



# Neurochemical alterations following the exposure to di-n-butyl phthalate in rats

Rami B. Kassab<sup>1</sup> · Maha S. Lokman<sup>1</sup> · Ehab A. Essawy<sup>2</sup>

Received: 5 July 2018 / Accepted: 8 November 2018 / Published online: 16 November 2018  
© Springer Science+Business Media, LLC, part of Springer Nature 2018

## Abstract

Due to its ability to cross blood brain barrier and placenta, dibutyl phthalate (di-n-butyl phthalate, DBP) is expected to cause severe side effects to the central nervous system of animals and humans. A little data is available about the potential DBP neurotoxicity; therefore, this work was designed to investigate the brain tissue injury induced by DBP exposure. Forty Wister albino rats were allocated randomly into 4 groups (10 rats each). Group 1 served as control and the rats administered with physiological saline (0.9% NaCl) orally for 12 weeks. Groups 2, 3 and 4 were orally treated with DPB (100, 250 and 500 mg/kg) respectively for 12 weeks. DBP-intoxicated rats showed a disturbance in the oxidative status in cerebral cortex, striatum and brainstem, as represented by the elevated oxidants [malondialdehyde (MDA), nitric oxide (NO), 8-hydroxy-2-deoxyguanosine (8-OHdG)] and the decreased anti-oxidant molecules [reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)]. DBP also enhanced a pro-inflammatory state through increasing the release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ). The increase of these cytokines was associated with the increase of pro-apoptotic proteins [Bcl-2 associated X protein (Bax) and caspase-3] and the decrease of the anti-apoptotic protein, B cell lymphoma 2 (Bcl-2). In addition, the levels of norepinephrine (NE), dopamine (DA) and acetylcholine esterase (AChE) activity were decreased. This was accompanied by the alterations in the major excitatory and inhibitory amino acids neurotransmitters levels. The present findings indicated that DBP could exert its neuronal damage through oxidative stress, DNA oxidation, neuroinflammation, activation of apoptotic proteins and altering the monoaminergic, cholinergic and amino acids transmission.

**Keywords** Dibutyl phthalate · Oxidative stress · Inflammation · Apoptosis · Neurotoxicity · Rats

## Introduction

Di-n-butyl phthalate (DBP) belongs to the phthalate esters which are ubiquitous environmental hazards and known to disturb the endocrine functions through affecting hormones synthesis and metabolism or competing with their receptors leading to the suppression of the hormonal response Tabb and Blumberg (2006). DBP is used commonly as plasticizer in different industries such as clothing, pharmaceuticals, toys, furniture, medical devices and cosmetics (Wojtowicz et al. 2017). Human exposed mainly to DBP through the

consumption of contaminated food, skin absorption and polluted air resulted from the manufacturing processes (Heudorf et al. 2007). Once inhaled or ingested, DBP is absorbed rapidly due to its lipophilic nature. DBP has the ability to cross placenta and blood brain barrier and accumulate in the brain tissue and other organs (Fujii et al. 2003; Kavlock et al. 2006). In addition, the existence of DBP in the body fluids has been reported (Calafat et al. 2006; Faniband et al. 2014).

Numerous reports connected between the exposure to DBP and the developmental, reproductive, neuronal, immune, cardiovascular and metabolic consequences in humans and animals (de Mello Santos et al. 2017; Gao et al. 2017; Mahaboob Basha and Radha 2017; Mariana et al. 2016). But still the precise mechanisms involved in DBP induce these health adverse events aren't clear. There is growing evidence that the imbalance between the oxidant/antioxidant systems following the ROS production play a fundamental role in DBP-induced toxicity (Zhou et al. 2011, 2010; Zhu et al. 2017). Zhu et al.

✉ Rami B. Kassab  
ramikassab@mail.muni.cz

<sup>1</sup> Zoology and Entomology Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

<sup>2</sup> Chemistry Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

(2017) reported that, oxidative stress caused renal fibrosis and dysplasia in adult rat offspring treated with DBP. Also Yan et al. (2016) confirmed the involvement of oxidative stress in DBP induces neurotoxicity and behavioral alterations in Kunming mice. Moreover, Wojtowicz et al. (2017) demonstrated that DBP induced neuronal damage via activating apoptotic cell death and ROS production in murine cortical neurons.

Furthermore, it has been recorded that, the prenatal treatment with DBP decreased number of neurons and modified the structure of hippocampus in the offspring rats leading to learning and memory dysfunctions (Li et al. 2013). Previous studies showed that, DBP affect the neurobehavioral and cognitive abilities in rats and humans (Li et al. 2009; Lien et al. 2015). Moreover, DBP disturbed the functions of nicotinic acetylcholine receptors via suppressing the calcium signaling in human neuroblastoma and bovine chromaffin cells (Liu et al. 2009; Lu et al. 2004). However, still little data is available describing the effect of DBP on the murine brains. Therefore, the objective of the current study is to characterize the changes in oxidant/antioxidant systems, inflammatory response, apoptotic proteins, monoamines, acetylcholine esterase activity, excitatory and inhibitory neurotransmitters which may follow the exposure to DBP in cerebral cortex, striatum and brainstem of rats.

## Materials and methods

### Chemicals and experimental animals

*n*-butyl phthalate (C<sub>6</sub>H<sub>4</sub>-1,2-[CO<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub>]<sub>2</sub>) was supplied from Sigma (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade. Double-distilled water was used as the solvent.

Forty Wistar strain albino rats (150–170 g) obtained from the NODCAR Animal House, NODCAR, Giza, Egypt, were used for the study. The rats were housed in wire mesh cages under standard conditions (temperature 25–29 °C, 12 h light and 12 h darkness cycles). Animals were fed with pelleted standard rat diet and water ad libitum. Generally, the study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animals.

Rats were divided randomly into 4 equal groups (10 rats each) as follow:

Group 1 served as normal control and the rats were received normal saline orally for 12 weeks. Groups 2, 3 and 4 were orally treated with DPB (100, 250 and 500 mg/kg) for 12 weeks according to Giribabu et al. (2014).

### Sample collection

Rats were sacrificed by decapitation 24 h after the last administration. Brain cortex, striatum and brainstem were rapidly dissected, thoroughly washed with isotonic saline and then weighed. Each brain tissue was homogenized in 75% aqueous HPLC grade methanol (10% w/v). The homogenate was spun at 4000 r.p.m. for 10 min for the determination of monoamines and amino acids, while for the estimation of the other biochemical investigations; brain tissue was homogenized in an ice-cold medium of 50 mM Tris-HCl (pH 7.4) to give a 10% (w/v) homogenate. After being centrifuged at 3000 r.p.m. for 10 min at 4 °C, the supernatants obtained from the homogenates was separated and stored at –80 °C. The total protein content of the homogenates in all experiments was estimated by the method of Lowry et al. (1951).

