Effect of piracetam on brain oxidative stress and tissue damage following toluene exposure in rats

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ARTICLE INFO

ABSTRACT

In this study, the effect of the nootropic drug piracetam on brain oxidative stress and histopathological changes in brain, liver and kidney following exposure to toluene in rats was examined. Piracetam (150 or 300 mg/kg, subcutaneously, daily) was given along with toluene (500 mg/kg, intraperitoneally, daily) for one week. The brain content of malondialdehyde (MDA), reduced glutathione (GSH) and the activity of paraoxonase-1 (PON-1), and butyrylcholinesterase (BChE) in brain homogenates were determined. Histopathology of the brain, liver and kidney and brain Bax immunohistochemistry were performed. Results showed that exposure to toluene resulted in increased brain lipid peroxidation (MDA) and NO along with decreased reduced glutathione. Toluene also inhibited PON-1, and BChE activities. The administration of piracetam had no significant effect on brain lipid peroxidation. The level of reduced glutathione was unchanged by piracetam but PON1 activity was increased by the lower dose of the drug. Piracetam showed no significant effect on BChE activity in toluene treated rats. Histopathological examination of the brain of toluene only treated rats showed degenerated neurons in cerebral cortex, marked neuronal vacuolation in hippocampus and focal hemorrhage. Bax 3 immunohistochemical staining showed cytoplasmic reactivity in degenerated neurons. Rats given piracetam showed decreased cortical cellularity, increased number of degenerated neurons and increased BAX staining. The higher dose of the drug caused sinusoidal hemorrhage and intertubular hemorrhage in kidney. Collectively, these results indicate that treatment with piracetam wan not able to decrease neuronal damage in rats exposed to toluene.

1.   Introduction

Occupational and environmental exposure to organic solvents is an important health problem. One such solvent is toluene or methylbenzene which is widely used in industry, gasoline, glues, adhesives, paint thinners and household cleaning agents (Eisenberg, 2003). Solvent abuse is associated with more toxic effects due to exposure to high levels of the toxicant. It is more prevalent among adolescents both in developed and undeveloped countries (Lubman et al., 2008; Elkoussi and Bakheet, 2011; Filley, 2013). Toluene is rapidly absorbed after inhalation and being

highly lipophyllic is distributed in tissues rich in lipids, especially the brain tissue, causing serious central nervous system toxicities including chronic toxic encephalopathy, cognitive and memory impairments, brain atrophy and dementia (Rosenberg et al., 1988; Fornazzari et al., 2003; Yucel et al., 2008; Aydin et al., 2009). The mechanism (s) underlying these toxic effects of toluene is not yet well defined but accumulating evidence suggest an important role for free radical mediated damage (Burmistrov et al., 2001; Kodavanti et al,., 2011; Atef et al., 2015; Abdel-Salam et al., 2016). The brain tissue in particular is considered highly vulnerable to oxidative injury. This is due to the fact that the brain which is only 2% of body weight consumes about 20% of basal O_2 consumption which is reflected in higher mitochondrial superoxide ($O_2^{\bullet-}$) production and also increased damage upon toxins-induced mitochondrial dysfunction and the consequent energy failure. Other risk factors are the presence of excitotoxic amino acids, and the autoxidizable neurotransmitters dopamine, noradrenaline and serotonin, high iron content in certain regions, rich content of highly polyunsaturated fatty acid side-chains, and modest antioxidant defenses particularly catalase levels (Halliwell, 1992; Floyd, 1999). Workers with paint thinner containing toluene were reported to have elevated serum malondialdehyde levels compared to controls (Halifeoglu et al., 2000). Animals studies showed that, exposure to toluene caused increased generation of ROS in crude mitochondrial fractions from rat cerebellum, striatum and hippocampus (Mattia et al., 1993), increased protein carbonyls in rat frontal cortex and cerebellum (Kodavanti et al., 2015) and increased MDA and NO together with decreased levels of GSH in rat brain (Abdel-Salam et al., 2016, 2019). Rats treated with toluene exhibited dead and apoptotic neurons, perineuronal vacuolations in cerebral cortex, and degeneration of cerebellar Purkinje cells (Abdel-Salam et al., 2016; 2019; Abdel-Wahhab et al., 2019; Hassan et al., 2020).

