AMELIORATIVE ROLE OF N-ACETYLCYSTEINE ON TOXIC POTENTIAL OF TITANIUM DIOXIDE NANOPARTICLES ON THE BRAIN OF ALBINO RATS

BY

Shereen A. Elkhateeb and Hossam F. Attia*

Departments of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Zagazig University

*Histology and Cytology, Faculty of Veterinary Medicine, Benha University**

ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) could affect biological targets like the brain. The aim of this study was to investigate the toxic effects of TiO_2 NPs on the brain of adult male rats and to evaluate the protective role of N- Acetylcysteine (NAC) on the affected parameters. Sixty adult male Albino rats were divided into 4 groups; control group, NAC group: rats received NAC (100 mg/kg), TiO_2 NPs group: rats received 1200 mg/kg TiO_2NPs in 1ml of 5% gum acacia solution), TiO_2NPs/ NAC groups rats received TiO_2 NPs and NAC as previously mentioned. The rats were gavaged once daily for 12 weeks. After 6 and 12 weeks, rats were sacrificed and their brains were obtained for estimation of brain malondialdehyde (MDA), glutathione (GSH) levels and histopathological examination. The results revealed time dependent significant increase of MDA and decreased GSH levels. Histopathological examination showed vacuoles in the brain tissues, pyknotic nuclei, necrosis in the nerve cells and fibrosis of the nerve fibers. Bcl_2 immunolocalization revealed time dependent weak reaction in the cytoplasm of nerve cells. These changes showed minimization after using NAC. The present results indicated that TiO_2NPs induced time dependent oxidative stress and apoptosis in rat brain which could be ameliorated by co-administration of NAC.

Keywords: *Titanium dioxide NPs, NAC, brain, oxidative stress, Bcl_2, rats.*

INTRODUCTION

Nanoparticles (NPs) are entering into the environment with the increasing development of nanotechnology. According to the National Nanotechnology Initiative of America, titanium dioxide nanoparticles (TiO_2 NPs) are among those most widely manufactured on a global scale (Liang et al., 2009). NPs are currently used in a wide range of applications including pigments, cosmetics, medicine, pharma-

ceuticals, food products and toothpaste because they provide whiteness and opacity (Fisher et al., 2001). However, these unique characteristics (such as small sizes, large surface per mass and high reactivity) allow TiO_2NPs to enter the human body easily, and then impose potential risks on human health (Warheit et al. 2007; Robertson et al., 2010).

In subacute toxicity study, Wang et al. (2007) reported that mice treated with TiO_2 NPs showed different pathological changes in the liver and kidneys. Also, there has been increasing incidence of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (Matés et al., 1999; Orringer et al., 2009). The exact etiology of these diseases is unknown, but environmental pollutants, including NPs, may be an important risk factor to various tissues including the brain (Takenaka et al., 2001; Burch, 2002).

Oxidative damage has been implicated in many degenerative and non degenerative diseases. Oxidative stress (OS) derived from the imbalance between reactive oxygen species (ROS) formation and individual antioxidant activity potentially leads to damage of lipids, proteins, and macromolecules such as DNA and RNA (Risom et al., 2005). Wang et al. (2008) found that the intra-nasally instilled TiO_2 NPs could migrate into the CNS, deposited in the hippocampus region causing oxidative stress, inflammation responses and changes in the release and metabolism of neurotransmitters.

N-Acetylcysteine (NAC) is a thiol-containing amino acid with free radical-scavenging properties, powerful neuroprotective and anti-oxidant actions (Atkuri et al., 2007; Sadowska et al., 2007). Little information about the toxicological effects of TiO_2 NPs on the brain tissue was reported. So, the aim of this study was to evaluate the potential toxic effects of TiO_2 NPs on the brain of adult male albino rats and to evaluate the ameliorative role of N-Acetylcysteine.

MATERIAL AND METHODS

A. Chemicals :

Nano-sized Titanium dioxide (TiO_2 NPs): Anatase form, particle size (25- 70 nm), surface area (20- 25) m^2/g, purity 99.9 was purchased from Sigma Aldrich Chemical Co., Germany.

Gum acacia: powder form was obtained from El- Nasr Co., Egypt.

N-Acetylcysteine (NAC) : Effervescent instant sachets, 200 mg each, were obtained from SEDICO, Co., Egypt.

B. Experimental Animals :

Sixty adult male albino rats (150-200 gm) were obtained from the Animal

House in Veterinary Medicine Faculty, Zagazig University. All animals were subjected to 14 days of passive preliminaries for adaptation to their new environment and to ascertain their physical wellbeing. They were housed in separate well-ventilated cages, under standard conditions, with free access to standard diet and water ad libitum. The experiment was conducted at the Animal House of Faculty of Medicine, Zagazig University in accordance with the guidelines of ethical committee for research on laboratory animals (National Research Council, 1996).

