growth rates were not altered by developmental exposure to BPA via the mother. Also, Ema et al. found that BPA treatment had no significant effects on righting reflex. Thus, it would appear that this early behavior is resistant to oral BPA treatment [62].

The data of the current work revealed that postnatal Zn and/or Se treatment ameliorated BPA induced deterioration in the physical and neurobehavioral development in pup rats indicated by acceleration in the time of appearance of some sign of physical and neurobehavioral maturation which were delayed by BPA (as downy hair, fur development, ear opening, palmar grasp and auditory startle), when Zn and/ or Se were co-administered along with BPA dams, while Zn either alone or in combination induced a significant decrease in the time of appearance of righting reflex and negative geotaxis 25° in rat pups as compared to BPA treated group. Perinatal or neonatal BPA exposure affects brain development and behaviors [66]. BPA can induce aggression, anxiety, cognitive deficits, and learning-memory impairment [67]. The role of Zn in brain function is poorly understood. Zn is essential pre- and postnatally for growth, maturation, and function. In early pregnancy, zinc is essential for cell multiplication and implantation of the embryo and for cell differentiation and organ formation. Zn deficiency causes teratology in all tissues and in later pregnancy, impairs neuronal replication and migration. It has been reported that Zn deficiency impairs calcium channels causing a decrease in intracellular calcium that suppresses gene expression of growth factors and synthesis of nucleic acids and proteins. Finally, it was found that whatever the mechanism, Zn deficiency in experimental animals cause poorly reversible impairments in learning and memory later in life, which appears associated with decreased neuronal survival. It is unknown if similar phenomena occur in humans. It is known, that maternal Zn deficiency causes a low fetal growth, which is a risk factor for coronary heart disease, type 2 diabetes mellitus, chronic lung disease, and obesity [68,69].

Selenium, an antioxidant and a main constituent of brain selenoproteins, may be particularly important for the maintenance of brain functions. Generally, Se is also recognized to be a trace element of great importance for human health which protects the cells from the harmful effects of free radicals [70]. Regarding antioxidant effects of selenium, it is believed that this trace element protects cells from the harmful effects of free radicals by inhibiting metal- mediated DNA damage. Its action is mediated through the glutathione peroxidases that inactivate hydroperoxides and hydrogen peroxide, potent lipid damaging factors [70-72].

In the present study, it was observed that exposure of BPA during lactation significantly decreased the number of correct choice of neonates in the T-maze. These data concluded that postnatal BPA exposure significantly induced learning- memory impairment in rat pups. These findings are compatible with numerous studies which demonstrated significant impairment of learning and memory during lactation or from beginning of gestation until weaning [73,74]. It has been shown that some immunohistochemical examination revealed that in mice that received prenatal and neonatal exposure to low and high doses of BPA, the level of choline acetyltransferase- like immunoreactivity (ChAT-IR) in many regions of the hippocampus dramatically decreased, when compared with the control [75]. In particular, the density of the cholinergic fiber was dramatically decreased in mice that received prenatal and neonatal exposure to low and high doses of BPA, when compared with the control [75]. It is widely accepted that cholinergic function in the hippocampus is important for learning and memory processes [73,76,77]. These results

strongly support the findings that memory impairment remarkably corresponded to dysfunction of the cholinergic neurons in the hippocampus of mice that received prenatal and neonatal exposure to BPA. Some of the other mechanisms for BPA have been proposed, BPA has been reported to act as an antagonist of thyroid receptors [73,78]. It is widely known that thyroid hormones are essential for brain development.

Several findings indicate that BPA induces oxidative stress in many organs [79,80]. BPA facilitates the production of reactive oxygen species (ROS) and thus results in neuronal damage [1,81]. Moreover, BPA and some bisphenols have been reported to cause a reduction in mitochondrial functions [82]. It is widely accepted that during development, neurons are highly sensitive to ROS. Although further studies are needed to clarify the effects of BPA on the developmental processes, the findings of these reports suggest that oxidative stress may contribute to the action of BPA. Also, it has been shown that hippocampus is particularly sensitive to BPA in animal models and in humans. *In vivo*, BPA exposure during pregnancy and lactation has been shown to decrease neuronal density in the hippocampus [83,84]. Several studies have reported alterations in hippocampal morphology with altered estradiol and testosterone induced spine synapse formation not only in rats but also in non- human primates following BPA exposure [1,85,86]. Synapse plasticity in the striatum is also perturbed after pre- and postnatal exposures with alteration of the function of dopaminergic receptors and disturbance in the developmental pattern of synaptic plasticity, which causes controlling deficits in motor behavior [86]. Some studies [87], reported that the exposure to BPA during pre- and postnatal developments has long-lasting effects on central dopaminergic systems linked with behavioural rewarding effects. *In vitro* exposure to BPA results in a marked influence on synaptogenesis and potentially neuronal vulnerability in hippocampal cultures [86,88]. The effects of BPA on synaptogenesis could be mediated through several mechanisms, including a number of receptor systems, such as estrogen (ER-□ and ER-B) and thyroid receptors (TRs). As synapse remodeling in the hippocampus is implicated in memory acquisition, retention and learning [89], it is likely that BPA exposure during critical periods of development could lead to altered cognitive function. Moreover, direct neuronal toxicity of BPA induced apoptotic cell death has been suggested to be through calcium, ROS *in vitro*. In contrast, NF-kappaB cascade was activated for survival signaling after BPA treatment [1]. Therefore the mechanisms leading to decrease of hippocampal volume and neuronal density seen in this study are likely multi-factorial not only with a direct toxicity of BPA on the developing neurons, but also with BPA's ability to alter synaptogenesis that would lead to increased developmental pruning of neurons unable to establish sufficient synaptic activity for survival. The decrease in hippocampal neuronal density and volume may reflect inadequate accelerated neuronal differentiation and maturation with an increase in the normal developmental process of programmed cell death [74,90].