The nootropic or cognitive enhancing drug piracetam (2-oxo-1-pyrrolidineacetamide) is widely used in several countries to improve cognitive functioning and treat memory disorders such as that associated with aging or caused by cerebrovascular insufficiency (Waegemans et al., 2002). The drug has also been shown to improve cognitive function after coronary artery bypass surgery (Holinski et al., 2008), improves blood flow and facilitates recovery from aphasia following stroke (Kessler et al., 2000). It demonstrated efficacy in treating hereditary cerebellar ataxia (Inci et al., 2008) and familial myoclonic epilepsy patients (Fedi et al., 2001). Piracetam facilitated learning, prevented the development of amnesia (Shorvon, 2001; Abdel-Salam and Nada, 2011), exerted an analgesic action (Abdel-Salam, 2006), and showed neuroprotective effects under different experimental conditions (Keil et al., 2006; He et al., 2014; Abdel-Salam et al., 2016; babar et al., 2020). It improved mitochondrial membrane potential and ATP production in PC12 cells exposed to nitric oxide, serum deprivation or respiratory chain complex inhibitors (Keil et al., 2006) and decreased cell damage in rat primary cortical neurons exposed to O_2 and glucose deprivation (He et al., 2014). In vivo, piracetam at 100 mg/kg decreased brain lipid peroxidation and improved neuronal damage in the $AlCl_3$ rat model of Alzheimer's disease (Abdel-Salam et al., 2016).

The aim of the present study was therefore to investigate the possible modulation by piracetam of the toluene-induced brain oxidative stress and neurodegeneration. Oxidative DNA damage was also reported in the liver and kidney of rats after toluene inhalation (Tokunaga et al., 2003). The study was extended to include histopathological study of the effect of toluene alone or with on the liver and kidney tissues.

2. Materials and Methods

2.1. Animals

Male Sprague-Dawley rats, obtained from Animal House of the National Research Centre, Cairo, weighing between 130-140 g were group-housed under temperature- and light-controlled conditions with standard laboratory rodent chow and water provided *ad libitum.* Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and

followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and chemicals

Toluene (Sigma-Aldrich, St Louis, MO, USA) and piracetam (Pharco Pharmaceuticals, Alexandria, ARE) were used. Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

2.3. Study design

Rats were randomly divided into four groups, six rats each. Group 1 received the vehicle (0.2 ml saline) daily. Group 2 received toluene in paraffin oil (vol/vol) in a dose 500 mg/kg (1.6 ml/kg), intrapreitoneally (i.p.) daily for 7 days. Groups 3 and 4 received toluene (500 mg/kg, i.p) along with piracetam (150 or 300 mg/kg, subcutaneously daily) for 7 days. Rats were then euthanized by decapitation under light ether anesthesia for tissue collection.

Half of the brain of each rat was quickly removed, washed with ice-cold phosphate buffered saline (PBS, pH 7.4), dissected out on an ice-cold plate, dissected into different regions (the cerebral cortex, striatum, and rest of the brain), weighed, and stored at−80 °C until the biochemical analyses were carried out. The brain tissues were homogenized with 0.1 M phosphate buffer saline at pH 7.4 to give a final concentration of 10 %w/v for the biochemical assays.

2.4. Biochemical analysis

2.4.1. Lipid peroxidation

Lipid peroxidation products in the brain homogenates was assayed by measuring the level of malondialdehyde (MDA) where the thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red colored complex having a peak absorbance at 532 nm (Nair and Turner, 1984).

2.4.2. Nitric oxide

The level of NO, measured as nitrite, was determined using the Griess reagent. Nitrite, a stable end-product of nitric oxide, is used as an indicator of the production of NO. In this assay, nitrate is converted to nitrite by nitrate reductase. The Griess reagent then reacts with nitrite forming a deep purple azo compound. The absorbance is read at 540 nm using a spectrophotometer (Archer, 1993).