C. Experimental design :

The rats were divided into 4 groups as follows:

- Group I (control group): 24 rats, were subdivided into two equal subgroups:

Subgroup A (negative control): rats received only regular diet and water to determine the basic values of performed tests for 12 weeks. Subgroup B (positive control group): Each rat received 1 ml of 5% gum acacia solution daily by gavage for 12 weeks.

- Group II (N-acetylcysteine treated group): 12 rats received 100 mg/kg body weight NAC once daily by gavage for 12 weeks (Jain et al., 2011).

- Group III (Titanium dioxide nanoparticles treated group): 12 rats received 1200 mg/kg body weight TiO_2 NPs by gavage (1/10 LD_{50}) in 1ml 5% gum acacia solution as a solvent once daily for 12 weeks.

The LD_{50} of TiO_2 NPs for rats is 1200 mg/kg body weight after oral administration (Wang et al., 2007).

- Group IV (Titanium dioxide nanoparticles and N-Acetylcysteine treated group) : 12 rats received (100 mg/kg NAC 1h before giving 1200 mg/kg TiO_2NPs) by gavage once daily for 12 weeks.

After 6 and 12 weeks (24 hours from the last dose), 6 rats from each group and subgroup were anesthetized by ether then sacrificed. The brain tissues were extracted for measurement of MDA content and GSH level and histopathological study.

D. Biochemical analysis :

The brain of each rat was divided into two parts; one part was wrapped with aluminum foil and kept frozen at -20°C till used and the other part was preserved for histopathological examination.

Preparation of brain homogenate

Brain tissues were homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer pH (7.4). Aliquots of homogenates from rat brain were used to determine lipid peroxidation and reduced glutathione. Estimation of tissue MDA levels and re-

duced glutathione (GSH) was done according to Okhawa et al. (1979) and Ellman (1959). The results were expressed as nmoles / g tissue.

E. Histopathological examination:

The brains of the sixty albino rats were collected from the different groups after 6 and 12 weeks. The samples were fixed in Bouin's solution, then dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin. The samples were blocked, then sliced into 5 μm in thickness and placed onto glass slides. The slides were stained by Haematoxylin and Eosin and PAS stains (Wilson and Gamble, 2002).

Immunohistochemical staining was conducted for detection of Bcl_2, the primary antibody used was mouse monoclonal Bcl_2 oncoprotein (N1587; Dako Corporation, Glostrup, Denmark). The cellular site of the reaction was cytoplasmic (Wang, 1995).

Immunohistochemical reaction was carried out using avidin biotin peroxidase system. The primary antibody used was rabbit polyclonal antibody (Sigma Laboratories). Universal kit used was avidin biotin peroxidase system produced by Nova-Castra Laboratories Ltd, UK. The same method was applied to prepare negative control sections but the primary antibody was not added. Mayer's Haematoxylin was added as counter stain. Tonsil was used as positive control tissue (Kiernan, 2008).

Statistical analysis :

Data was represented as mean ± SD. The differences were compared for statistical significance by ANOVA and post hoc Tukey's tests. Difference was considered significant at $p < 0.05$.The statistical analysis was performed using Epi-Info version 6.1 (Dean et al., 2000).

RESULTS

1. Biochemical results: (Table 1)

Comparison between the negative and positive control groups regarding oxidative stress markers (MDA and GSH) revealed no significant difference ($P > 0.05$) after 6 and 12 weeks, so the negative control was used for comparison with other groups of the study. Rats that received NAC alone showed no significant difference ($P > 0.05$) regarding the mean values of MAD content and GSH level in the brain when compared with the negative control group all over the study period .

a. Effects of TiO_2 NPs on brain MDA content and GSH level:

Table (1) demonstrated that there was a significant time- dependent increase in the mean values of MDA content ($P < 0.05$) in the brain of rats treated with TiO_2 NPs for 6 and 12 weeks (126±6.13&

164±14.6 respectively) in comparison with those of the negative control group (P < 0.05). Regarding GSH level, there was a significant time-dependent decrease in the mean values (52.3±5.4 & 30.8±4.52) in rats treated with TiO_2 NPs for (6 & 12 weeks) respectively in comparison with those of the negative control group (p> 0.05).

b. Role of NAC on MDA content and GSH level in brain tissues of rats received TiO_2 NPs:

Table (1) showed that co-administration of NAC with TiO_2NP (group IV) significantly displayed beneficial effects on MDA and GSH (P < 0.05) by returning their mean values close to those of the control group. Rats treated with TiO_2NP and NAC for 6 and 12 weeks (group IV) showed a significant decrease in the mean values of MDA (81.1±4.59& 86.8±2.75) respectively in comparison with those treated with TiO_2 NPs (group III) for 6 & 12 weeks (126±6.13& 164±14.6) respectively. Concerning GSH level, group IV showed a significant increase in the mean values of GSH level after 6&12 weeks (99.7±4.74& 96.6±5.5) respectively in comparison with those treated with TiO_2NP (group III) for 6 & 12 weeks. This improvement was partial because the mean value in group IV (at the end of 12 weeks) 96.6±5.5 was still lower than those of the negative control group (107±3.95, p > 0.05).