The present work showed that Zn either alone or in combination significantly increased correct choices in the neonatal T- maze. In previous study, it was shown that Zn administration improved spatial learning and memory in rat pups after pre- and postnatal [91]. Our results are in harmony with numerous studies which demonstrated that Zn supplementation may improves behavioral deficits such as cognitive impairment induced by various insults such as maternal Zn deficiency [92], and following traumatic brain injury [93]. Zn deficiency induces several neurobehavioral abnormalities as anxiety and impairments in learning and memory during fetal life, lactation and early postnatal period, and it was shown that Zn administration improves spatial learning and memory in rat pups during pre- and postnatal [91,94,95]. Also, it was found that Zn has an important role in cognition and memory via its function as a neuronal messenger, modulator of synaptic transmission and cortical plasticity [96]. Zn is a key modulators of neuronal excitability via it is important role for myelination and for the release of the neurotransmitters GABA and glutamate also, the neuromodulatory effect of Zn may be connected with its antioxidant properties. Thus the protective effect of Zn supplementation on cognitive function may also depend on its anti-oxidative action. It has been revealed that the essential trace element Zn plays an important role in the control of both developmental and adult neurogenesis [97-99]. The findings of the present work are in harmony with other findings [91].

Data obtained from the current work suggested that postnatal BPA exposure induced a significant reduction in locomotor activity in rat pups as reflected by a significant reduction in the ambulation frequency, and showed significant elevation in emotionality and exploratory activities in rat pups as reflected by a significant elevation in rearing frequency and latency time in rat pups. The current results are supported by the results of previous studies which showed that oral administration of lower doses of BPA like 0.05 mg/kg decreased the locomotor activity, in the open-field test [100]. Another study suggests that both gestational and lactational exposures to BPA increased anxiety- and depression-like behaviors of adult mice of both sexes [101]. In addition gestational exposure exhibited a stronger effect on anxiety-like state in females. The altered levels of AMPA and NMDA receptors in the hippocampus and amygdala may be associated with BPA-induced behavioral changes [101].

A number of animal studies reported that BPA exposure during gestational period affects brain development and behaviors. Perinatal or neonatal BPA exposure alters brain sexual differentiation [58,66]. BPA can induce aggression, anxiety, cognitive deficits, and learning-memory impairment [67,102]. Perinatal exposure to BPA increases anxiety-like behavior and elevates dopamine levels in male, but not female, mice [103]. BPA exposure during organogenesis and breastfeeding upregulates dopamine receptor function, whereas exposure at other gestational periods does not, suggesting the presence of critical developmental windows of BPA toxicity [104]. In both rodents and nonhuman primates, BPA has adverse effects on the brain even at relatively low exposure levels [58,105]. Furthermore, a number of studies have shown behavioral modifications after exposure to BPA during gestation and/or lactation at low and high doses (40–400 g/kg/day), such as masculinization behavior in female pups or hyperactivity [106,107]. These behavioral changes suggest some effects of BPA on the developing central nervous system (CNS). The effect of BPA on the CNS may be of increased importance in the fetal and postnatal brain development and appears now as a public health concern [2]. To date, there have been few *in vivo* studies that have examined the impact of BPA exposure during gestation and lactation on brain development [74,108]. Finally, several studies in rodents have investigated neurotoxic endpoints and suggested that BPA treatment during development can cause alterations in brain development and behavior at doses that are relevant to human exposure [109].