2.4.3. Reduced glutathione

Brain reduced glutathione (GSH) was determined in homogenates according to Ellman et al. (1959).The procedure is based on the reduction of Ellman′s reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, which is intense yellow in color and determined spectrophotometrically at 412 nm.

2.4.4. Paraoxonase activity

Arylesterase activity of paraoxonase was measured in supernatants using phenylacetate as a substrate and the formation of phenol was measured by monitoring the increase in absorbance at 270 nm and 25°C with a spectrophotometer. (Eckerson et al., 1983).

2.4.5. Butyrylcholinesterase activity

Butyrylcholinesterase activity (BChE) was measured spectrophotometrically in supernatants using commercially available kit (Biodiagnostics, Egypt) according to the method of Ellman et al. (1961).

2.5. Histopathology

For the histopathological study, the brain was immediately removed, placed in 10% formaldehyde, dehydrated in graded alcohol and embedded in paraffin, the paraffin blocks were serially sectioned at 5 μm thickness. Afterwards, they were stained with routine hematoxylin-eosin stain for pathological examination and morphologic assessment.

2.6. Bax immunostaining

Paraffin sections were processed for immunostaining using Bax (0.7 ml. of antibody prediluted 0.05 mol/L Tris-HCl, pH 7.6 containing stabilizing protein and 0.015 mol/L sodium azide – Thermo Fisher Scientific. UK) and EconoTek HRP Anti-Polyvalent (DAP) ready-to use (ScyTek Laboratories inc. USA) detection system as follows: sections on Citoglas adhesion microscope slides were deparaffinized using xylene then rehydrated with distilled water. To reduce nonspecific background staining due endogenous peroxidase the slides were incubated in hydrogen peroxide for 5 minutes then washed in buffer 2 times. For more blockage of nonspecific back ground staining the slides were incubated in super block for 10 minutes then washed in buffer 1 time. Tissue sections were boiled in 10 mM citrate buffer (Lab vision corporation. USA) for 20 minutes followed by cooling in room temperature for 20 minutes. Primary antibody was applied and incubated for 30 minutes at room temperature then washed in buffer 4 times. EconoTek biotinylated Anti-polyvalent was applied and incubated for 30 minutes at room temperature then washed in buffer 4 times. EconoTek HRP was applied and incubated for 30 min at room temperature then rinse in buffer 4 times. DAB was prepared by adding 4 drops (200 μl) DAB chromogen to DAB substrate (5 ml) then applied to tissue sections and incubated for 10 minutes. Finally counter stained in hematoxylin for 3 minutes.

Bax stained sections were examined at high power for immunohistochemical expression and were divided into those that were negative (no immunoreactivity in any cells) and positive (membrane and/or cytoplasm immunoreactivity present).

2.7 Statistical analysis

Data are expressed as mean ± SE. Data were analyzed by one-way analysis of variance, followed by Duncan's multiple range test for post hoc comparison of group means using SPSS software (SPSS Inc., Chicago, USA). Effects with a probability of $p < 0.05$ were considered to be significant.

3. Results

3.1. Brain biochemistry

3.1.1. Lipid peroxidation

Malondialdehyde levels were increased by 53.7% in the cortex from 21.6 ± 1.5 nmol/g.tissue in the vehicle group to 33.2 ± 1.4 nmol/g.tissue in the toluene only group. It increased by toluene in the striatum and rest of brain tissue by 57.6% and 65.4%, respectively, compared with the vehicle group. No significant effects in brain MDA levels were observed in rats treated with toluene and piracetam compared with the toluene control group (Fig. 1).

3.1.2. Nitric oxide

Toluene significantly increased NO level by 54.2% in the cortex from 19.1 ± 1.3 μmol/g.tissue in the vehicle group to 29.7 ± 1.2 μmol/g.tissue in the toluene only group. It increased by toluene in the striatum and rest of brain tissue by 46% and 45.8%, respectively, compared with the vehicle group. Nitric oxide was significantly decreased in the cerebral cortex by 20.5% (23.6 ± 1.0 vs.