### Determination of monoamines and free amino acids

The HPLC system consisted of quaternary pump; a column oven, Rheodine injector and 20 µl loop, UV variable wavelength detector. The report and chromatogram taken from data acquisition program purchased from chemstation. The sample was immediately extracted from the trace elements and lipids by the use of solid phase extraction CHROMABOND column NH<sub>2</sub> phase cat. No.730031. the sample was then injected directly into an AQUA column 150 mm 5 µ C18, purchased from Phenomenex, USA under the following conditions: mobile phase 20 mM potassium phosphate, pH 2.5, flow rate 1.5 mL/min, UV 190 nm. Norepinephrine (NE), dopamine (DA), and serotonin (5-HT) were separated after 12 min. The resulting chromatogram identified each monoamine position and concentration from the sample as compared to that of the standard purchased from Sigma Aldrich, and finally, the determination of the content of each monoamine as µg per gram brain tissue was calculated according to Pagel et al. (2000). Free amino acid neurotransmitters were detected by using the precolumn PITC derivatization technique employed by Henrikson and Meredith (1984).

### Oxidative stress markers in brain tissue

Malondialdehyde (MDA) was estimated using the method described by Ohkawa et al. (1979). Nitric oxide (NO) was measured colorimetrically, using Griess reagent, according to the method described by Green et al. (1982). The protocol by Ellman (1959) was used for the determination of reduced glutathione (GSH). For the estimation of 8-hydroxy-2-deoxyguanosine (8-OHdG), the isolation and hydrolysis of brain DNA was performed using the method of Lodovici et al. (1997).

## Endogenous antioxidant enzymes in brain tissue

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) were assayed based on the protocols described by (Nishikimi et al. 1972), (Aebi 1984), (Paglia and Valentine 1967), and (Factor et al. 1998), respectively.

## Inflammatory markers in brain tissue

The concentration of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) in the homogenates of brain were estimated using commercial ELISA kits (R&D System, Minneapolis, MN, USA) according to the manufacturers' procedures.

## Estimation of apoptotic markers in brain tissue

Brain homogenates were made in lysis buffer and analyzed using a colorimetric caspase-3 assay kit (Sigma-Aldrich Co. USA) according to the manufacturer's instructions. The concentrations of caspase-3 in brain lysates were calculated with the help of the calibration curve generated using known amounts of standards. B cell lymphoma 2 (Bcl-2) and Bcl-2 associated X protein (Bax) levels were measured in the brain tissue lysates by ELISA kits, (LifeSpan BioSciences, Inc., Seattle, WA, USA). The procedure was performed according to instructions of manufacturer. Levels were expressed as ng/mg tissue protein.

## Statistical analysis

One-way analysis of variance (ANOVA) was used for the statistical analysis with post-hoc Tukey's test. Results are expressed as the mean  $\pm$  SD (standard deviation). Differences were considered statistically significant at  $p$  values  $< 0.05$ .

## Results

The oral treatment with DBP (100, 250 and 500 mg/kg) elicited a delirious effects in the oxidant-antioxidant system in the examined brain regions. ANOVA revealed that, the oral administration with DBP for 12 weeks produced a significant increase ( $p < 0.05$ ) in the levels of MDA and NO in cerebral cortex, striatum and brainstem. In addition, 8-OHdG level was increased in cerebral cortex and striatum, but in brainstem, this increase was recorded only in rats treated with 500 mg/kg as compared to the control group. Meanwhile, the content of GSH was markedly decreased (Fig. 1a-d). DBP exposed rats showed a disturbance in the activities of endogenous antioxidant enzymatic system in all tested brain areas. The treatment

for 12 weeks with DBP caused a significant decline ( $p < 0.05$ ) in the activities of SOD, CAT, GPx and GR in comparison with the control levels in the cerebral cortex, striatum and brainstem (Fig. 2a-d).

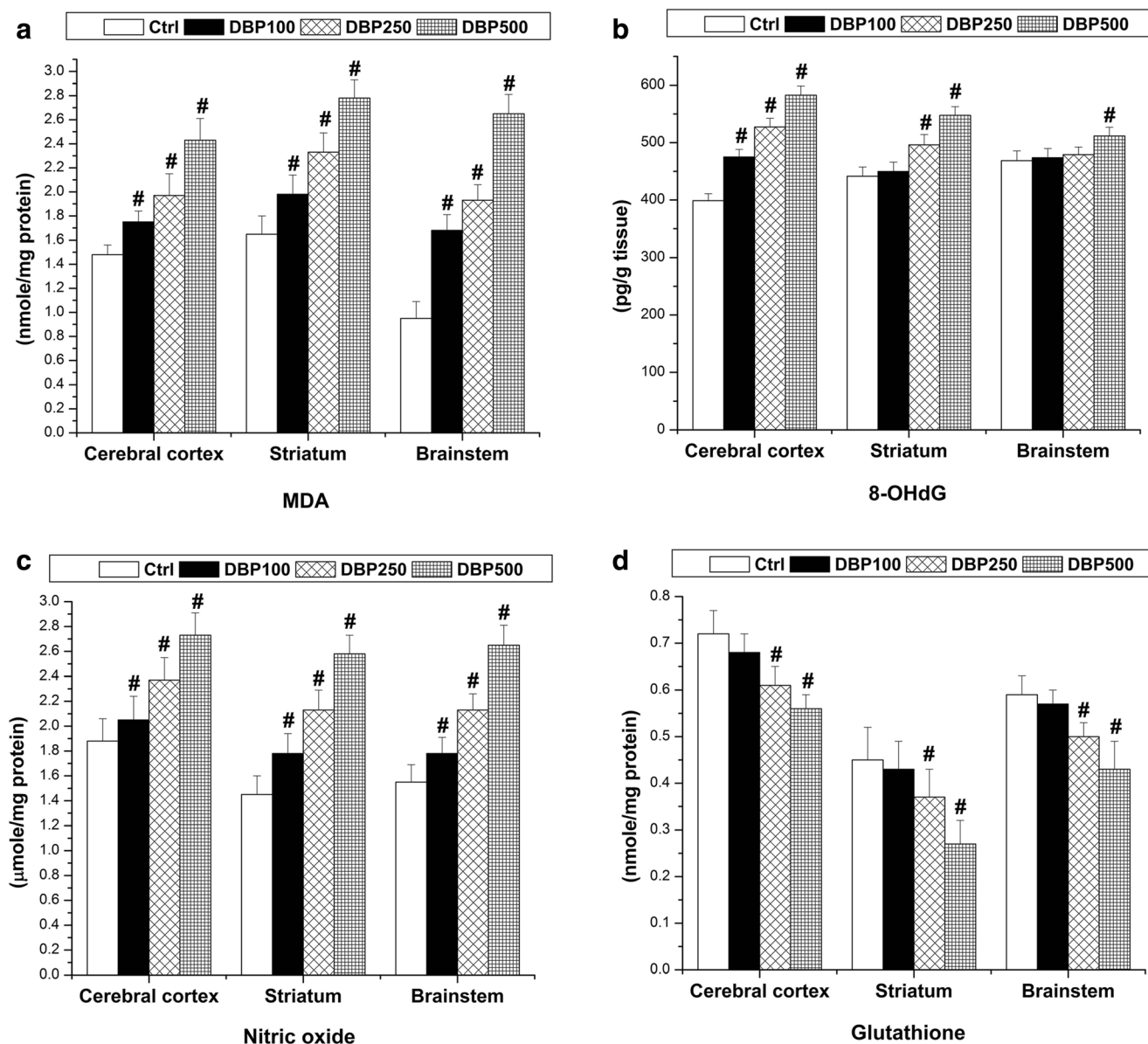
To estimate the potential inflammatory response following the exposure to DBP, the cytokines namely, TNF- $\alpha$  and IL-1 $\beta$  were measured in the selected brain areas. In comparison to the control group, the tested inflammatory mediators elevated significantly ( $p < 0.05$ ) in the cerebral cortex, striatum and brainstem (Fig. 3a, b).