The data of the present work revealed that postnatal Zn and/ or Se administration significantly increased the ambulation while decreased rearing frequency and latency time of rat pups. These results indicate that Zn and/ or Se administration inhibited BPA induced alterations in the locomotor, exploratory activities as well as in the emotional behavior. The role of Zn in the exploratory

behavior is poorly understood. In another study using open field Sobhanirad et al. [18]. and Piechal et al. [110]. found that both the exploratory and locomotor activity were increased in rats by high dose of zinc methionine. It was proposed that Zn deficiency is linked with nervous system disorders, including mental disturbances and loss of sensory acuity [22]. Zn deprivation may also result in increased stress response as well as altered emotionality and depression-induced behavior (aggression and anxiety) along with reduced attention and deterioration of learning and memory [23,24]. Previous study showed that Zn supplementation in rats in the prenatal and early postnatal period improves spatial learning, cognitive function, and locomotor activity of the offspring. Another study also demonstrated the correlation between Zn content in the hippocampus and spatial memory improvement in neonatal rats subjected to supplementation [91]. Previous study showed that Se produced an antidepressant-like action in the mouse. The antidepressant-like action of Se seems most likely to be mediated through an interaction with the dopaminergic system [111]. Deficiency of Se in mice have reported decreased activity in the OPT and increased anxiety demonstrated as decreased entry to the center of the field. This suggests that cerebellar function is especially sensitive to reduced Se [112].

Results of the current study showed that postnatal BPA exposure significantly decrease the brain oxidative stress markers (SOD, GP_x and CAT) enzyme activities. The present findings are in harmony with numerous studies which demonstrated that BPA (25–50 mg/kg/day, I.P. injection) has been shown to enhance oxidative stress and lipid peroxidation promoting the cellular death in several organs (e.g. brain, liver, and kidney) of exposed rodents [74,113]. Another studies showed association between BPA exposure and oxidative stress, which might contribute to some of its toxic effects [114,115]. Several findings indicate that BPA increased the production of ROS and thus results in neuronal damage [1,81]. Oxidative stress can induce mitochondrial damage, and damaged mitochondria can generate more ROS. Accumulation of oxidative damage in the mitochondria induces mitochondrial dysfunction [82]. It is widely accepted that during development, neurons are highly sensitive to ROS. Although further studies are needed to clarify the effects of BPA on the developmental processes, the findings of these reports suggest that oxidative stress may contribute to the action of BPA. Finally, the findings of some studies suggest that BPA appears to induce physiological responses such as DNA methylation via epigenetic mechanisms [116,117]. Ho et al. [118], reported that neonatal exposure to BPA increases phosphodiesterase-4 expression, which results from early and increased DNA hypomethylation Yaoi et al. [119], showed that exposure to low doses of BPA alters DNA methylation in the forebrain of a mouse fetus.

Data of the present work revealed that postnatal Zn and/ or Se administration showed a significant elevation in the brain oxidative stress markers (SOD, GP_x and CAT) enzyme activities. The present findings are in harmony with numerous studies which demonstrated that administration of Se attenuated lipid peroxidation and ameliorated the biochemical changes [120]. Se deficiency has been associated with decreased GPx activity in the brain of laboratory animals thus; Se deficiency may be linked to a reduced antioxidant capacity in the brain [121,122]. Also, Zn acts as antioxidant [123], or a cofactor for the antioxidant enzyme and dietary Zn intake has been shown to exert beneficial effects against oxidative damage to the cellular membranes and atherogenic action [26,115]. Moreover, Zn and/ or Se supplementation significantly enhanced the deleterious effect of BPA on antioxidant enzymes, lipid peroxidation and free radical formation. It was concluded that Zn and/ or Se supplementation significantly protect against BPA-induced oxidative stress, which represent the main contributing factor to its neurotoxicity [26,99].

Results of the present work showed that BPA exposure during lactation significantly decreased the brain NE and 5-HT level, while significantly elevated the brain DA content. These data are in accordance with previous studies which demonstrated that BPA exposure during early life has been shown to affect the dopamine system [124,125]. Also, the number of midbrain dopamine neurons in monkeys was reduced during gestational exposure to BPA [126]. It has been reported that BPA may affect tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis [127]. Therefore, it was concluded that BPA exposure was associated with an alteration of the dopaminergic system which may account for some of the neurological deficits, such as anxiety-like behaviors [103,128].

Moreover, another finding suggests that alteration in the functions of the dopaminergic system by prenatal and neonatal exposure to BPA is attributable to changes in the functions of the dopamine D3 receptor. Many previous findings suggest that prenatal exposure to BPA affects the development of the monoaminergic system. Matsuda et al. [103] reported that prenatal exposure to BPA alters the concentration of monoamines in the brain. In addition, Tian et al. [102], showed that prenatal and neonatal exposure to BPA increases dopamine D2 receptor binding in the caudate putamen of mice. Therefore, these findings indicate that prenatal and neonatal exposure to not only high doses but also low doses of BPA may dramatically alter neuronal transmission, including dopaminergic transmission in the adult brain.

Exposure to BPA during either organogenesis or lactation significantly enhanced the morphine-induced rewarding effects [104], and resulted in the up regulation of dopamine receptor-induced G-protein activation in the mouse limbic forebrain [104]. In general, during organogenesis of the brain, particularly during cerebral development, it is well known that rapid proliferation, differentiation, or migration of the nerve cells and glial cells occur [73,129]. In addition, the functional development of the CNS occurs most rapidly during lactation [129]. Therefore, these results strongly support our present results that organogenesis and lactation are the most sensitive periods during which BPA exposure influences the development of the CNS. Some findings suggest that exposure to BPA during organogenesis could affect differentiation or migration of neuronal stem cells. In addition, exposure to BPA during lactation affects the functional development of the CNS, including synaptogenesis and construction of the neuronal network. Taken together, these results may indicate that although BPA treatment of adult animals do not affect reproductive functions and social behaviors, exposure during the prenatal and neonatal stages, especially during organogenesis and lactation, induces toxicity in the developing neurons in the midbrain [73]. The results of another study suggest that prenatal and lactation exposure to low doses of BPA might modulate the NE, GABA and Glu systems, resulting in behavioral alterations [130].