29.7 ± 1.2 μmol/g.tissue) following treatment with the lower dose of piracetam compared with the toluene control group (Fig. 2).

3.1.3. Reduced glutathione

Reduced glutathione levels were significantly decreased by 27.3% from 3.3 ± 0.11 μmol/g.tissue in the vehicle group to 2.5 ± 0.06 μmol/g.tissue in the toluene control group. Reduced glutathione was significantly decreased by toluene in the striatum and rest of brain tissue 25.1% and 30.3%, respectively, compared with the vehicle group. Piracetam had no significant effects on brain GSH in toluene-treated rats (Fig. 3).

3.1.4. Paraoxonase-1

Paraoxonase-1 activity was significantly inhibited by 44.7% and 77.4% toluene in cerebral cortex (6.9 ± 0.22 vs. 13.2 ± 0.87 kU/l) and rest of brain tissue (3.2 ± 0.15 vs. 14.1 ± 0.78 kU/l), respectively, compared with the vehicle group. Paraoxonase-1 activity was significantly increased in cerebral cortex and rest of brain tissue by 32.0% and 78.1% following treatment with 150 mg/kg piracetam compared with the toluene control group (Fig. 4).

3.1.5. Butyrylcholinesterase

Administration of toluene caused significant inhibition of BChE activity by 38.5% compared with the vehicle group (146.3± 6.8 vs. 238.0 ± 9.0 U/l). Piracetam treatment had no significant effect on BChE activity in brain of toluene intoxicated rats (Fig. 5).
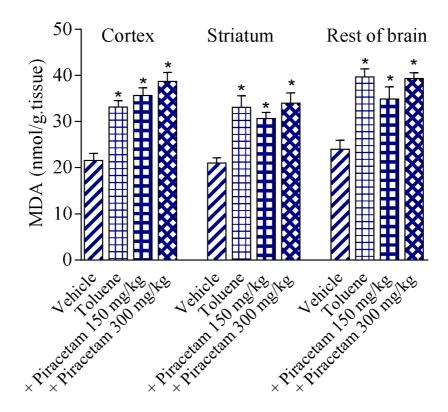

Fig 1. The effect of piracetam on brain malondialdehyde (MDA) activity after toluene administration. Data are mean ±SEM. *P<0.05 compared with the vehicle group.
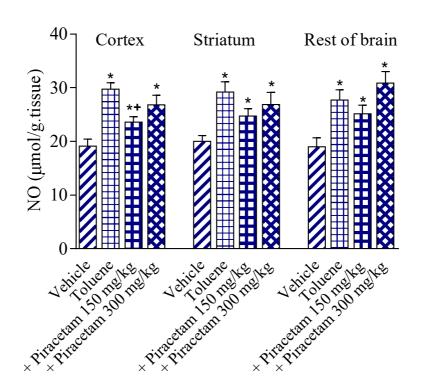
Fig 2. The effect of piracetam on brain nitric oxide (NO) after toluene administration. Data are mean ±SEM. *P<0.05 compared with the vehicle group.
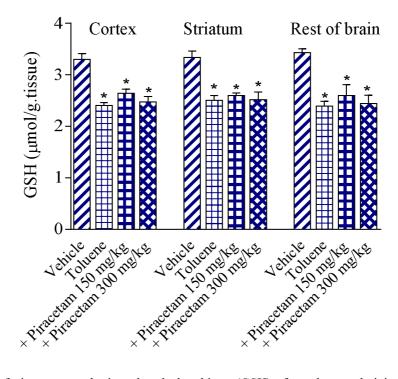