To understand the molecular mechanism which may involved in DBP intoxication, our findings showed a significant up regulation in the expression of pro-apoptotic proteins (Bax and caspase-3) while the level of anti-apoptotic protein (Bcl-2) was down regulated when compared to the control group in all tested brain tissues (Fig. 4a-c).

To evaluate the effect of DBP on the monoaminergic system, the levels of NE, DA and 5-HT were estimated in the cortical, striatal and brainstem homogenates. The results in Fig. 4 illustrated that the levels of NE and DA were decreased significantly ( $p < 0.05$ ), while the content of 5-HT didn't changed following the chronic treatment with DBP as compared to the normal levels. Interestingly, the cholinergic activity was also inhibited in cerebral cortex and striatum and mid-brain in a dose dependent effect (Fig. 5a-d). Parallel to the effect on the monoaminergic and cholinergic systems, the content of the major excitatory and the inhibitory amino acids were evaluated in the current study. The treatment with the selected DBP doses for 12 weeks elevated the levels of glutamate in the cerebral cortex only, while aspartate was increased in cerebral cortex, striatum and brainstem. In contrast, the levels of GABA were significantly decreased in all examined brain tissues, while glycine content didn't changed when compared to the control values (Fig. 6a-d).

## Discussion

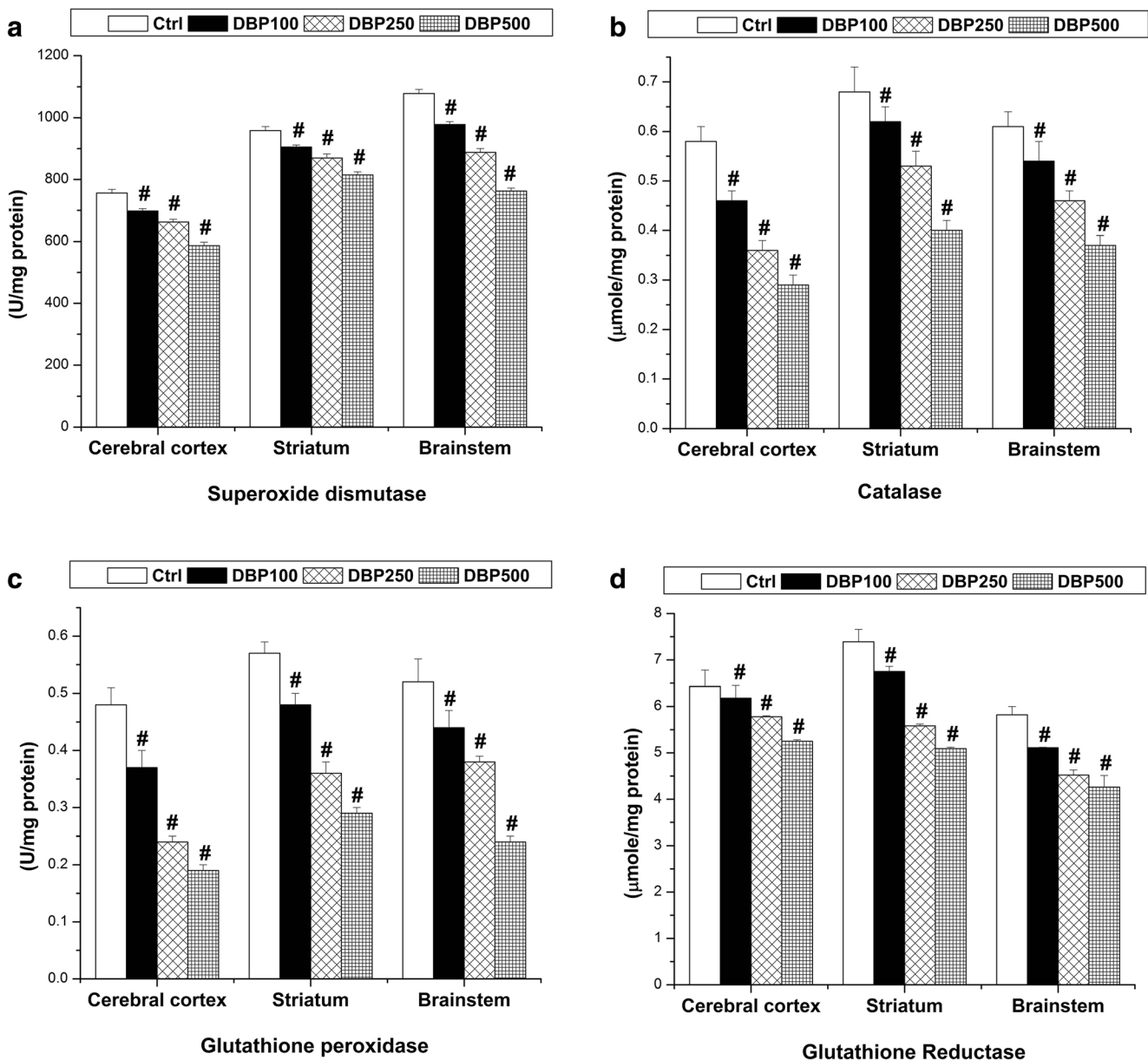
Phthalates are universal environmental contaminants causing several health problems in humans and animals. DBP is the second most common used phthalate compound in the industrial products as a plasticizer and solvent (Schettler 2006). Few reports focused on the neurochemical changes following DBP exposure, therefore, we aimed to evaluate the potential neurotoxicity which may follow the exposure to DBP through estimating the levels of oxidative status, pro-inflammatory cytokines, apoptotic proteins, monoaminergic system, acetylcholine esterase activity, excitatory and inhibitory amino acids in cerebral cortex, striatum and brainstem of Wister male albino rats. Our findings recorded a disturbance in the oxidative status in all studied brain tissues as a results of chronic DBP intoxication, as evidenced by the elevation of MDA, NO and 8-OHdG levels and the suppression of GSH content, this was