Data of the present work revealed that postnatal Zn and/ or Se administration showed a significant elevation in the brain NE and 5-HT level, while it showed a significant reduction in the brain DA content. Zn is necessary for hormones transport and production of neurotransmitter precursors that will eventually affect the brain functions [18,19]. Also, Zn is important for proper function of GABA, aspartate, and norepinephrine [131]. Deficits of this nutrient during early development result in adverse effects

on brain structure and function [132]. Dietary Zn deficiency during pregnancy and lactation leads to the impairment of learning and memory function in offspring [133,134]. Zn is a cofactor in enzymes that mediate protein and nucleic acid biochemistry [68]. Fetal and neonatal Zn deficiency results in decreased brain DNA, RNA, and protein content [135,136]. Importantly, insulin-like growth factor I and growth hormone receptor gene expression are regulated by Zn [137]. Neuronally, presynaptic boutons are dependent on adequate Zn for delivery of neurotransmitters to the synaptic cleft [136,138]. Selenium will influence compounds with hormonal activity (and neurotransmitters) in the brain, and this is postulated to be the reason that Se affects moods in humans and behavior in animals. Severe Se deficiency or malfunction of Se transporting protein, selenoprotein P, causes degeneration of special group of GABAergic neurons leading to impaired neuronal function. This may result in such disorders as motor abnormalities, including seizures, and cognitive impairments like affected learning. The reason is the abundance of the GABA-utilising neurons in the corresponding brain regions – hippocampus, cerebral cortex and cerebellum. The pathway of DA might be also Se dependent; Se has neuroprotection in the nigrostriatal pathway but under higher concentrations, it exerts toxicity towards dopaminergic neurons. Recent findings also point to acetylcholine neurotransmission involvement. Mechanism of Se interaction with the neuronal signalling is complex and not fully understood up-to-now. It may be not limited to the antioxidant properties of selenoproteins but also to inflammation, and other signalling mechanisms like protein modifications and ion permeability of cell membranes as well as, alteration of calcium homeostasis and brain cholesterol metabolism [139,140].

CONCLUSION

Postnatal BPA exposure caused alterations and delaying in different aspects of physical as well as neurobehavioral development. Some behavioral functions as learning, locomotors and exploratory activities have been delayed while others as emotionality have been increased. Zinc and selenium was shown to protect against BPA- induced these alterations in rat pups. The protective effect of Zn either alone or in combination was more pronounced than Se concerning learning and some reflexes, while the protective effect of Zn and Se combination was more pronounced than each of them alone regarding brain monoamines level and oxidative stress markers. Consequently, Zn and Se co-administration could be re recommended during lactation especially at high risk conditions of BPA exposure as in low socioeconomic status.

REFERENCES

1. Lee S, et al. Signaling pathways of bisphenol A-induced apoptosis in hippocampal neuronal cells: role of calcium-induced reactive oxygen species, mitogen-activated protein kinases, and nuclear factor-kappa B. J Neurosci Res. 2008;86:2932-2942.
2. Vandenberg LN, et al. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine Review. 2009;30:75–95.
3. Goodson A, et al. Migration of Bisphenol-A from can coatings effects of damage, storage conditions and heating. Food Additives and Contaminants. 2004;21:1015–1026.
4. Elobeid MA and Allison DB. Putative environmental-endocrine disruptors and obesity: a review. Curr Opin Endocrinol Diabetes Obes. 2008;15:403-408.
5. Newbold R, et al. Prenatal exposure to bisphenol a at environmentally relevant doses adversely affects the murine female reproductive tract later in life. Environ Health Perspect. 2009;117:879–885.
6. Calafat AM, et al. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ Health Perspect. 2009;117:639-644.
7. Fernandez MF, et al. Bisphenol-A and chlorinated derivatives in adipose tissue of women. Reprod Toxicol. 2007;24:259-64.
8. Takahashi O and Oishi S. Disposition of orally administered 2,2-Bis(4-hydroxyphenyl) propane (Bisphenol-A) in pregnant rats and the placental transfer to fetuses. Environmental Health Perspective. 2000;108:931-935.
9. Markey CM, et al. In utero exposure to Bisphenol-A alters the development and tissue organization of the mouse mammary gland. Biology of Reproduction. 2001;65:1215–1223.
10. Markey CM, et al. Endocrine disruptors: from Wingspread to environmental developmental biology. The Journal of Steroid Biochemistry and Molecular Biology. 2002;83:235–244.
11. Richter C, et al. In vivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol. 2007;24:199–224.
12. Hajszan T and Leranth C. Bisphenol A interferes with synaptic remodeling. Front Neuroendocrinol. 2010;31:519–530.
13. Eilam-Stock T, et al. Bisphenol-A impairs memory and reduces dendritic spine density in adult male rats. Behav Neurosci. 2012;126:175–185.
14. Xu X, et al. Perinatal exposure to bisphenol-A inhibits synaptogenesis and affects the synaptic morphological development in offspring male mice. Chemosphere. 2013;91:1073–1081.
15. Groff T. Bisphenol A: invisible pollution. Curr Opin Pediatr. 2010;22:524–529.
16. Von Goetz N, et al. Bisphenol A: how the most relevant exposure sources contribute to total consumer exposure. Risk Anal. 2010;30:473–487.