Fig 3. The effect of piracetam on brain reduced glutathione (GSH) after toluene administration. Data are mean ±SEM. *P<0.05 compared with the vehicle group.
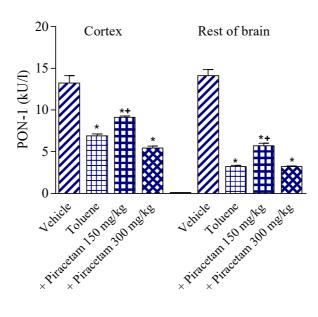
Fig 4. The effect of piracetam on brain paraoxonase-1 (PON-1) activity after toluene administration. Data are mean ±SEM. *P<0.05 compared with the vehicle group.+P<0.05 compared with the toluene only group.
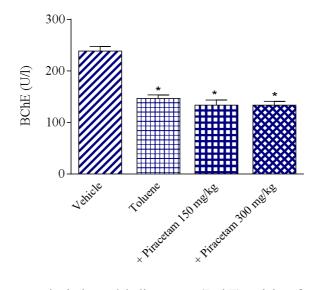

Fig 5. The effect of piracetam on brain butyrylcholinesterase (BChE) activity after toluene administration. Data are mean ±SEM. *P<0.05 compared with the vehicle group.

3.2.   Histopathology

3.2.1. Brain histopathology

In vehicle treated rats, the brain tissue showed averagely cellular cortex with normal looking neurons and glial cells distributed against fibrillary stroma (Figs.6A & 6B). Rats treated with toluene showed markedly vacuolated neurons in the hippocampal region with normal cortex cellularity yet occasional degenerated neurons were observed and focal background hemorrhage (Figs. 6C-6F). The brain tissue in the group treated with toluene and 150 mg/kg piracetam showed increased number of degenerated neuron displaying small dark nuclei and vacuolated cytoplasm. The background stromal vessels were increased (Figs. 7A & 7B). The brain of rats given toluene and 300 mg/kg piracetam showed decreased cortical cellularity with numerous degenerated neurons and foci of background hemorrhage and edema (Figs. 7C & 7D).

3.2.2. Bax expression

In the vehicle group, the Bax immunohistochemical staining was negative (Fig. 9A). In toluene only treated rats, the Bax immunohistochemical staining showed cytoplasmic reactivity in the occasionally degenerated neurons (Fig. 9B). In the group treated with toluene and 300 mg/kg piracetam, the Bax staining showed cytoplasmic staining in many degenerated neurons (Fig. 9C).

3.2.3. Liver histopathology

Vehicle treated rats showed liver with preserved lobular architecture, with each lobule showing a central vein with radial arrangement of hepatocytes and preserved sinusoids. The portal area showed normal bile ducts and vasculature (Fig. 9A). In toluene only treated rats, the liver showed unremarkable histopathological changes (Fig. 9B). Following treatment with 150 mg/kg piracetam, the liver tissue showed minimally congested sinusoids with no observable changes in the hepatocytes (Fig. 9C). In rats treated with 300 mg/kg piracetam, the liver showed more sinusoidal hemorrhage (Fig. 9D).

3.2.4. Kidney histopathology

In the vehicle group, the kidney showed normal looking glomeruli with average mesangial cellularity and patent capillaries. The tubules had normal appearance (Fig. 10A). In toluene only treated rats, the kidney showed unremarkable histopathological changes (Fig. 10B). In the group treated with toluene and 150 mg/kg pircaetam, the kidney showed minimal intertubular RBC'S, but glomerular structure was not affected (Fig. 10C). Following treatment with 300 mg/kg piracetam, intertubular hemorrhage in the kidney was more than that seen in group given the lower dose of the drug (Fig. 10D).
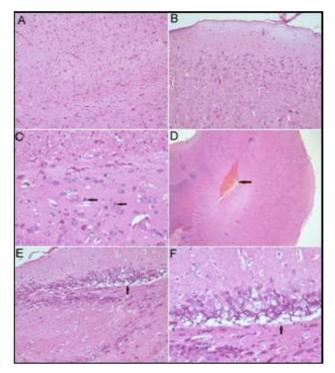