Table (1) : Statistical analysis of the changes in the brain MDA content and GSH level of the studied rats after 6 and 12 weeks.

Groups / Parameters	Group I (negative control) (n: 12)		Group II (NAC treated) (n: 12)		Group III (TiO_2 treated) (n: 12)		Group IV (TiO_2 /NAC) (n: 12)		F	P
Duration in weeks	6	12	6	12	6	12	6	12		
MDA nmol/gm tissue	80.1± 3.52	79.8± 3.67	76.9± 3.76	75.4± 3.25	126± 6.13[a]	164± 14.6[ac]	81.1± 4.59[b]	86.8± 2.75[b]	147.9	<0.001
GSH nmol/gm tissue	107± 3.95	108± 3.35	110± 2.53	112± 2.12	52.3± 5.4[a]	30.8± 4.52[ac]	99.7± 4.74[b]	96.6± 5.5[ab]	319.4	<0.001

n = number of rats

a: Significant difference (p<0.05) when compared with negative control group

b: Significant difference (p<0.05) when compared with group III at 6 and 12 weeks.

c: Significant difference (p<0.05) when compared with TiO_2 treated rats for 6 weeks.

2. Histopathological results: (Figures I, II and III)

Histopathological examination of control and NAC groups revealed normal structure of the brain. The gray mater of the adult male albino rats appeared with its well organized regularly arranged six layers which consisted of nerve cells with different sizes and shapes. The normal pattern of the white mater is formed of homogenously stained nerve fibers running down the cortex (Figure 1). The nerve cells appeared in different shapes and sizes distributed throughout the gray mater (Figure 2). Positive immunostain for Bcl_2 appeared in the cytoplasm of nerve cells and mesothelial cells of the pia mater (Figure 3). After 6 weeks of TiO_2 NPs administration, the brain sections showed fine disorganization of the cortical layers (Figure 4). There were degenerated nerve cells that collected together forming vacuoles (Figure 5). The cytoplasm of some nerve cells showed faint positive reaction for Bcl_2, while other cells showed negative reaction (Figure 6).

Rats received TiO_2 NPs and NAC for 6 weeks showed return of brain tissues towards normal morphology as evidenced by remarkable regression of the degenerative changes induced by TiO_2 NPs only. There were congested blood vessels between the nerve cells and some inflammatory cells were accumulated around them (Figures 7& 8). The nerve cells reacted positively with the immunostain for Bcl_2 (Figure 9).

The nerve cells of the brain tissue after 12 weeks of TiO_2NPs administration revealed marked cortical layer disorganization, vacuolated foci with pyknotic nuclei. The vacuoles collected together forming large size vacuole compared with those of the control rats (Figure 10).The nerve cells showed wide spread necrosis and there was fibrosis in the nerve fibers that spread in large areas of the brain sections (Figure 11). Immunohistochemical stained sections revealed negative reaction in most of the cytoplasm of nerve cells (Figure 12).

In addition, concomitant use of NAC with TiO_2 NPs showed progressive improvement in the architecture of the brain and remarkable regression of the total degenerative changes. There were no large vacuoles, only some small vacuoles were seen around the nerve cells (Figure 13). Nerve fibers fibrosis and nerve cells necrosis showed remarkable improvement (Figure 14). Immunostaining for Bcl_2 showed positive reaction of the cytoplasm of many nerve cells (Figure 15).