17. Caulfield LE, et al. Potential contribution of maternal zinc supplementation during pregnancy to maternal and child survival. Am J Clin Nutr. 1998;68:499S–508S.
18. Piechal A, et al. Influence of long-term zinc administration on spatial learning and exploratory activity in rats. Biol Trace Elem Res. 2016;172:408–418.
19. Golub MS, et al. Developmental zinc deficiency and behavior. J Nutr. 1995;125:2263S–2271S.
20. Prasad AS. Impact of the discovery of human zinc deficiency on health. J Trace Elem Med Biol. 2014;28:357–363.
21. Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. Adv Nutr. 2013;4:176–190.
22. Black MM. Zinc deficiency and child development. Am J Clin Nutr. 1998;68:464S–469S.
23. Młyniec K, et al. Essential elements in depression and anxiety. Pharmacol Rep. 2014;66:534–544.
24. Hagmeyer S, et al. Behavioral impairments in animal models for zinc deficiency. Front Behav Neurosci. 2014;8:443.
25. Maret W. Molecular aspects of human cellular Zinc homeostasis: Redox control of Zinc potentials and Zinc signals. Biometals. 2009;22:149–157.
26. Rogalska J, et al. Enhanced Zinc consumption prevents cadmium-induced alterations in lipid metabolism in male rats. Chemico-Biological Interactions. 2009;177:142-152.
27. Brzóska MM, et al. Effect of Zinc supplementation on bone metabolism in male rats chronically exposed to Cadmium. Toxicol. 2007;237:89-103.
28. Rayman MP. The importance of selenium to human health. Lancet. 2000;356:233-241.
29. Schweizer U, et al. The neurobiology of selenium: lessons from transgenic mice. J Nutr. 2004;134:707-710.
30. Mostert V. Selenoprotein P: properties, functions, and regulation. Arch Biochem Biophys. 2000;376:433-8.
31. Savarino L, et al. Serum concentrations of zinc and selenium in elderly people: results in healthy nonagenarians/centenarians. Exp Gerontol. 2001;36:327-39.
32. Brenneisen P, et al. Selenium, oxidative stress, and health aspects. Mol Aspects Med. 2005;26:256–267.
33. Rayman MP. Selenium in cancer prevention: a review of the evidence and mechanism of action. Proc Nutr Soc. 2005;64:527–542.
34. Schrauzer GN. Anticarcinogenic effects of selenium. Cell Mol Life Sci. 2000;57:1864–1873.
35. Trueba GP, et al. Oxygen free radical and antioxidant defense mechanism in cancer. Front Biosci. 2004;9:2029–44.
36. Kobayashi K, et al. Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance inF1 rat offspring. Ind Health. 2002;40:375-81.
37. Rossowska MJ and Nakamoto T. Caffeine decreases zinc and metallothionein levels in heart of newborn and adult rats. Pediatr Res. 1992;32:330-2.
38. Hefnawy AE, et al. Effect of pre- and postpartum selenium supplementation in sheep. Journal of Animal and Veterinary Advances. 2008;7:61-67.
39. Alder S and Zbinden G. Methods for the evaluation of physical, neuromuscular and behavioral development of rats in early postnatal life. In: Neubert D, Merker HJ, Kwasigroch TE, (eds). Method in prenatal toxicology: Thieme, Stuttgart. 1977;175-185.
40. Vorhees CV. Behavioral teratogenicity testing as a method of screening for hazards to human health: a methodological proposal. Neurobehav Toxicol Teratol. 1983;5:469-474.
41. Zbinden G. Experimental methods in behavioral teratology. Arch Toxicol. 1981;48:69-88.
42. Hamed MR, et al. Influence of protein malnutrition on prenatal toxicity of fluoxetine. EJHM. 2009;35:229-245.
43. Volosin M, et al. Influence of adrenocorticotrophic hormone on the behaviour in the swim test of rats treated chronically with desipramine. J Pharm Pharmacol. 1988;40:74-76.
44. Vorhees CV. Some behavioral effects of maternal hypervitaminosis A in rats. Teratology. 1974;10:269-273.
45. Lo Pumo R, et al. Long-lasting neurotoxicity of prenatal benzene acute exposure in rats. Toxicol. 2006;223:227-234.
46. Lazarini CA, et al. Effects of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. Neurotoxicol Teratol. 2001;23:665-673.
47. Van den Buuse M and de Jong W. Differential effects of dopaminergic drugs on open-field behavior of spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. J Pharmacol Exp Ther. 1989;248: 1189-1196.
48. Cunha JM and Masur J. Evaluation of psychotropic drugs with a modified open field test. Pharmacol. 1978;16:259-267.
49. Welch BL and Welch AS. Differential activation by restraint stress of a mechanism of converse brain catecholamines and serotonin in mice differing in excitability. Nature. 1968;575-600.

