

Effect of Ginkgo Biloba Leaves Extract on Some Formaldehyde Induced Toxicity in hippocampus of Adult Male Albino Rats

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Abstract Formaldehyde (FA) heavily impacts the everyday consumer products. It is widely used in the construction, textile, furniture, medical, chemical, and pharmaceutical industries. Exposure to FA poses a significant threat to public health. It can cause severe central nervous system impairment. Extract from the leaves of Ginkgo biloba exerts a novel spectrum of biological, pharmacological and therapeutic properties against oxidative stress, so it was of special concern to investigate the role of Ginkgo biloba leaves extract (GBE) on the neurotoxic effects of FA on hippocampus of adult male albino rats. Thirty adult male albino rats were used in the present study. They were divided into 5 equal groups: Group I negative control, Group II received distilled water by intraperitoneal (i.p.) injection. Group III received GBE (300 mg/kg/day) orally. Group IV received FA (0.2 mg/kg/day i.p. and Group V received 0.2 mg/kg FA + 300 mg/kg GBE. After the end of the study (4weeks), all rats were sacrificed and brain were dissected out and hippocampus subjected to estimation of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) catalase (CAT) and malondialdehyde (MDA) levels. Histological examination of the hippocampus and immunohistochemical staining for Glial fibrillary acidic protein (GFAP) were also done. The results of this study revealed that, the levels of SOD, CAT and GSH-Px were significantly decreased, while MDA level in hippocampus tissue were significantly increased in rats treated with FA compared to those of the controls. Microscopic examination of neurons of the hippocampus in this group revealed picnotic nuclei, vacuolar degeneration and neuronal loss. Immunostaining showed areas with significantly increased GFAP immunopositivity. Concomitant administration of GBE with FA resulted in a significant increase in antioxidant enzymes activity (SOD, GSH-Px and CAT), decreased MDA, and improvement of histopathological changes induced by FA. Also a significant reduction in expression of GFAP was observed. It was concluded that short term administration of FA induced neurotoxicity on the hippocampus of adult male albino rats, with oxidant stress and lipid peroxidation which may be a molecular mechanism involved in FA induced neurotoxicity. Furthermore, these effects were decreased by the concomitant use of GBE. It is recommended to use GBE for amelioration of toxic manifestations of formaldehyde in exposed population.

Introduction

Formaldehyde (FA), a member of the aldehyde family, is a common environmental pollutant (Luo et al., 2012). The body can encounter environmental FA, since a number of commonly used products contain either formaldehyde or formaldehyde-releasing substances (de Groot et al., 2009). It is widely used in many products, including disinfectants, cosmetics, antiseptics and fungicides. It is also used in the manufacture of plastics, resins and building

materials, such as particle board, plywood, floor coverings and office furniture, and it is emitted by cigarette smoking (Maiellaro et al., 2014). Hair smoothing products containing methylene glycol (or other FA donors or releasers) require the use of heat. Heat leads to volatilization of both FA gas as well as methylene glycol vapors increasing the potential for hair stylist and consumer exposure to FA from

methylene glycol formulated in keratin products (Golden and Valentini, 2014).

Formaldehyde is toxic to the majority of organisms due to its non-specific reactions with macromolecules such as proteins, nucleic acids and lipids to form crosslinks, thereby leading to the loss of their biological functions (Zeng et al., 2014). As an effective cross-linking agent FA is used in certain vaccines to inactivate viruses and to detoxify bacterial toxins while not materially affecting antigenicity (Mitkusa et al., 2013). Formaldehyde can be added illegally as a food preservative and antimicrobial agent (Yeh et al., 2013). It is added to milk in the production of cheese and dairy products (Afifi and Hanon, 2011)

The term "formalin" refers to solutions of formaldehyde dissolved in water. Methanol is added as a stabilizer to prevent polymerization of formaldehyde into paraformaldehyde (Doane and Sarenbo, 2014). Now formaldehyde had been definitely the most common known indoor and outdoor air pollutant (Tang et al., 2012; Meng et al., 2014).