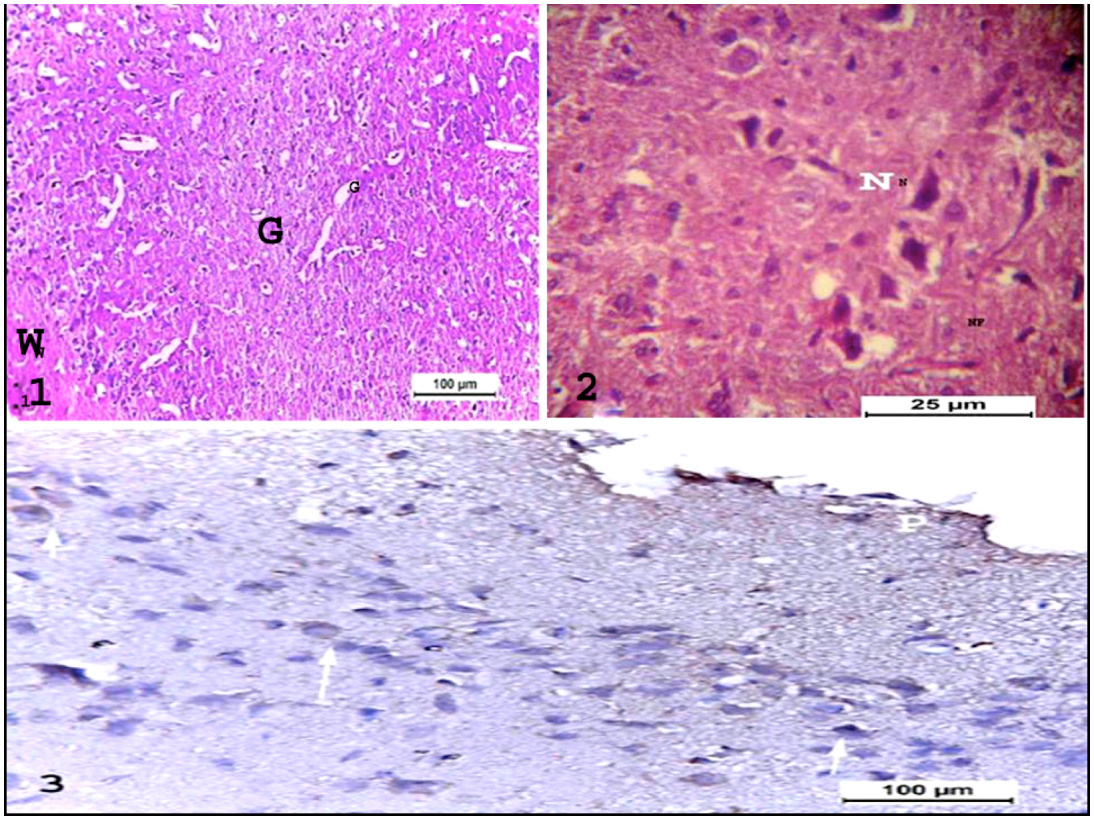

Figure (I) : Photomicrographs of brain in control rat showing the gray (G) and white mater (W) in part (1) H&E X100; the nerve cells distributed in the gray mater (N) and fine nerve fibers between them, most of the nerve cells in the form of small and large pyramidal cells in part (2) H&E X 400; positive Bcl_2 reaction in the cytoplasm of nerve cells and the mesothelial cells of the pia mater (arrows) in part (3) Bcl_2X 200.