50. Walther MM, et al. Pheochromocytoma: evaluation, diagnosis, and treatment. World J Urol. 1999;17:35-39.

51. Kopf D, et al. Clinical management of malignant adrenal tumors. J Cancer Res Clin Oncol. 2001;127:143-155.

52. Endo A, et al. Cardiac and plasma catecholamine responses to exercise in patients with type 2 diabetes: prognostic implications for cardiac cerebrovascular events. Am J Med Sci. 2000;320:24-30.

53. Attanasio A, et al. Ontogeny of circadian rhythmicity for melatonin, serotonin, and N-acetylserotonin in humans. J Pineal Res. 1986;3:251–256.

54. Nishikimi M, et al. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972;46:849-854.

55. Mannervik B. Glutathione peroxidase. Methods Enzymol. 1985;113:490–495.

56. Clairborne A. Catalase activity. In: RA Greenwald (ed). Handbook of methods for oxygen radical research. Boca Raton, Florida: CRC Press. 1985;283-284.

57. Kelly EA, et al. The effects of postnatal exposure to low-dose bisphenol-A on activity-dependent plasticity in the mouse sensory cortex. Front Neuroanat. 2014;22:8:117.

58. Inadera H. Neurological effects of bisphenol A and its analogues. Int J Med Sci. 2015;12:926-936.

59. Vandenberg LN, et al. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect. 2010;118:1055-1070.

60. Edlow AG, et al. Fetal bisphenol A exposure: concentration of conjugated and unconjugated bisphenol A in amniotic fluid in the second and third trimesters. Reprod Toxicol. 2010;34:1-7.

61. Sun Y, et al. Determination of bisphenol A in human breast milk by HPLC with column-switchnig and fluorescence detection. Biomed Chromatogr. 2004;18:501-507.

62. Ema M, et al. Rat two-generation reproductive toxicity study of bisphenol A. Reprod Toxicol. 2001;15:505-23.

63. Mendl M and Paul ES. Parental care, sibling relationships and the development of aggressive behaviour in two lines of wild house mice. Behaviour. 1990;116:11–41.

64. Cameron EZ. Is suckling behaviour a useful predictor of milk intake? A review. Anim Behav. 1998;56:521–532.

65. Palanza PL. Exposure to a low dose of bisphenol a during fetal life or in adulthood alters maternal behavior in mice. Environmental Health Perspectives. 2002;1100:415–422.

66. Patisaul HB, et al. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotoxicol Teratol. 2006;28:111-118.

67. Xu XH, et al. Perinatal exposure to bisphenol-A impairs learning-memory by concomitant down-regulation of N-methyl-D-aspartate receptors of hippocampus in male offspring mice. Horm Behav. 2010;58:326-333.

68. Sandstead HH. Zinc: essentiality for brain development and function. Nutr Rev. 1985;43:129–37.

69. Sandstead HH. Zinc is essential for brain development and function. The Journal of Trace Elements in Experimental Medicine. 2003;16:165–173.

70. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J Trace Elem Med Biol. 2004;18:113-21.

71. Abdel-Hamid GA. Effect of vitamin E and selenium against aluminum- -induced nephrotoxicity in pregnant rats. Folia Histochem Cytobiol. 2013;51:312-9.

72. Arthur J R. The glutathione peroxidases. Cell Mol Life Sci. 2000;1825–1835.

73. Keisuke M, et al. Effects of prenatal and neonatal exposure to bisphenol a on the development of the central nervous system. Biomol Ther. 2010;18:125-134.

74. Kunza N, et al. Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation. Int J Devl Neurosci. 2011;29:37–43.

75. Miyagawa K, et al. Memory impairment associated with a dysfunction of the hippocampal cholinergic system induced by prenatal and neonatal exposures to bisphenol-A. Neurosci Lett. 2007;418:236-241.

76. Miyamoto M, et al. Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 1987;419:19-31.

77. Dutar P, et al. The septohippocampal pathway: structure and function of a central cholinergic system. Physiol Rev. 1995;75:393-427.

78. Moriyama K, et al. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab. 2002;87:5185-5190.

79. Bindhumol V, et al. Bisphenol A induces reactive oxygen species generation in the liver of male rats. Toxicol. 2003;188:117-124.