Thus, this ubiquitously present compound can enter the human body by inhalation, ingestion, or entry through the skin. (Tulpule and Dringen, 2013). Formaldehyde dehydrogenase catalyze the oxidation of formaldehyde in the tissues into formic acid and the highly toxic methanol (Katsnelson et al., 2013). Therefore formaldehyde serves as a well-documented source of methyl groups, which are transferred via tetrahydrofolate into the one-carbon pool for incorporation via biosynthesis into various macromolecules damaging it (Golden and Valentini, 2014).

The effect of FA on people is well known: irritation of the eyes and upper respiratory tract, headache, nausea, drowsiness, and allergic skin reactions. Prolonged exposure to FA can cause serious health effects such as immune system disorders, as well as asthma and nasopharyngeal cancer (Wang et al., 2012a). Many studies have been undertaken on the impacts of FA on the central nervous system. Epidemiological data showed that histology technicians exhibited reduced performance on story memory, digit span, increased errors on trails and they presented excessive fatigue and difficulty in remembering. Animal studies showed interventional effects of FA exposure on psychology and cognition (Liu et al., 2010). Tang et al. (2012) reported that FA is a neurotoxic molecule, capable of damaging the hippocampus of rats causing behavioral, learning and memory disorders

Oxidative damage was one of the most critical effects of formaldehyde exposure. It occurred in various organs of animals which were exposed to formaldehyde (Tang et al., 2013).

The use of traditional medicine is wide-spread throughout the world and plants remain a large source

of natural antioxidants that might serve as leads for the development of novel drugs (Mengome et al., 2014).

Ginkgo biloba (Ginkgoaceae) is an important and widely used herb of the Chinese traditional medicine. It was known to have a variety of biological and pharmacological properties that made it useful as a cardioprotective, antiasthmatic, and antidiabetic agent (Jain et al., 2011). In addition, it had been shown to possess neuroprotective properties against hypoxia, ischemia, seizure activity and peripheral nerve damage. In accordance, it was shown – in clinical researches – to be effective in treating dementia in Alzheimer's disease and in enhancing memory and cognition and improving the capacity of geriatric patients to cope with the stressful demands of daily life (Abousetta et al., 2014).

Ginkgo biloba leaf extract (GBE) is available as film-coated tablets, oral liquids, and injectable solutions. In Europe GBE is primarily regulated as herbal medicine, but in the US as a dietary supplement. The pharmacological modes of action include antioxidant effects, radical scavenging, inhibition of platelet activating factor, alterations in membrane fluidity (signal transduction), and inhibition of glucocorticoid synthesis (Diamond and Bailey, 2013 ; Pereir et al., 2013).

The identified pharmacodynamic effects of GBE are clearly linked to terpene trilactones [ginkgolides A, B, and C and bilobalide] and flavonoid glycosides [quercetin, kaempferol, isorhamnetin] (Xiong et al., 2014).

The present study has been designed to explore the effect of GBE against some of FA toxicities on the hippocampus of adult male albino rats.

Material and methods

Material

1-Chemicals

- Formaldehyde (FA): formalin 37% was obtained from Merck, Darmstadt, Germany.
- Ginkgo biloba leaf extract (GBE): commercially obtained as Gencofar tablets (Pharco Pharmaceuticals).
- Kits for immunohistochemistry were supplied by Dako, Carpinteria CA, USA.

2-Animals

The study was conducted on thirty adult male albino rats. Their weight ranged from 180 - 200 gm each. The rats were obtained from the breeding animal house, faculty of Veterinary Medicine, Zagazig University. All rats were housed under similar conditions. The rats were fed commercial rodent pellets and given water ad libitum throughout the experiment.

Ethical consideration of the study

The study was conducted according to the guide for care and use of laboratory animals. All

ethically approved considerations for animal housing and handling were considered.

The experimental protocol used followed the regulations for administration and for painless scarification of the experimental animals. The animals were acclimated in the animal house for a week before the start of the study.

3-Experimental design

The rats were divided into five equal groups.

Group I (Negative control)

The animals of this group left on ordinary rat diet and didn't receive any chemicals and were used to determine the basic values of the tested parameters.

Group II (Distilled water group)

The rats in this group was given 0.5 ml distilled water daily by intraperitoneal (i.p.) injection and used as positive control group.