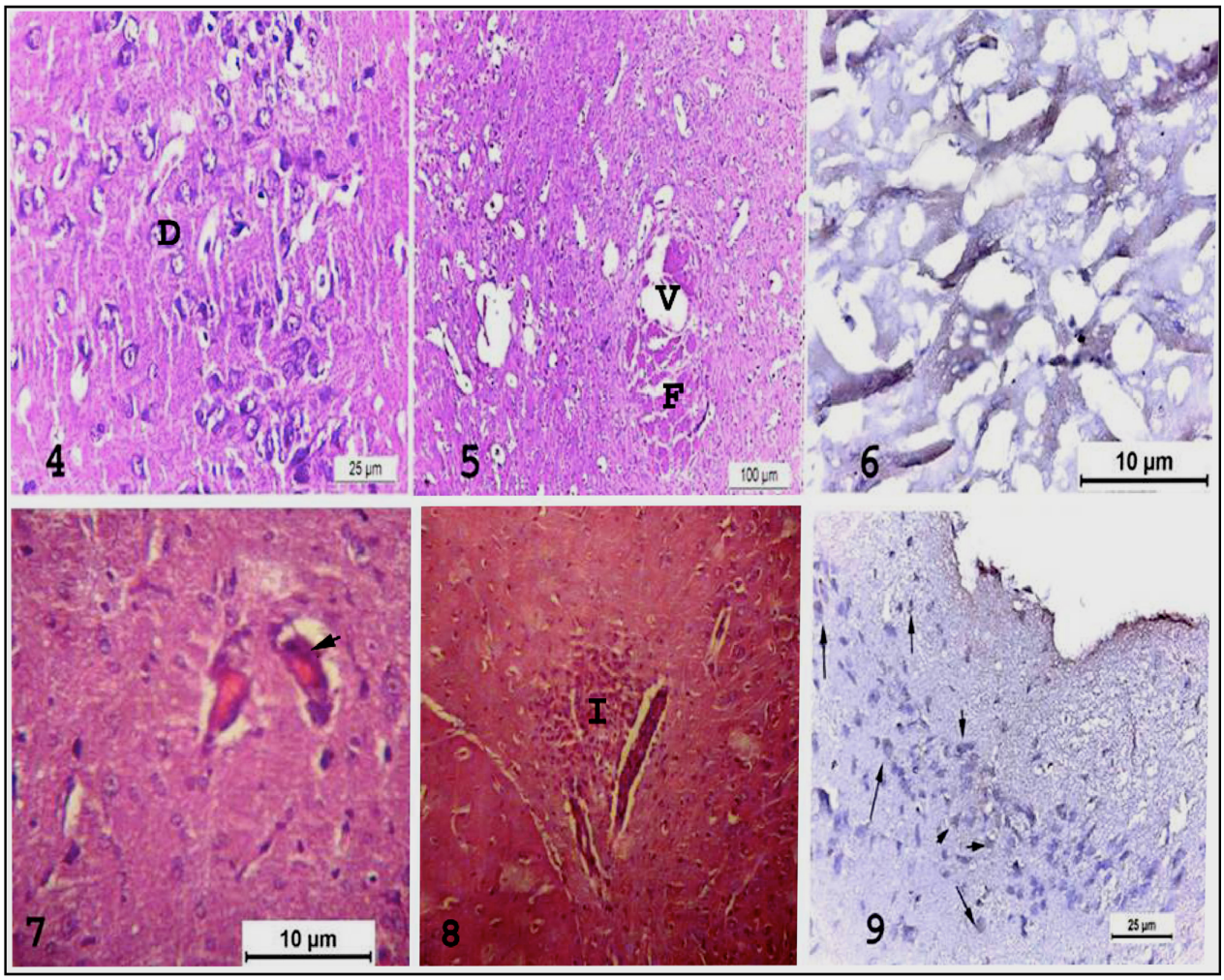

Figure (II) : Photomicrographs of the brain of rats given TiO_2 NPs/ (TiO_2 NPs /NAC) for 6 weeks. part (4): fine degeneration of the nerve cells (D). H &E X 200, part (5): degenerated cells are replaced by variable size vacuoles (V), some nerve fibers showed fibrosis (F). PAS stain X200, part (6): faint positive Bcl_2 reaction (arrow) in the cytoplasm of some nerve cells X400, part (7): The brain of the rat after 6 weeks of (TiO_2 NPs /NAC) administration showing congestion in the microvasculature of the cerebrum and small blood vessels (arrow). "H&E stain X200" , part (8): some inflammatory cells infiltrated around nerve cells (I). "H &E stain X200 ", part (9): positive Bcl_2 reaction (arrow) in the cytoplasm of nerve cells "Bcl_2 X100".