80. Kabuto H, et al. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. Environ Res. 2003;93:31-35.

81. Ooe H, et al. Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1. Toxicol Sci. 2005;88:114-126.

82. Nakagawa Y and Tayama S. Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Arch Toxicol. 2000;74:99-105.

83. Choi IS, et al. Multiple effects of bisphenol A, an endocrine disrupter, on GABA (A) receptors in acutely dissociated rat CA3 pyramidal neurons. Neurosci Res. 2007;59:8–17.

84. Ogiue-Ikeda M, et al. Rapid modulation of synaptic plasticity by estrogens as well as endocrine disrupters in hippocampal neurons. Brain Res Rev. 2008;57:363–375.

85. Leranth C, et al. Bisphenol A prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex of ovariectomized nonhuman primates. Proc Natl Acad Sci USA. 2008;105:14187–14191.

86. Zhou R, et al. Deficits in development of synaptic plasticity in rat dorsal striatum following prenatal and neonatal exposure to low-dose bisphenol A. Neurosci. 2009;159:161–171.

87. Ikemoto S, et al. Rewarding effects of AMPA administration into the supramammillary or posterior hypothalamic nuclei but not the ventral tegmental area. J Neurosci. 2004;24:5758–5765.

88. Sato K, et al. Effects of 17beta-estradiol and xenoestrogens on the neuronal survival in an organotypic hippocampal culture. Neuroendocrinology. 2002;76:223–234.

89. Silva AJ. Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. J Neurobiol. 2003;54:224–237.

90. Kubo T, et al. Bisphenol A, an environmental endocrine-disrupting chemical, inhibits hypoxic response via degradation of hypoxia-inducible factor 1alpha (HIF-1alpha): structural requirement of bisphenol A for degradation of HIF-1alpha. Biochem Biophys Res Commun. 2004;318:1006–1011.

91. Piechal A, et al. Maternal zinc supplementation improves spatial memory in rat pups. Biol Trace Elem Res. 2012;147:299-308.

92. Yu X, et al. Effects of maternal mild zinc deficiency and different ways of zinc supplementation for offspring on learning and memory. Food Nutr Res. 2016;60:29467.

93. Cope EC, et al. Use of zinc as a treatment for traumatic brain injury in the rat: effects on cognitive and behavioral outcomes. Neurorehabil Neural Repair. 2012;26:907-13.

94. Halas ES, et al. Learning and memory impairment in adult rats due to severe zinc deficiency during lactation. Physiol Behav. 1983;30:371–381.

95. Chowanadisai W, et al. Maternal zinc deficiency reduces NMDA receptor expression in neonatal rat brain, which persists into early adulthood. J Neurochem. 2005;94:510–519.

96. Nakashima AS and Dyck RH. Zinc and cortical plasticity. Brain Res Rev. 2009;59:347–373.

97. Hardy AB, et al. Regulation of glucagon secretion by zinc: lessons from the beta cell-specific Znt8 knockout mouse model. Diabetes Obes Metab. 2011;13:112–17.

98. Levenson CW and Morris D. Zinc and neurogenesis: making new neurons from development to adulthood. Adv Nutr. 2011;2;96-100.

99. Ali AA, et al. Protective effect of zinc against postnatal lead-induced alterations in the neurobehavioral development of normally-fed and protein malnourished rats. J Neuro Neurosci. 2016;7:117.

100. Kuwahara R, et al. Bisphenol A does not affect memory performance in adult male rats. Cell Mol Neurobiol. 2016;34:333-42.

101. Xu X, et al. Gestational and lactational exposure to bisphenol-A affects anxiety- and depression-like behaviors in mice. Horm Behav. 2012;62:480-90.

102. Tian YH, et al. Prenatal and postnatal exposure to bisphenol a induces anxiolytic behaviors and cognitive deficits in mice. Synapse. 2010;64:432-439.

103. Matsuda S, et al. Effects of perinatal exposure to low dose of bisphenol A on anxiety like behavior and dopamine metabolites in brain. Prog Neuropsychopharmacol Biol Psychiatry. 2012;39:273-279.

104. Narita M, et al. Changes in central dopaminergic systems and morphine reward by prenatal and neonatal exposure to bisphenol-A in mice: evidence for the importance of exposure period. Addict Biol. 2007;12:167-172.

105. Nakagami A, et al. Alterations in male infant behaviors towards its mother by prenatal exposure to bisphenol A in cynomolgus monkeys (Macaca fascicularis) during early suckling period. Psychoneuroendocrinology. 2009;34:1189-1197.

106. Ishido M, et al. Bisphenol A causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurosci Res. 2004;76:423–433.

107. Palanza P, et al. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ Res. 2008;108:150–157.

108. Facciolo RM, et al. Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst(2). Environ Health Perspect. 2002;110:397–402.

109. Clancy B, et al. Translating developmental time across mammalian species. Neurosci. 2001;105:7-17.

110. Sobhanirad S, et al. Assessment of changes in anxiety and exploratory behaviors following zinc supplementation in rats. Res J Biol Sci. 2008;3:1415–1418.