**Fig. 1** Effects of oral administration of di-n-butyl phthalate (DBP) on MDA, NO, 8-OHdG and GSH levels in cerebral cortex, striatum and brainstem of rats. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test

accompanied with inactivation of the endogenous antioxidant and detoxifying enzymes including SOD, CAT, GPx and GR. It is well known that brain is the most susceptible organ to oxidative stress as a result of consuming oxygen in large quantity, contains unsaturated fatty acids which are labile to peroxidation; in addition, brain has a lower activity of the antioxidant defense enzymes when compared to the other organs (Uttara et al. 2009). There are several reports confirmed the crucial role of oxidative stress in the progression of neurodegenerative diseases (Escudero-Lourdes 2016; Kassab and El-Hennamy 2017). MDA is widely used as a lipid peroxidation

biomarker in cells and tissues (Al-Olayan et al. 2015). The elevation in MDA levels has been attributed to the formation of reactive oxygen species (ROS) in the brain tissue after the exposure to phthalates in a dose dependent effect (Peng 2015; Yan et al. 2016; Zuo et al. 2014). NO is an important intracellular and extracellular biological mediator controlling different mechanisms in the nervous, cardiovascular and the immune systems (Aktan 2004). The hazard of NO comes from the interaction with superoxide radical ( $O_2^-$ ) to produce peroxynitrite ( $ONOO^-$ ) which is highly cytotoxic agent (Shaw et al. 2005). The increase in NO level is also observed

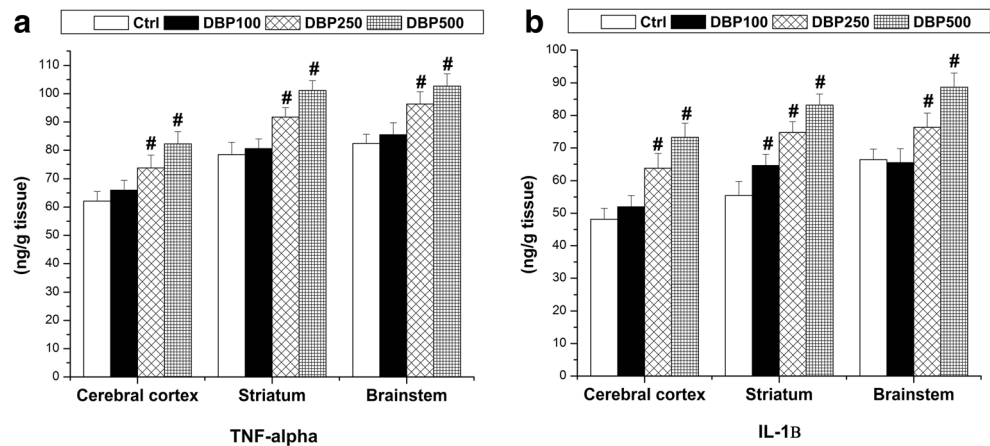


**Fig. 2** Effect of di-n-butyl phthalate (DBP) intoxication for 12 weeks on SOD, CAT, GPx and GR activities in cerebral cortex, striatum and brainstem of rats. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test

by Yavasoglu et al. (2014) following the exposure to butyl cyclohexyl phthalate in mice, the authors attributed this behavior to the over expression of iNOS resulted from oxidative damage. The increased MDA and NO in the present study may reflect the overproduction of reactive oxygen species and reactive nitrogen species in the examined brain tissue. 8-OHdG is a sensitive marker for DNA oxidative damage (Al Omairi et al. 2018). A positive correlation between phthalate exposure and 8-OHdG concentration in humans and animals has been reported (Franken et al. 2017; Lee et al. 2007; Rocha

et al. 2017; Shono and Taguchi 2014). GSH is a non-enzymatic antioxidant plays an important role in protecting the cells and tissues through scavenging ROS (Al-Olayan et al. 2016). It has been reported that the decrease in the content of GSH is associated with the increase in ROS production and the elevation in lipid peroxidation which causes overconsumption of functional thiol (-SH) groups in the enzymatic and non-enzymatic system (Zuo et al. 2014). Yan et al. (2016) found that the treatment with DBP enhances ROS generation, a drastic elevation in lipid peroxidation levels and

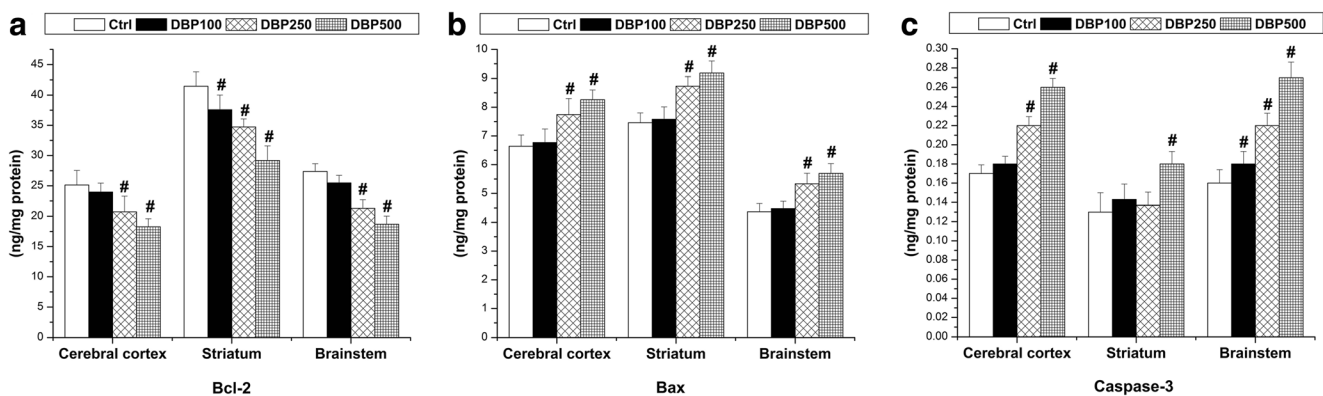
**Fig. 3** Levels of TNF- $\alpha$  and IL-1 $\beta$  in cerebral cortex, striatum and brainstem of rats following the oral administration of di-n-butyl phthalate (DBP) for 12 weeks. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test