Group III (GBE group)

The rats in this group were given GBE 300 mg/kg/day by gastric gavage (Naidu et al., 2000). The human therapeutic dose ranges from 80 to 720 mg/d for durations of 2 weeks to 2 years (Diamond and Bailey, 2013). The higher dose used in the study was due to the fact that GBE is a potent inducer of hepatic xenobiotic biotransformation enzymes in the rats (Sugiyama et al., 2004). Gencofar tablets contain 40 mg of GBE. One and half tablets (60 mg/ rat) were ground well and suspended in 2ml distilled water, then the suspension was administered to the rat by gastric gavage.

Group IV (FA group)

The rats of this group received FA 0.2 mg/kg/day by i.p injection [1/10 of LD50 of FA] (Odeigah, 1997). According to the manufacture; formalin is 37% by weight and 40% by volume. So, 1ml formalin contains 0.4 g FA. One ml of formalin was diluted with distilled water up to 1000 ml. In the new concentration 1ml contained 0.4 mg FA. One ml then was diluted with distilled water up to 5 ml and every rat received 0.5ml by i.p. injection. Doane and Sarenbo, (2014) stated that ingestion of small amounts of 36% formalin causes corrosive damage to the pharyngeal mucosa, epiglottis and esophagus leading to nausea, vomiting and discomfort, with a substantial risk of bleeding and perforation. So the i.p. route was chosen in the present study.

Group V (FA + GBE group)

The rats of this group was given GBE 300 mg/kg orally + FA 0.2 mg/kg/day i.p.

The period of the study was 4 weeks. Twenty four hours after the last treatment, rats were anaesthetized by ether inhalation and were sacrificed. The brain were quickly dissected out and washed free of blood with ice cold saline. Hippocampus region was taken. The hippocampus is located in the medial temporal lobe, underneath the cortical surface. In the

rat, the two hippocampi resemble a pair of bananas, joined at the stems by the hippocampal commissure that crosses the midline under the anterior corpus callosum. Different neuronal cell types are neatly organized into layers in the hippocampus (Pearce, 2001; Amaral and Lavenex, 2006). Some of the tissue specimens were used for oxidant enzymes assays while the rest of hippocampus region were subjected to histopathological examination and immunohistochemical staining for Glial fibrillary acidic protein (GFAP).

Methods

I- Biochemical analysis

Preparation of brain tissue samples

This was done according to (Farbiszewski et al., 2000): tissues were homogenized in a four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a homogenizer for 2 min at 5000 rpm, Levels of malondialdehyde (MDA) , and CAT activity were determined in this homogenate. Also some of the homogenate was taken, centrifuged and its supernatant was separated. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 g for 30 min, the supernatant was taken and used in the SOD activity measurement.

SOD activity determination

Super oxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a Spectrophotometer. Activity was expressed as units per mg protein.

CAT activity determination

The enzyme catalase (CAT; EC 1.11.1.6) converts H₂O₂ into water. The CAT activity in plasma and tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H₂O₂, the substrate of the enzyme (Johansson and Borg, 1988). Activity was expressed as units per mg protein.

Determination of glutathione peroxidase activity

Glutathione peroxidase (GSH-Px, EC 1.6.4.2) activity was measured by the method of Paglia and Valentine (1967). The enzyme reaction in the tube containing NADPH, reduced glutathione (GSH), sodium azide and glutathione reductase was initiated by addition of H₂O₂, and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was expressed as units per mg protein.

Measurement of MDA levels

The tissue MDA levels were determined by the method of Draper and Hadley (1990) based on the reaction of MDA with thiobarbituric acid (TBA) at 95 °C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 95 °C for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and supernatant was reacted with 0.67% TBA in a boiling water-bath for 15 min. After cooling, the absorbance was read at 532 nm. Results were expressed as nmol/mg tissue.

II- Histopathological study

Hippocampus samples were fixed in 10% formaline saline. Paraffin sections of 5µm thickness were prepared, stained with haematoxylin (Hx) and eosin (E) stain and examined by light microscope (Bancroft, and Steven, 2002).