Fig 6. Representative photomicrographs of H&E stained brain sections of rat treated with: **(A&B)** Vehicle showing normal cerebral cortex showing glial and neuron cells (x100). **(C)** Toluene showing focal neuronal degeneration (arrow) (x400). (D) Toluene brain showing parenchymal hemorrhage (arrow) (x40). **(E &F)** Toluene showing vacuolations of pyramidal neurons (arrow), thinning of dentate gyrus layer and cytoplasmic vacuolations in the hippocampal region (x100 & x400).
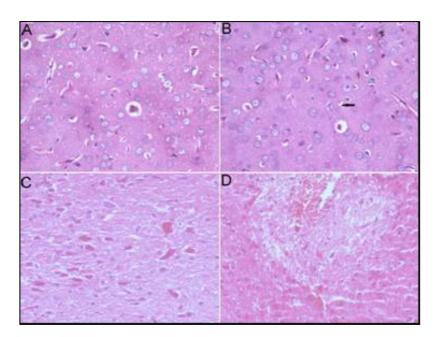
Fig 7. Representative photomicrographs of H&E stained brain sections of rat treated with: **(A)** Toluene + piracetam 150 mg/kg showing minimal vacuolations in the hippocumbal region with vascular proliferation and focal edema (x400). **(B)** Toluene + piracetam 150 mg/kg showing some apoptotic neurons (arrow) (x400). **(C)** Toluene + piracetam 300 mg/kg showing decreased cortical cellularity and numerous degenerated neurons (X100). **(D)** Toluene + piracetam 300 mg/kg showing stromal hemorrhage and edema (x100).
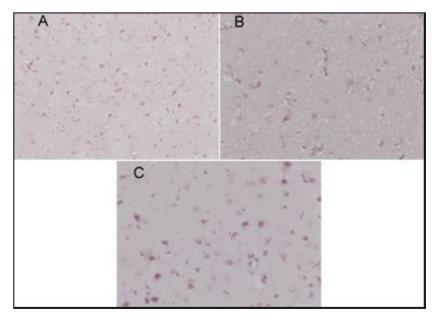

Fig 8. Representative photomicrographs of Bax stained brain sections of rat treated with **(A)** Vehicle showing no staining (x100). **(B)** Toluene showing focal staining with Bax (x100). **(C)** Toluene + piracetam 300 mg/kg showing numerous neurons showing Bax cytoplasmic staining.
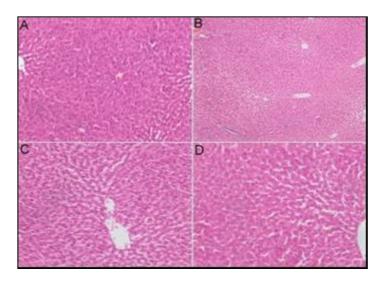
Fig 9. Representative photomicrographs of H&E stained liver sections of rat treated with **(A)** Vehicle showing normal looking liver (x200). **(B)** Toluene showing no effect on liver (x40). **(C)** Toluene + piracetam 150 mg/kg: minimal sinusoidal hemorrhage in the liver (x200). **(D)** Toluene + piracetam 300 mg/kg: little hemorrhage seen in liver sinusoids (x200).
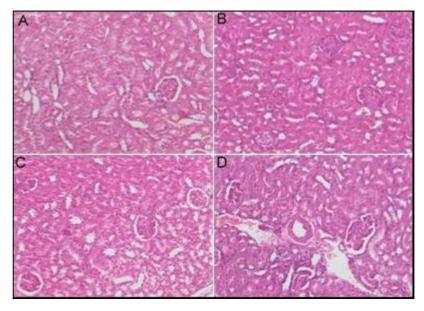

Fig 10. Representative photomicrographs of H&E stained kidney sections of rat treated with **(A)** Vehicle showing normal looking kidney and liver (x200). **(B)** Toluene: no effect on kidney (x200). **(C)** Toluene + piracetam 150 mg/kg: normal looking kidney structures (x200). **(D)** Toluene + piracetam 300 mg/kg: little hemorrhage seen in the kidney tubule (x200).