111. Oliveira CES, et al. The antidepressant-like action of a simple selenium-containing molecule, methyl phenyl selenide, in mice. Eur J Pharmacol. 2012;690:119–123.

112. Watanabe C and Satoh H. Effects of prolonged selenium deficiency on open field behavior and Morris water maze performance in mice. Pharmacol Biochem Behav. 1995;51:747-752.

113. Aydogan M, et al. The effect of vitamin C on bisphenol A, nonylphenol and octylphenol induced brain damages of male rats. Toxicol. 2008;249:35–39.

114. Hong YC, et al. Community level exposure to chemicals and oxidative stress in adult population. Toxicol Lett. 2009;184:139-144.

115. Ahmed A, et al. The possible protective effects of some antioxidants against growth retardation and malformations induced by bisphenol-A in rats. Life Sci J. 2013;10.

116. Baccarelli A and Bollati V. Epigenetics and environmental chemicals. Curr Opin Pediatr. 2009;21:243-251.

117. Bromer JG, et al. Bisphenol-A exposure in utero leads to epigenetic alterations in the developmental programming ofuterine estrogen response. FASEB J. 2010;24:2273-80.

118. Ho SM, et al. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res. 2009;66:5624-5632.

119. Yaoi T, et al. Genome-wide analysis of epigenomic alterations in fetal mouse forebrain after exposure to low doses of bisphenol A. Biochem Biophys Res Commun. 2008;376:563- 567.

120. Lakshmi BV, et al. Protective effect of selenium against aluminum chloride-induced Alzheimer's disease: behavioral and biochemical alterations in rats. Biol Trace Elem Res. 2015;165:67-74.

121. Sanchez V, et al. Differential effect of dietary selenium on the long-term neurotoxicity induced by MDMA in mice and rats. Neuropharmacology. 2003;44:449-461.

122. Gladyshev VN. Selenoproteins and selenoproteomes. In: Hatfield DL, Berry MJ, Gladyshev VN (eds). Selenium: its molecular biology and role in human health. Springer, New York. 2006;99-114.

123. Catania AS, et al. Vitamins and minerals with antioxidant properties and cardiometabolic risk: controversies and perspectives. Arq Bras Endocrinol Metabol. 2009;53:550-559.

124. Prasanthi RP, et al. Calcium and zinc supplementation protects lead (Pb)-induced perturbations in antioxidant enzymes and lipid peroxidation in developing mouse brain. Int J Dev Neurosci. 2010;28:161-167.

125. Masuo Y and Ishido M. Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. J Toxicol Environ Health B Crit Rev. 2011;14:346-349.

126. Elsworth JD, et al. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. Neurotoxicol. 2013;35:113-120.

127. Ishido M, et al. Mesencephalic neurodegeneration in the orally administered bisphenol A-caused hyperactive rats. Toxicol Lett. 2007;173:66-72.

128. Cox KH, et al. Gestational exposure to bisphenol A and cross-fostering affect behaviors in juvenile mice. Horm Behav. 2010;58:754-761.

129. Temple S. The development of neural stem cells. Nature. 2011;414:112-117.

130. Ogi H, et al. Alterations of neurotransmitter norepinephrine and gamma-aminobutyric acid correlate with murine behavioral perturbations related to bisphenol A exposure. Brain Dev. 2015;37:739-46.

131. Koh JY. Zinc and disease of the brain. Mol Neurobiol. 2001;24:99-106.

132. Uriu-Adams JY and Keen CL. Zinc and reproduction: Effects of zinc deficiency on prenatal and early postnatal development. Birth Defects Res B Dev Reprod Toxicol. 2010;89:313–325.

133. Gardner JM, et al. Zinc supplementation and psychosocial stimulation: Effects on the development of undernourished Jamaican children. Am J Clin Nutr. 2005;82:399–405.

134. Yu X, et al. Effects of maternal mild zinc deficiency and zinc supplementation in offspring on spatial memory and hippocampal neuronal ultra-structural changes. Nutrition. 2013;29:457-461.

135. Duncan J and Hurley L. Thymidine kinase and DNA polymerase activity in normal and zinc deficient developing rat embryos. Proc Soc Exp Biol Med. 1978;159:39–43.

136. Monk C, et al. Maternal prenatal distress and poor nutrition– mutually influencing risk factors affecting infant neurocognitive development. J Child Psychol Psychiatry. 2013;54:115–130.

137. McNall A, et al. The impaired growth induced by zinc deficiency in rats is associated with decreased expression of hepatic insulin-like growth factor I and growth hormone receptor genes. J Nutr. 1995;125:874–9.

138. Frederickson C and Danscher G. Zinc-containing neurons in hippocampus and related CNS structures. Prog Brain Res. 1990;83:71–84

139. Whanger PD. Selenium and the brain: a review. Nutr Neurosci. 2001;4:81-97.

140. Solovyev ND. Importance of selenium and selenoprotein for brain function: From antioxidant protection to neuronal signaling. J Inorg Biochem. 2015;153:1–12.