III- Immunohistochemistry for Glial fibrillary acidic protein (GFAP)

Immunohistochemistry was performed by following the method of Ramos-Vara et al.(2008). The paraffin sections were processed by Streptavidin-biotin complex (Strep ABC) paraffin sections were deparaffinized in xylene, hydrated and then placed in phosphate buffered saline (PBS; pH 7.6). Antigen retrieval was performed by boiling for 15 minutes in citrate buffer (0.01 M). Sections were treated with 3% hydrogen peroxide for 5 minutes to quench endogenous peroxidase activity, rinsed with deionized water and then washed with PBS. Sections were incubated first with 1% pre-immune rabbit serum to decrease non-specific staining and then with a monoclonal antibody against GFAP (Dako, Carpinteria CA, USA) at 23 °C in a moist chamber for 1 hour. Detection of the antibody was performed using a biotin-streptavidin detection system (Bio-Genex, San Ramon CA, USA) with 3-amino 9-ethyl carbazole (AEC) as chromogen (Dako, Carpinteria CA, USA). Sections were counterstained with Mayer's hematoxylin, sections were evaluated using a light microscope.

IV-Microscopical evaluation for GFAB stained cells

The number of positive neuronal cells was assessed. Measurements were done within 10 fields for each specimen at X400 magnification. This was achieved by using the Leica Q 500 Image analyzer computer system in the Histology department, Faculty of Medicine, Zagazig University.

V- Statistical analysis

All the grouped data were statistically evaluated with SPSS, version 10 software. Testing methods included

one-way analysis of variance (ANOVA) for comparisons between more than two groups followed by least significant difference (LSD) test for comparison between two groups. Percentage values were analyzed by Chi square test. The level of significance was set at $p < 0.05$. All the results were expressed as mean \pm S.D.

Results

Biochemical results

No statistically significant changes were observed in the studied biochemical parameters between negative control, distilled water group and GBE group.

Formaldehyde treated rats showed a statistically significant decrease in the activities of SOD, GSH-Px and CAT in hippocampus tissues as compared to their corresponding values in control group. They also showed statistically significant increase in MDA level with $P < 0.05$. The rats that were exposed to FA together with GBE administration had significantly increased SOD, GSH-Px and CAT enzyme levels and decreased MDA level as compared to their corresponding values in FA treated group with $P < 0.05$. Rats treated with FA + GBE showed non-significant change in the levels of SOD, GSH-Px, CAT and MDA in comparison with the control group (Table I).

Histopathological results

In the control group and the group treated with GBE the morphology of neurons in the hippocampus tissue was nearly normal (Figure 1a) and (Figure 1c) respectively. The most consistent findings in histological sections in FA treated group were the degenerative changes and neuronal loss, shrunken cytoplasm and extensively dark pycnotic nuclei (Figs. 1b). In FA + GBE treated group, the severity of degenerative changes in the cytoplasm and nucleus of hippocampus tissues were less than those in FA group (Figure 1d). Statistical analysis of these pathological changes by Chi square revealed that FA treated rats showed significantly increased changes compared to other groups with $P < 0.05$ (Table II).

Immunohistochemical staining of GFAP results

The result of GFAP immunohistochemistry has been shown in Figure 2. The number of GFAB positive astrocytes was increased in formaldehyde exposed rats (Figure 2b). In GBE+FA treated group, the GFAP-positive cells were less than FA group (Figure 2d). Statistical analysis of the GFAB results by ANOVA revealed significant increase in GFAB positive astrocytes in formaldehyde treated rats as compared to other groups of the study with $p < 0.05$ (Table III).

Table (I): A statistical comparison between the negative control group I, group III (received GBE 300 mg/kg/day orally), group IV (received FA 0.2 mg/kg/day by i.p injection) and group V(received FA+ GBE in the previously mentioned doses) as regard mean values of SOD, GSH-Px,CAT and MDA in the hippocampus of the adult male albino rats all over the period of the study (4 weeks) by (ANOVA test followed by LSD).