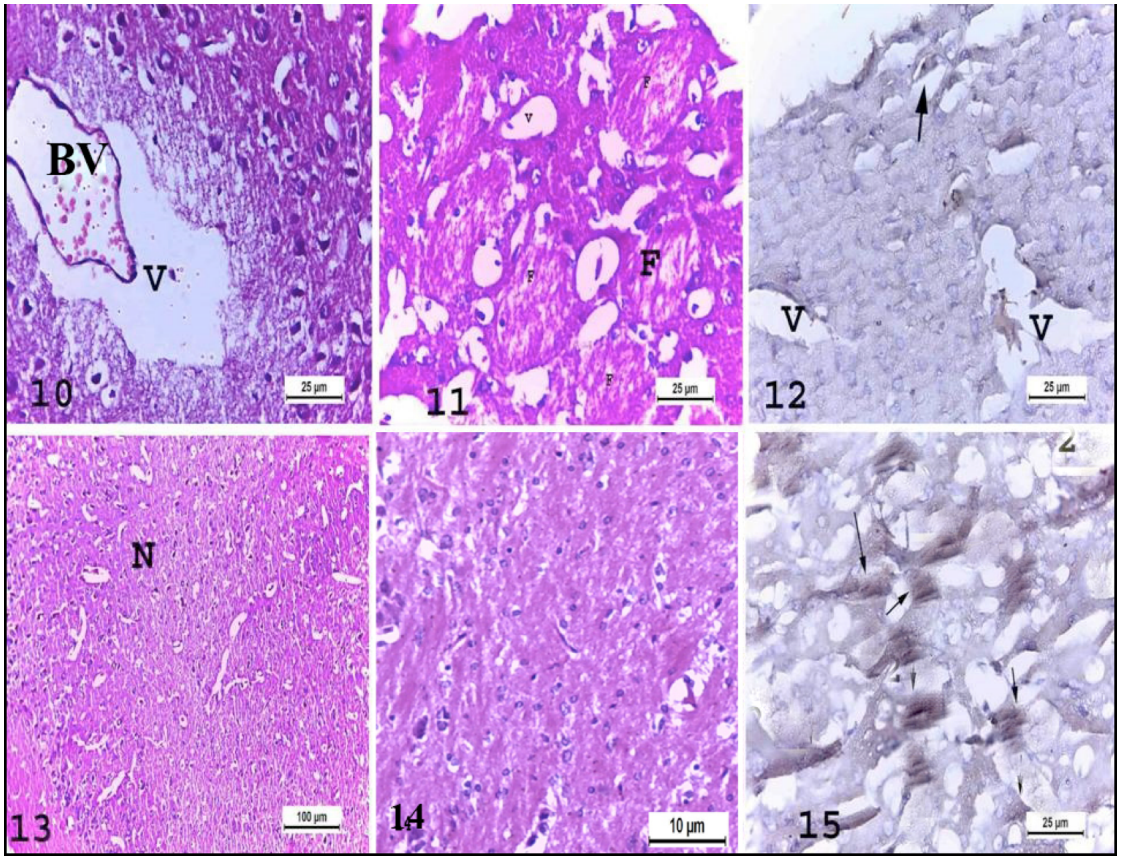

Figure (III) : Photomicrographs of the brain of rats given (TiO_2NPs) / (TiO_2 NPs/ NAC) for 12 weeks. Part (10) shows dilated blood vessel (BV) and variable size vacuoles; some of them collected together forming large vacuole (V." H &E stain X200"; part (11): The nerve cells showed necrosis with fibrosis in the nerve fibers (F)."PAS stain X200", part (12): weak Bcl_2 reaction (arrow) in the cytoplasm of nerve cells. "Bcl_2 X200",part (13): The brain of rat received (TiO_2 NPs/NAC) for 12 weeks showed decrease in the number of the vacuoles in the gray matter "H &E stain X100", part (14): The nerve cells and fibers showed improvement and disappearance of fibrosis. "PAS stain X100", part (15): Positive Bcl_2 reaction (arrow) in the cytoplasm of nerve cells "Bcl_2 X200".

DISCUSSION

The increased biological activity of nanoparticles could be useful to penetrate cells for drug delivery. However, undesirable effects of nanoparticles could include generation of oxidative stress and/or impairment of antioxidant defense responses (Ma et al., 2010). This work evaluated the role of NAC on TiO_2 NPs induced oxidative stress and brain damage in experimental animals. The present study supported the results of Li et al. (2010) and Hu et al. (2011) about the ability of these NPs to translocate into the brain, irrespective of the route of exposure. The effects of TiO_2 NPs on the rats' brains occurred at the cellular and molecular levels.

The results of this study showed time dependent significant oxidative stress as evidenced by increased MDA, the end product of lipid peroxidation and decreased GSH level in the brain of rats treated with TiO_2 NPs when compared with the control rats. These findings coincided with those of Long et al. (2007); Wang et al. (2008) and Ma et al. (2010). The overproduction of ROS would break down the balance of the oxidative/ antioxidative system in the brain, resulting in lipid peroxidation.

Moreover, it has been reported that TiO_2 NPs could be phagocytized by neurons and microglia, which then released ROS (Long et al., 2006). There are large amounts of polyunsaturated fatty acids (PUFA) in the brain, which play an important role in the brain structures and functions. However, PUFA are easy to be invaded by ROS and cause impairment of cellular functions (Matés, 2000). Also, the brain is highly vulnerable to OS because of its high metabolic rate, reduced capacity for cellular regeneration, low levels of endogenous scavengers (e.g., vitamin C, catalase, superoxide dismutase) and high cellular concentration of OS targets i.e. lipids, nucleic acids, and proteins (Takenaka et al., 2001, Kreyling et al., 2002).

The results of the present study coincided with Wang et al. (2007) who reported that exposure of mice to TiO_2 NPs revealed vacuoles in the neurons of brain sections and interpreted these findings as fatty degeneration induced in the brain tissue. Long et al. (2007) reported that exposure of immortalized mouse microglia to TiO_2 NPs, resulted in immediate and prolonged release of ROS and upregulation of inflammatory, apoptotic, and cell cycling pathways.

The molecular mechanism of apoptosis of nerve cells was previously detected by Hu et al. (2011) who stated that TiO_2 NPs significantly induced apoptosis as evidenced by activated caspase-3 and -9, inhibited Bcl_2, promoted the levels of Bax

and cytochrome C and contributed this to-TiO_2 NPs induced accumulation of reactive oxygen species in the mice hippocampus.

The contribution of oxidative stress to cytotoxic responses elicited by TiO_2NPs was discussed in many studies (Fabian et al., 2008; Park et al., 2008; Jin et al., 2011 and Zhu et al., 2012). They stated that the ROS generation could lead to cellular toxicity if the level of ROS production overwhelms the antioxidant defense of the cell or induces the mitochondrial apoptotic mechanisms. In agreement with the previous theory, our results were associated with biochemical criteria of oxidative stress that might interpret the cellular damage in the form of degeneration and apoptosis in brain sections.

Concomitant use of NAC along with TiO_2 NPs significantly restored the values of MDA and GSH. Brain sections revealed regression of the degenerative changes of the nerve cells. Immunostaining by Bcl_2 showed positive reaction in the cytoplasm of nerve cells.

These findings were in accordance with Zafarullah et al. (2003) who stated that NAC had promoted the cell growth and survival in response to ROS-induced injuries which normally lead to growth arrest and apoptosis. Van de Poll et al. (2006) and Atkuri et al. (2007) stated that NAC is an antioxidant with free radical-scavenging properties and is a source of cysteine, the precursor of de novo GSH synthesis. So, administration of NAC replenishes intracellular GSH levels. On the same context, Xue et al. (2011) stated that NAC strongly inhibited ROS production in TiO_2 NPs treated cells and suppressed TiO_2NPs induced apoptosis.