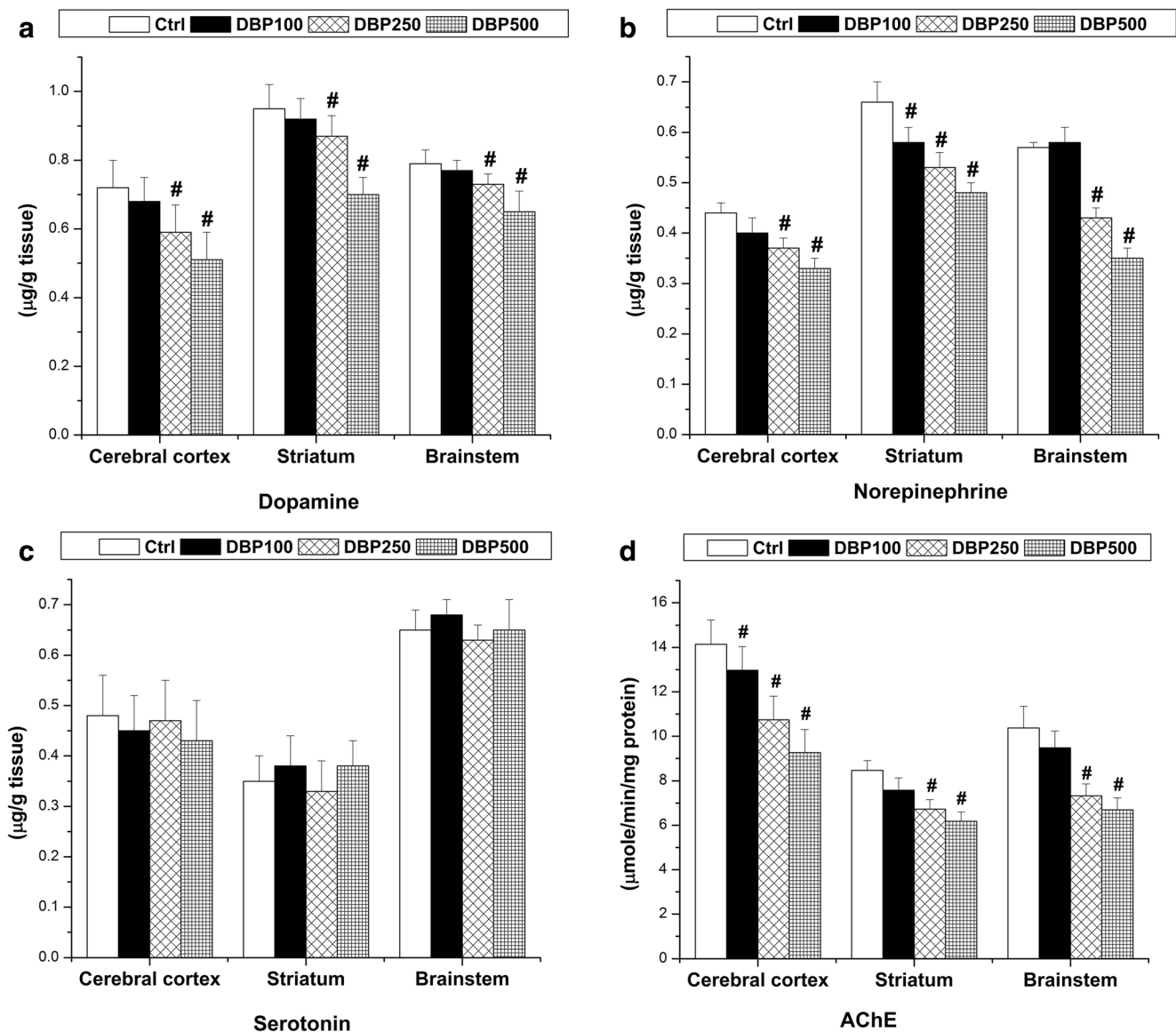
depletion in GSH pool leading to neuronal injury in the mice brain. Numerous reports linked between the disturbance in the antioxidant enzymes and the exposure to phthalates in experimental animals (Wang and Karmaus 2017; Yan et al. 2016; Yavasoglu et al. 2014; Zhou et al. 2010). The decrease in the enzymatic antioxidants activity namely; SOD, CAT, GPx and GR in the present study could be due to the accumulation of ROS (Birben et al. 2012). Collectively, the depletion of the assessed enzymatic and non-enzymatic antioxidant molecules in the brain tissue of rats treated with DBP, may explain why the defense system is failed to cope with the elevation of ROS/RNS which may produce neuronal injury.

Neuroinflammation has been strongly associated with the alterations in the oxidative status. This hypothesis was confirmed in our experiment, as demonstrated by the marked elevation of TNF- $\alpha$  and IL-1 $\beta$  levels in the cortical, striatal and brainstem homogenates after DBP exposure. TNF- $\alpha$  and IL-1 $\beta$  are characterized as a pro-inflammatory cytokines participating in the systemic inflammation, proliferation, differentiation, and apoptosis (Elkhadragy et al. 2018). Environmental endocrine disruptors at low doses have been

implicated in the disturbance of the cytokines release pattern, activating a pro inflammatory state leading to apoptotic cascade through the activation of nuclear factor - $\kappa$ B (Chighizola and Meroni 2012; Peng 2015; Teixeira et al. 2016). In addition, Kovacic and Edwards (2010) attributed the increase of these mediators to the ROS overproduction following phthalates intoxication. Programmed cell death is regulated by several protein including Bcl-2, Bax and Caspase-3. Bcl-2 has anti apoptotic effect, enhancing cellular survival and found mainly in the outer membrane of mitochondria. Bax is widely distributed in the cytoplasm stimulate the opening of calcium ion channels which in turn increase the release of cytochrome c. whereas, caspase-3 activates DNA fragmentation through proteolytic pathway (Elmore 2007). Our findings showed that Bcl-2 expression was down regulated, while the expression of Bax and caspase-3 were up regulated in all studied brain regions after DBP treatment for 6 weeks. Wojtowicz et al. (2017) demonstrated that DBP-induced neuronal damage in mouse neocortical neurons via enhancing ROS production, activating lactate dehydrogenase and caspase-3 and attributed its



**Fig. 4** Effects of oral administration of di-n-butyl phthalate (DBP) on apoptotic proteins (Bcl-2, Bax and caspase-3) levels in cerebral cortex, striatum and brainstem of rats. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test