4. Discussion

In this study, the nootropic drug piracetam was investigated for its ability to modulate brain oxidative stress and neuronal damage evoked by the repeated administration of the organic solvent toluene in rats. Our results show that piracetam failed to prevent toluene-induced neurotoxicity, although an improvement in some oxidative stress parameters indicated by decreased NO level and increased PON-1 activity, was observed with the lower dose of the drug. Moreover, the brain of toluene treated rats given the high dose of piracetam showed numerous neurons showing Bax

cytoplasmic staining. The Bcl2-associated X protein (Bax) is a pro-apoptotic protein in the mitochondrial apoptotic pathway (Oltvai et al., 1993; Brunelle and Letai, 2009). We also found that piracetam did not protect against the histopathological alterations in liver or kidney of toluene-treated rats.

The neurotoxic effects of toluene are ascribed to the increased generation of reactive oxygen and nitrogen species and consequent oxidative damage of cell biomolecules (Mattia et al., 1993; Burmistrov et al., 2001; Kodavanti et al,., 2011, 2015; Atef et al., 2015; Abdel-Salam et al., 2016, 2019). Our present findings are consistent with the above notion and with other studies that demonstrated increase in reactive oxygen species and lipid peroxidation and depletion o reduced glutathione in brain of rats following exposure to toluene (Atef et al., 2015; Abdel-Salam et al., 2016, 2019). In this study, rats treated with toluene for one week exhibited significant increase in the lipid peroxidation end product malondialdehyde, indicating oxidative damage to cell membrane lipids. There was also decreased brain level of the antioxidant and free radical scavenger reduced glutathione. The tripeptide glutathione (γ-L-glutamyl-L-cysteinylglycine) is the brain's most important antioxidant and the ratio of its reduced form (GSH) and the oxidized form (glutathione disulfide or GSSG) is largely a determinant of the redox state of the cell. GSH serves as a scavenger where it reacts non-enzymatically with superoxide ($O_2•^-$), nitric oxide (NO•), hydroxyl radical (OH•) and peroxynitrite ($ONOO^-$). It also detoxifies hydroperoxides, peroxynitrite, and lipid peroxides through the enzymatic reactions of GSH peroxidases (GPX) and peroxiredoxins (Wu et al., 2004; Franco et al., 2007). The decrease in GSH in brain of toluene intoxicated rats could therefore reflect its consumption by the increase in reactive oxygen and nitrogen species. In addition, the toxicant-induced mitochondrial dysfunction and decreased metabolic regeneration of reducing equivalents will result in decreased regeneration of GSH (Monks et al., 1999).

In the present study, there was a marked increase in the level nitric oxide in the brain rats intoxicated with toluene. This finding is in agreement with previous studies (Atef et al., 2015; Abdel-Salam et al., 2016, 2019; Hassan et al., 2020). In brain, the free radical NO• is an important signaling molecule (Sobrevia et al., 2016). The increased release of nitric oxide, however, can be neurotoxic and has been linked to the development of neurodegenerative diseases (Yuste et al., 2015). The neurotoxic effects of NO is ascribed to its ability to react with $O_2•^-$ producing the strong oxidant peroxynitrite ($ONOO^-$) and also with oxygen forming NO_2 and N_2O_3 capable of inducing lipid peroxidation, DNA oxidation, and oxidation or nitrosylation of thiols (Wink et al., 1999). Damage to the mitochondrial respiratory chain leads to energy depletion and cell death (Brown, 2010).

Our results also demonstrate that toluene can cause marked inhibition of brain PON-1 activity which is in agreement with previous studies (Abdel-Salam et al., 2016, 2019). Serum PON-1 was also significantly depressed after toluene treatment in rats (Abdel-Salam et al., 2016). The PON-1 enzyme is important in the detoxification of organophosphorus insecticides, nerve agents, and many other xenobiotics (La Du, 1992). Studies showed that the catalytic efficiency of the enzyme largely determines the individual's susceptibility to organophosphate insecticides (Costa et al., 2013) and also increases the risk for developing neurodegeneration in Parkinson's and Alzheimers's diseases (Baltazar et al., 2014). A decrease in PON-1 activity was also observed in a number of neurological disorders (Menini and Gugliucci, 2013). PON-1 is endued with both an anti-oxidant and anti-inflammatory properties (Furlong et al., 2016). Based on the results of this study and others (Abdel-Salam et al., 2016,2019) workers exposed to toluene or those who abuse the solvent will possibly have reduced enzyme activity, which will increase the risk for developing neurodegeneration.