CONCLUSION

In conclusion, the present study showed that $1/10^{th}$ LD_{50} TiO_2NPs induced detrimental effects on brain tissue including; oxidative stress, histopathological changes and apoptosis that were improved by concomitant administration of NAC.

RECOMMENDATIONS

Upon the increasing applications of TiO_2NPs products, it is recommended that NAC must be given daily to people working and dealing with titanium to avoid oxidative stress and brain toxicity. Follow up studies and more researches are needed about TiO_2 NPs toxicity at different doses and durations.

REFERENCES

Atkuri, K. R.; Mantovani, J. J. and Herzenberg, L.A. (2007): "N-Acetylcysteine-a

safe antidote for cysteine/glutathione deficiency". Curr. Opin. Pharmacol., 7(4):355-359.

Burch, W.M. (2002): "Passage of inhaled particles into the blood circulation in humans ". Circulation, 106:e141–e142.

Ellman, G.L. (1959): "Tissue sulfhydryl groups". Arch. Biochem. Biophys., 82: 70–77.

Dean, A.G., Dean, J.A., Coulombier, D. et al. (2000): Epi-Info (6.1): A word processing data base and statistical program for epidemiology and microcomputer office. Center for Disease Control, Atlanta, Georgia, USA.

Fabian, E.; Landsiedel, R., Ma-Hock, L. et al. (2008): "Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats." Arch. Toxicol., 82:151–157.

Fisher, J.; Egerton, T. and Othmer, K. (2001): Titanium compounds, inorganic. In: Encyclopedia of Chemical Technology. New York, John Wiley & Sons.

Hu, R.; Zheng, L.; Zhang ,T. et al. (2011): "Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles ". J. Hazard. Mater., 15; 191 (1-3): 32-40.

Jain, S.; Kumar, C.H. M.; Suranagi, U. D. et al. (2011): "Protective effect of N-acetylcysteine on bisphenol A-induced cognitive dysfunction and oxidative stress in rats". Food and Chemical Toxicology, 49: 1404-1409.

Jin, C.; Tang, Y.; Yang, F.G. et al. (2011): "Cellular toxicity of TiO_2 nanoparticles in anatase and rutile crystal phase". Biol. Trace Elem. Res., 141 (1-3):3-15.

Kiernan, J.A. (2008): Histological and Histochemical Methods. Theory and Practice.4th ed., UK: Butterworth- Heinemann: Oxford.

Kreyling, W.G.; Semmler, M.; Erbe F. et al. (2002): "Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low". J. Toxicol. Environ. Health, 65:1513–1530.

Liang, G.; Pu, Y.; Yin, L. et al. (2009): "Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress". J. Toxicol. Environ. Health, 72 (11): 740–745.

Li,Y.; Li, J. ; Yin, J. et al. (2010): "Systematic influence induced by 3nm titanium dioxide following intratracheal instillation of mice". J. Nanosci. Nanotech., 10 (12): 8544–8549.

Long, T.C.; Saleh, N.; Tilton, R.D. et al. (2006): "Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2) implications for nanoparticle neurotoxicity". Environ. Sci. Technol., 40:4346–4352.

Long, T.C.; Tajuba, J.; Sama, P. et al. (2007): "Nano-TiO_2 stimulates ROS in brain microglia and damages neurons in vitro". Environ. Health Perspect., 115:1631–1637.

Ma, L.; Liu, J.; Li, N.; et al. (2010): "Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity". Biomaterials, 31 (1):99-105.

Matés, J.M.; Pérez-Gómez, C. and Núñez de Castro, I. (1999): "Antioxidant enzymes and human diseases ". Clin. Biochem., 32:595-603.

Matés, J.M. (2000): "Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology". Toxicologist, 153:83–104.

National Research Council (1996): Guide for the use and care of laboratory animals. Washington, DC: National Academy Press, Office of Laboratory Animal Welfare. (7^{th} ed.) Available at http:// www.nap.edu/openbook .php? record_id =5140

Okhawa, H.; Ohishi, N. and Yagi, K. (1979): "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". Anal. Biochem., 95: 351–358.

Orringer, D.A.; Koo, Y.E. and Chen, T. (2009): "Small solutions for big problems: The application of nanoparticles to brain tumor diagnosis and therapy". Clin. Pharmacol. Ther., 85:531–534.

Park, E.J.; Yi, J.; Chung, K.H. et al. (2008): "Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells".Toxicol. Lett., 180: 222–229.

Risom, L.; Møller, P. and Loft, S. (2005):"Oxidative stress-induced DNA damage by particulate air pollution". Mut. Res., 592 (1): 119–137.

Robertson, T.A.; Sanchez, W.Y. and Roberts, M.S. (2010): "Are commercially available nanoparticles safe when applied to the skin"? J. Biomed. Nanotechnol., 6:452-468.

Sadowska, A.M.; Manuel-Y-Keenoy, B. and De Backer, W.A. (2007): "Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: A review". Pulm. Pharmacol. Therapeutics, 20(1):9–22.