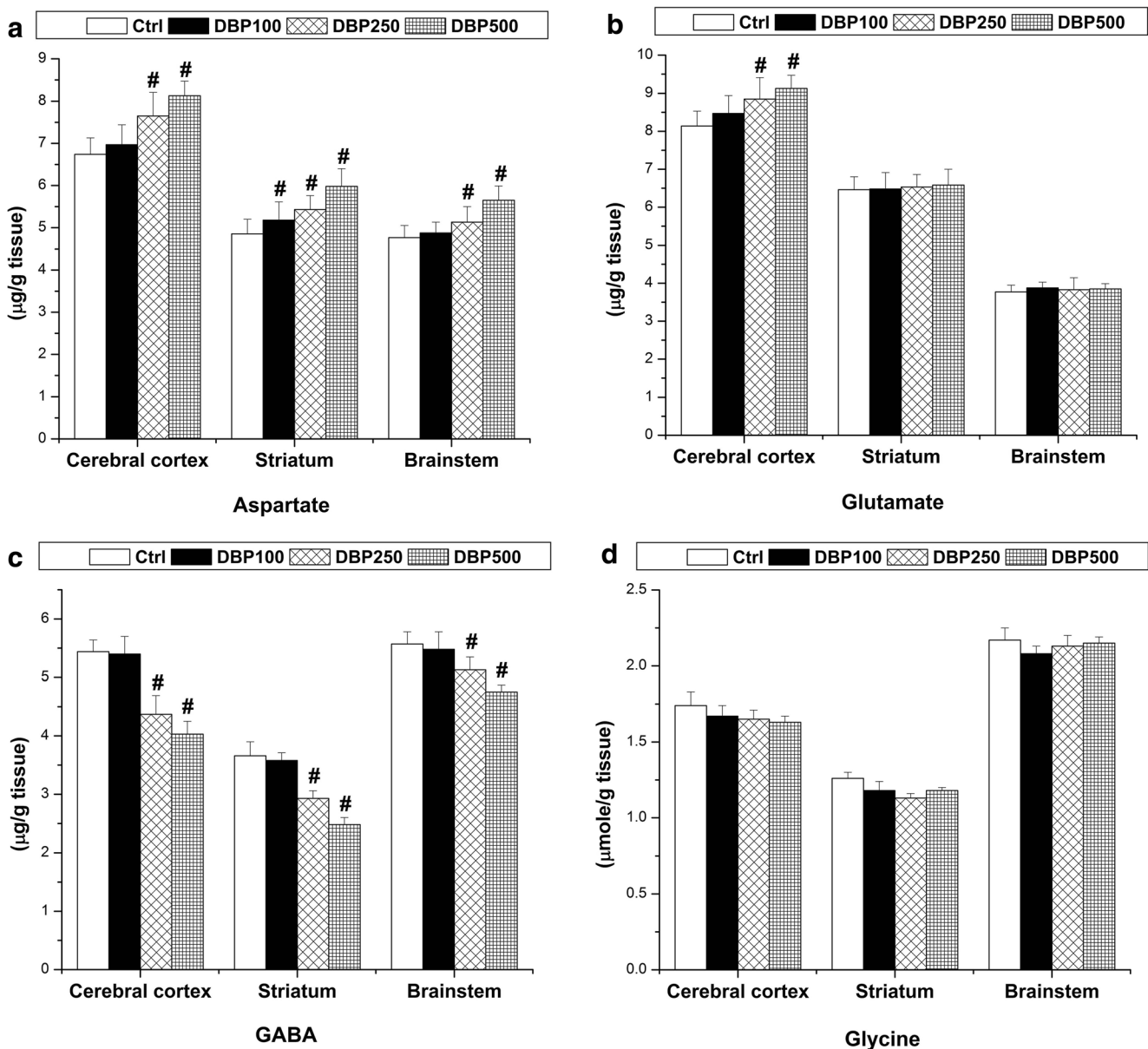


**Fig. 5** The content of monoamines (DA, NE and 5-HT) and AChE activity in cerebral cortex, striatum and brainstem of rats following di-n-butyl phthalate (DBP) exposure. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test

apoptotic effect to the over expression of aryl hydrocarbon receptors. Additionally, Li et al. (2013) found that prenatal exposure to DBP activated significantly caspase-3 in the hippocampi of rats. Moreover, diisononyl phthalate exposure elevated ROS levels and caspase-3 activity in mice brain tissues (Peng 2015). Furthermore, the expression of Bax and caspase-3 were up regulated, but Bcl-2 remained unchanged in mice brain following the treatment with a mixture of phthalates and arsenate (Mao et al. 2016).

Neurotransmitters are chemical messengers produced from nerve cells and have multiple pivotal roles in the nervous system (Marc et al. 2011). The estimation of the neurotransmitters plays an important role to understand

the development of several neurological diseases and to evaluate the treatment strategies efficiency (Cook 2008). In our experiment, NE and DA content were declined in the tested brain regions after DBP exposure but 5-HT levels unaltered. DBP also suppressed significantly AChE activity in the brain tissue in the present study. Moreover, the excitatory amino acids levels (aspartate and glutamate) were significantly increased, while the major inhibitory amino acids GABA, was decreased. Mice treated with benzyl butyl phthalate for two weeks showed a disturbance in learning and memory functions and impaired neurotransmission (Min et al. 2014). In contrast to our findings, Carbone et al. (2010) recorded



**Fig. 6** The content of amino acid transmitters (aspartate, glutamate, GABA and glycine) in cerebral cortex, striatum and brainstem of rats following di-n-butyl phthalate (DBP) exposure. Data are expressed as the mean  $\pm$  SD ( $n = 10$ ). # $p < 0.05$  vs. the control group using Tukey post hoc test

a decrease in aspartate and an increase in GABA contents in rat treated with di-(2-ethylhexyl) phthalate. We suggest that, the alterations in the monoaminergic, AChE and amino acids transmitters in the current study might be due to the imbalance between the oxidants and the antioxidants and the activation of apoptotic cell death in the brain tissue following DBP exposure.

## Conclusion

DBP-exposed rats showed a disturbance in the oxidative status, as represented by the elevated oxidants (MDA, NO,

8-OHdG) and the decreased antioxidant defense system (GSH, SOD, CAT, GPx and GR). DBP also enhanced a pro-inflammatory state through increasing the release of TNF- $\alpha$  and IL-1 $\beta$ . The increase of these cytokines was associated with the increase of pro-apoptotic proteins (Bax and caspase-3) and the decrease of the anti-apoptotic protein (Bcl-2). In addition, the levels of NE and DA were decreased. AChE activity was also suppressed. This was accompanied by the alterations in the major excitatory and inhibitory amino acids neurotransmitters in the cerebral cortex, striatum and brainstem of adult male Wistar albino rats. However, further studies are required to explain the molecular mechanisms involved in DBP-induced neuronal damage.



## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126
- Aktan F (2004) iNOS-mediated nitric oxide production and its regulation. *Life Sci* 75:639–653. <https://doi.org/10.1016/j.lfs.2003.10.042>
- Al Omairi NE, Radwan OK, Alzahrani YA, Kassab RB (2018) Neuroprotective efficiency of *Mangifera indica* leaves extract on cadmium-induced cortical damage in rats. *Metab Brain Dis* 33:1121–1130. <https://doi.org/10.1007/s11011-018-0222-6>
- Al-Olayan EM, El-Khadragy MF, Abdel Moneim AE (2015) The protective properties of melatonin against aluminium-induced neuronal injury. *Int J Exp Pathol* 96:196–202. <https://doi.org/10.1111/iep.12122>
- Al-Olayan EM, El-Khadragy MF, Omer SA, Shata MT, Kassab RB, Abdel Moneim AE (2016) The beneficial effect of cape gooseberry juice on carbon tetrachloride- induced neuronal damage. *CNS Neurol Disord Drug Targets* 15:344–350
- Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidative stress and antioxidant defense. *World Allergy Organ J* 5:9–19. <https://doi.org/10.1097/WOX.0b013e3182439613>
- Calafat AM, Brock JW, Silva MJ, Gray LE Jr, Reidy JA, Barr DB, Needham LL (2006) Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate. *Toxicology* 217:22–30. <https://doi.org/10.1016/j.tox.2005.08.013>
- Carbone S, Szwarcfarb B, Ponzio O, Reynoso R, Cardoso N, Deguiz L, Moguilevsky JA, Scacchi P (2010) Impact of gestational and lactational phthalate exposure on hypothalamic content of amino acid neurotransmitters and FSH secretion in peripubertal male rats. *Neurotoxicology* 31:747–751. <https://doi.org/10.1016/j.neuro.2010.06.006>
- Chighizola C, Meroni PL (2012) The role of environmental estrogens and autoimmunity. *Autoimmun Rev* 11:A493–A501. <https://doi.org/10.1016/j.autrev.2011.11.027>
- Cook I (2008) Biomarkers in psychiatry: potentials, pitfalls, and pragmatics. *Prim Psychiatry* 15:54–59
- de Mello Santos T, da Silveira LTR, Rinaldi JC, Scarano WR, Domeniconi RF (2017) Alterations in prostate morphogenesis in male rat offspring after maternal exposure to Di-n-butyl-phthalate (DBP). *Reprod Toxicol* 69:254–264. <https://doi.org/10.1016/j.reprotox.2017.03.010>
- Elkhadragy MF, Kassab RB, Metwally DM, Almeer R, Abdel-Gaber R, al-Olayan EM, Essawy EA, Amin HK, Abdel Moneim AE (2018) Protective effects of *Fragaria ananassa* methanolic extract in a rat model of cadmium chloride-induced neurotoxicity. *Biosci Rep*. <https://doi.org/10.1042/BSR2018086>
- Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* 82:70–77
- Elmore S (2007) Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35:495–516. <https://doi.org/10.1080/01926230701320337>
- Escudero-Lourdes C (2016) Toxicity mechanisms of arsenic that are shared with neurodegenerative diseases and cognitive impairment: role of oxidative stress and inflammatory responses. *Neurotoxicology* 53:223–235. <https://doi.org/10.1016/j.neuro.2016.02.002>
- Factor VM, Kiss A, Voitach JT, Wirth PJ, Thorgeirsson SS (1998) Disruption of redox homeostasis in the transforming growth factor- $\alpha$ /c-myc transgenic mouse model of accelerated hepatocarcinogenesis. *J Biol Chem* 273:15846–15853
- Faniband M, Lindh CH, Jonsson BA (2014) Human biological monitoring of suspected endocrine-disrupting compounds. *Asian J Androl* 16:5–16. <https://doi.org/10.4103/1008-682X.122197>
- Franken C, Lambrechts N, Govarts E, Koppen G, den Hond E, Ooms D, Voorspoels S, Bruckers L, Loots I, Nelen V, Sioen I, Nawrot TS, Baeyens W, van Larebeke N, Schoeters G (2017) Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents. *Int J Hyg Environ Health* 220:468–477. <https://doi.org/10.1016/j.ijheh.2017.01.006>
- Fujii M, Shinohara N, Lim A, Otake T, Kumagai K, Yanagisawa Y (2003) A study on emission of phthalate esters from plastic materials using a passive flux sampler. *Atmos Environ* 37(39–40):5495–5504
- Gao M, Dong Y, Zhang Z, Song W, Qi Y (2017) Growth and antioxidant defense responses of wheat seedlings to di-n-butyl phthalate and di(2-ethylhexyl) phthalate stress. *Chemosphere* 172:418–428. <https://doi.org/10.1016/j.chemosphere.2017.01.034>
- Giribabu N, Sainath SB, Sreenivasula Reddy P (2014) Prenatal di-n-butyl phthalate exposure alters reproductive functions at adulthood in male rats. *Environ Toxicol* 29:534–544. <https://doi.org/10.1002/tox.21779>
- Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 126:131–138
- Heinrikson RL, Meredith SC (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. *Anal Biochem* 136:65–74
- Heudorf U, Mersch-Sundermann V, Angerer J (2007) Phthalates: toxicology and exposure. *Int J Hyg Environ Health* 210:623–634. <https://doi.org/10.1016/j.ijheh.2007.07.011>
- Kassab RB, El-Hennamy RE (2017) The role of thymoquinone as a potent antioxidant in ameliorating the neurotoxic effect of sodium arsenate in female rat. *Egyptian J Basic Appl Sci* 4:160–167. <https://doi.org/10.1016/j.ejbas.2017.07.002>
- Kavlock R et al (2006) NTP-CERHR expert panel update on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate. *Reprod Toxicol* 22:291–399
- Kovacic P, Edwards C (2010) Integrated approach to the mechanisms of thyroid toxins: electron transfer, reactive oxygen species, oxidative stress, cell signaling, receptors, and antioxidants. *J Recept Signal Transduct Res* 30:133–142. <https://doi.org/10.3109/10799891003702678>
- Lee E, Ahn MY, Kim HJ, Kim IY, Han SY, Kang TS, Hong JH, Park KL, Lee BM, Kim HS (2007) Effect of di(n-butyl) phthalate on testicular oxidative damage and antioxidant enzymes in hyperthyroid rats. *Environ Toxicol* 22:245–255. <https://doi.org/10.1002/tox.20259>
- Li Y, Zhuang M, Li T, Shi N (2009) Neurobehavioral toxicity study of dibutyl phthalate on rats following in utero and lactational exposure. *J Appl Toxicol* 29:603–611. <https://doi.org/10.1002/jat.1447>
- Li XJ, Jiang L, Chen L, Chen HS, Li X (2013) Neurotoxicity of dibutyl phthalate in brain development following perinatal exposure: a study in rats. *Environ Toxicol Pharmacol* 36:392–402. <https://doi.org/10.1016/j.etap.2013.05.001>
- Lien Y et al (2015) Prenatal exposure to phthalate esters and behavioral syndromes in children at 8 years of age: Taiwan maternal and infant cohort study. *Environ Health Perspect* 123:95–100
- Liu PS, Tseng FW, Liu JH (2009) Comparative suppression of phthalate monoesters and phthalate diesters on calcium signalling coupled to nicotinic acetylcholine receptors. *J Toxicol Sci* 34:255–263
- Lodovici M, Casalini C, Briani C, Dolara P (1997) Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology* 117:55–60
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Lu KY, Tseng FW, Wu CJ, Liu PS (2004) Suppression by phthalates of the calcium signaling of human nicotinic acetylcholine receptors in human neuroblastoma SH-SY5Y cells. *Toxicology* 200:113–121. <https://doi.org/10.1016/j.tox.2004.03.018>