Consistent with our previous studies, toluene caused significant inhibition of brain BChE (Atef et al., 2015; Abdel-Salam et al., 2019). We have also demonstrated inhibition of BChE in serum of

rats following exposure to toluene (Abdel-Salam et al., 2016), suggesting that BChE measurements could be a useful marker for exposure to toluene (Abdel-Salam et al., 2016). Other studies showed an inhibitory effect for toluene on brain AChE in synaptosomal membranes in vitro (Engelke et al., 1992) and in rat brain in vivo (Atef et al., 2015). The inhibition of cholinesterases by toluene of might be of relevance to the cognitive changes seen in those who are abusers of the solvent.

In the present study, we found that piracetam was not able to protect against the biochemical alterations and neuronal damage in brain of toluene exposed rats. Piracetam has gained wide acceptance as a cognitive and memory enhancing drug both in experimental animals and humans (Lenègre et al., 1988; Scheuer et al., 1999; Waegemans et al., 2002; Abdel-Salam and Nada, 2011; Croisile et al.,1993). The beneficial effects of piracetam are evident in aged but not young animals, suggesting that the drug improves the aging-induced alterations in brain structures affecting cognitive functioning. These effects of piracetam include increased plasma membrane fluidity, improved erythrocytes rheology and reduced platelet aggregation leading to improvement in vascular perfusion and improved mitochondrial function and dynamics (Stockmans et al., 1998; Müller et al., 1999; Stockburger et al., 2013). When given to mice at 500 mg/kg daily for 2 weeks (Pilch & Müller, 1988) or to rats at 300 mg/kg for 5 weeks (Scheuer et al., 1999), muscarinic cholinergic receptor density increased in frontal cortex of aged but not young animals. Very few studies suggested a neuroprotective action for piracetam (Keil et al., 2006; He et al. 2014; Abdel-Salam et al., 2011, 2016). In their study, Keil et al. (2006) treated mice with piracetam 100-500 mg/kg/day for two weeks and reported improved mitochondrial function in dissociated brain cells from aged but not young mice treated with sodium nitroprusside. The activities of the enzymes glutathione peroxidase (GPx), glutathione reductase and superoxide dismutase (SOD) that showed increase in aged mice were decreased by treatment with piracetam. In vitro, piracetam was reported to reduce cell damage in rat primary cortical neurons subjected to oxidative stress via an antioxidant mechanism that increased SOD and GPx and decreased MDA (He et al. 2014). Other studies provided evidence that the high doses of the drug are devoid of antioxidant effects in vivo (Abdel-Salam et al., 2011, 2016; Nguemo, et al., 2019) which can be explained by the action of piracetam in increasing brain release of monoamine neurotransmitters (Stancheva et al., 1991; Budygin et al. 1996) and consequently the generation of free radicals. Piracetam which is a cyclic derivative of γ-aminobutyric acid (GABA) has also been reported to increase N-methyl-d-aspartate (NMDA)-type glutamate receptors receptor density in mice brain (Scheuer et al., 1999). The sustained activation of these receptors results in excitotoxic neuronal death (Choi et al., 1988) by a process that involves the release of superoxide by NADPH oxidase isoform NOX2 (Reyes et al., 2012).

5.   Conclusion

In summary, the findings in the present study indicate that piracetam, a widely used drug to improve cognitive functioning in the elderly did not protect against neuronal damage induced by toluene. The study does not preclude the possibility that the drug might prove of value in treating toluene-induced cognitive impairment.

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