Takenaka, S.; Karg, E.; Roth, C. et al.

(2001): "Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats". Environ. Health Perspect., 109:547–551.

Van de Poll, M.C.G.; Dejong, C.H.C. and Soeters, P.B. (2006): "Adequate range for sulfur-containing amino acids and biomarkers for their excess: lessons from enteral and parenteral nutrition". J. of Nutrition, 136 (6):1694–1700.

Wang, E. (1995): "Senescent human fibroblasts resist programmed cell death and failure to suppress Bcl_2 is involved". Cancer Res., 55: 2284–2292.

Wang, J.; Zhoua, G. and Chena, C. (2007): "Acute toxicity and bio-distribution of different sized titanium dioxide particles in mice after oral administration". Toxicology Letters, 168, 176–185.

Wang, J.X.; Liu, Y.; Jiao, F. et al. (2008): "Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO_2 nanoparticles". Toxicologist, 254: 82–90.

Warheit. D.B.; Hoke, R.A.; Finlay, C. et al. (2007): "Development of a base of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management ". Toxicol. Lett., 171 : 99–110.

Wilson, I. and Gamble, M. (2002): The hematoxylins and eosins. In: Theory and Practice of Histological Techniques. Bancroft, J. D. and Gamble, M. (eds.). Churchill Livingston, Elsevier Science Limited, London, UK, 5th ed., P.P. 125- 138.

Xue, C.; Liu, W.; Wu, J. et al. (2011): "Chemoprotective effect of N-acetylcysteine (NAC) on cellular oxidative damages and apoptosis induced by nano titanium dioxide under UVA irradiation". Toxicol. in Vitro, 25 (1):110-116.

Zafarullah, M.; Li, W.Q.; Sylvester, J. et al. (2003): "Molecular mechanisms of N-acetylcysteine actions". Cell. Mol. Life Sci., 60 (1):6-20.

Zhu, Y.; Eaton, J.W. and Li, C. (2012): "Titanium Dioxide (TiO_2) nanoparticles preferentially induce cell death in transformed cells in a Bak/Bax-independent fashion". PLOS ONE, 7(11): e50607.

الدور التحسينى لمركب ان- استيل سيستايين علي سمية ثاني أكسيد التيتانيوم النانوية على المخ فى الجرذان البيضاء

المشتركون فى البحث

د. شيرين الخطيب و د. حسام عطية*

من قسمى الطب الشرعى والسموم الاكلينيكية - كلية الطب - جامعة الزقازيق

والأنسجة والخلايا - كلية الطب البيطرى - جامعة بنها*

ثاني أكسيد التيتانيوم النانوية هي مادة متناهية الصغر واسعة الاستخدام والتي من الممكن أن تؤثر على أهداف حيوية مثل المخ. تهدف هذه الدراسة إلى تقييم الآثار السامة لثاني أكسيد التيتانيوم النانونية على مخ ذكور الجرذان البالغة و دورالمركب إن-استيل سيستايين فى الوقاية من هذه الآثار.أجريت الدراسة على عدد ستين من ذكور الجرذان البيضاء البالغة، تم تقسيمهم إلى أربع مجموعات، مجموعة ضابطة ، مجموعة إن-استيل سيستايين أعطوا ١٠٠مج/كج، مجموعة ثاني أكسيد التيتانيوم النانوية أعطوا ١٢٠٠مج/كج ,مجموعة ثاني أكسيد التيتانيوم النانوية / إن-استيل سيستايين أعطوا المادتين بنفس الجرعة كما فى المجموعتين السابقتين. وقد استمرت الدراسة لمدة ١٢ أسبوع أعطيت فيها الجرذان الجرعات السابقة مرة واحدة يوميا بالفم. وتم ذبح الجرذان بعد ستة أسابيع وبعد ١٢ أسبوع لأخذ المخ وقياس (المالونداياالدهيد و الجلوتاثيون) و تم عمل دراسة هيستوباثولوجية وهيستوكيمائية مناعية لأنسجه المخ.

ولقد أوضحت النتائج حدوث زيادة واضحة فى المالونداياالدهيد ونقص واضح فى الجلوتاثيون المختزل في الجرذان مقارنة بالمجموعة الضابطة وأن هذه التغيرات معتمدة على الوقت. كذلك أوضحت الدراسة حدوث تغيرات واضحة فى أنسجة المخ تتمثل في ظهور فقيعات وفجوات مختلفة الحجم مع تخثر الخلايا وموت بعضها و تليفات و أظهر الفحص الهستوكيميائى المناعى وجود تفاعل ضعيف فى سيتوبلازم الخلايا العصبية ومع إعطاء إن-استيل سيستايين لوحظ تحسن جزئي . ونخلص من هذه الدراسة أن ثاني أكسيد التيتانيوم النانوية له آثار سمية على المخ وأن هذه الآثار معتمدة على الوقت و قد تقل هذه الآثار باستخدام إن-استيل سيستايين.