- Mahaboob Basha P, Radha MJ (2017) Gestational di-n-butyl phthalate exposure induced developmental and teratogenic anomalies in rats: a multigenerational assessment. *Environ Sci Pollut Res Int* 24:4537–4551. <https://doi.org/10.1007/s11356-016-8196-6>
- Mao G, Zhou Z, Chen Y, Wang W, Wu X, Feng W, Cobbina SJ, Huang J, Zhang Z, Xu H, Yang L, Wu X (2016) Neurological toxicity of individual and mixtures of low dose arsenic, mono and di (n-butyl) phthalates on sub-chronic exposure to mice. *Biol Trace Elem Res* 170:183–193. <https://doi.org/10.1007/s12011-015-0457-6>
- Marc DT, Ailts JW, Campeau DC, Bull MJ, Olson KL (2011) Neurotransmitters excreted in the urine as biomarkers of nervous system activity: validity and clinical applicability. *Neurosci Biobehav Rev* 35: 635–644. <https://doi.org/10.1016/j.neubiorev.2010.07.007>
- Mariana M, Feiteiro J, Verde I, Cairrao E (2016) The effects of phthalates in the cardiovascular and reproductive systems: a review. *Environ Int* 94:758–776. <https://doi.org/10.1016/j.envint.2016.07.004>
- Min A, Liu F, Yang X, Chen M (2014) Benzyl butyl phthalate exposure impairs learning and memory and attenuates neurotransmission and CREB phosphorylation in mice. *Food Chem Toxicol* 71:81–89. <https://doi.org/10.1016/j.fct.2014.05.021>
- Nishikimi M, Appaji N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 46:849–854
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358
- Pagel P, Blome J, Wolf HU (2000) High-performance liquid chromatographic separation and measurement of various biogenic compounds possibly involved in the pathomechanism of Parkinson's disease. *J Chromatogr B Biomed Sci Appl* 746:297–304
- Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–169
- Peng L (2015) Mice brain tissue injury induced by diisononyl phthalate exposure and the protective application of vitamin E. *J Biochem Mol Toxicol* 29:311–320. <https://doi.org/10.1002/jbt.21700>
- Rocha BA, Asimakopoulos AG, Barbosa F Jr, Kannan K (2017) Urinary concentrations of 25 phthalate metabolites in Brazilian children and their association with oxidative DNA damage. *Sci Total Environ* 586:152–162. <https://doi.org/10.1016/j.scitotenv.2017.01.193>
- Schettler T (2006) Human exposure to phthalates via consumer products. *Int J Androl* 29:134–139; discussion 181–135. <https://doi.org/10.1111/j.1365-2605.2005.00567.x>
- Shaw CA, Taylor EL, Megson IL, Rossi AG (2005) Nitric oxide and the resolution of inflammation: implications for atherosclerosis. *Mem Inst Oswaldo Cruz* 100 Suppl 1:67–71. <https://doi.org/10.1590/S0074-02762005000900012>
- Shono T, Taguchi T (2014) Short-time exposure to mono-n-butyl phthalate (MBP)-induced oxidative stress associated with DNA damage and the atrophy of the testis in pubertal rats. *Environ Sci Pollut Res Int* 21:3187–3190. <https://doi.org/10.1007/s11356-013-2332-3>
- Tabb MM, Blumberg B (2006) New modes of action for endocrine-disrupting chemicals. *Mol Endocrinol* 20:475–482. <https://doi.org/10.1210/me.2004-0513>
- Teixeira D, Marques C, Pestana D, Faria A, Norberto S, Calhau C, Monteiro R (2016) Effects of xenoestrogens in human M1 and M2 macrophage migration, cytokine release, and estrogen-related signaling pathways. *Environ Toxicol* 31: 1496–1509. <https://doi.org/10.1002/tox.22154>
- Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 7:65–74. <https://doi.org/10.2174/157015909787602823>
- Wang JI, Karmaus WJ (2017) Oxidative stress-related genetic variants may modify associations of phthalate exposures with asthma. *Int J Environ Res Public Health* 14. <https://doi.org/10.3390/ijerph14020162>
- Wojtowicz AK, Szychowski KA, Wnuk A, Kajta M (2017) Dibutyl phthalate (DBP)-induced apoptosis and neurotoxicity are mediated via the aryl hydrocarbon receptor (AhR) but not by estrogen receptor alpha (ERalpha), estrogen receptor beta (ERbeta), or peroxisome proliferator-activated receptor gamma (PPARgamma) in mouse cortical neurons. *Neurotox Res* 31:77–89. <https://doi.org/10.1007/s12640-016-9665-x>
- Yan B, Guo J, Liu X, Li J, Yang X, Ma P, Wu Y (2016) Oxidative stress mediates dibutyl phthalate-induced anxiety-like behavior in Kunming mice. *Environ Toxicol Pharmacol* 45:45–51. <https://doi.org/10.1016/j.etap.2016.05.013>
- Yavasoglu NU, Koksall C, Dagdeviren M, Aktug H, Yavasoglu A (2014) Induction of oxidative stress and histological changes in liver by subacute doses of butyl cyclohexyl phthalate. *Environ Toxicol* 29: 345–353. <https://doi.org/10.1002/tox.21813>
- Zhou D, Wang H, Zhang J, Gao X, Zhao W, Zheng Y (2010) Di-n-butyl phthalate (DBP) exposure induces oxidative damage in testes of adult rats. *Syst Biol Reprod Med* 56:413–419. <https://doi.org/10.3109/19396368.2010.509902>
- Zhou D, Wang H, Zhang J (2011) Di-n-butyl phthalate (DBP) exposure induces oxidative stress in epididymis of adult rats. *Toxicol Ind Health* 27:65–71. <https://doi.org/10.1177/0748233710381895>
- Zhu YP, Chen L, Wang XJ, Jiang QH, Bei XY, Sun WL, Xia SJ, Jiang JT (2017) Maternal exposure to di-n-butyl phthalate (DBP) induces renal fibrosis in adult rat offspring. *Oncotarget* 8(19):31101–31111. <https://doi.org/10.18632/oncotarget.16088>
- Zuo HX et al (2014) Di-(n-butyl)-phthalate-induced oxidative stress and depression-like behavior in mice with or without ovalbumin immunization. *Biomed Environ Sci* 27:268–280. <https://doi.org/10.3967/bes2014.001>