Parameter	Group I (negative control)		Group III (GBE)		Group IV (FA)		Group V (FA+GBE)		f	P
	Mean ± S.D		Mean ± S.D		Mean ± S.D		Mean± S.D			
SOD (units/mg protein.)	14.2 ± 2.33		12.8 ± 1.29		7.7 ± 1.15 *		14.4 ± 2.70		13.43	<0.05
GSH-Px (units/mg protein.)	74.7 ± 1.28		69.4 ± 1.20		50.0 ± 1.21 *		72.5 ± 4.81		16.36	<0.05
CAT (units/mg protein)	20.5 ± 1.91		25.4 ± 1.30		12.5 ± 0.89 *		19.0 ± 1.24		15.13	<0.05
MDA (nmol/mg tissue)	9.3 ± 0.97		8.4 ± 1.12		22.5 ± 2.83 *		10.3 ± 1.29		14.89	<0.05

(*) Significantly different from other groups in the same raw ($P < 0.05$).

N.B: number of rats in each group is six rats.

Table (II): A statistical comparison between the negative control group I, group III (received GBE 300 mg/kg/day orally), group IV (received FA 0.2 mg/kg/day by i.p injection) and group V(received FA+ GBE in the previously mentioned doses) as regard the histopathological changes in the hippocampus of the adult male albino rats all over the period of the study (4 weeks) by Chi square.

Pathology	Group I (negative Control)		Group III (GBE)		Group IV (FA)		Group V (FA+GBE)		P
	No	%	No	%	No	%	No	%	
Degenerated neuron	0	0	0	0	5	83.3*	1	16.7	<0.05
Shrunken cytoplasm	0	0	1	16.7	4	66.7*	1	16.7	<0.05
Pycnotic nuclei	0	0	1	16.7	4	66.7*	1	16.7	<0.05

(*) Significantly different from other groups in the same raw ($P < 0.05$).

N.B: number of rats in each group is six rats.

Table (III): A statistical comparison between the negative control group I, group III (received GBE 300 mg/kg/day orally), group IV (received FA 0.2 mg/kg/day by i.p injection) and group V(received FA+ GBE in the previously mentioned doses) as regard mean number of GFAB positive astrocytes in the hippocampus of the adult male albino rats all over the period of the study (4 weeks) by (ANOVA test followed by LSD).

Parameter	Group I (negative control)		Group III (GBE)		Group IV (FA)		Group V (FA+GBE)		f	P
	Mean ± S.D (cells per mm ²)		Mean ± S.D (cells per mm ²)		Mean ± S.D (cells per mm ²)		Mean± S.D (cells per mm ²)			
GFAB positive astrocytes	17.2 ± 2.33		16.5 ± 1.29		65. 6 ± 1.15 *		23.7 ± 1.70		18.48	<0.05

(*) Significantly different from other groups ($P < 0.05$).

N.B: number of rats in each group is six rats.

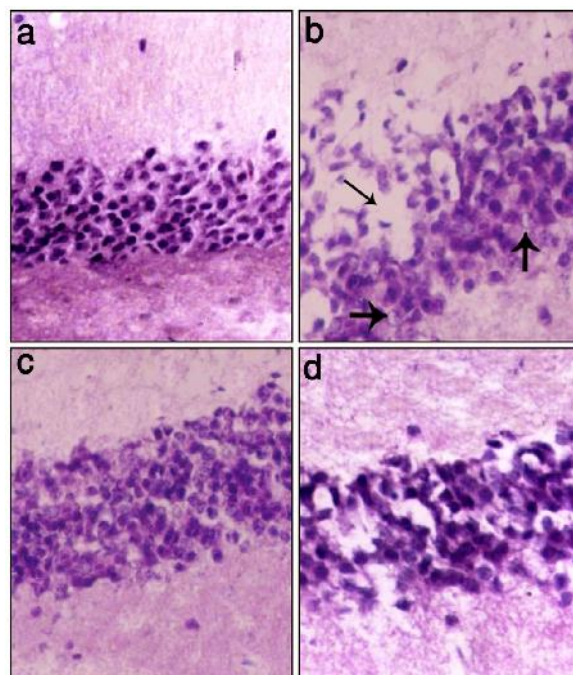


Figure 1: A photomicrograph of hippocampus region of the rat brain of (a) control group showing normal histological picture (b) FA treated group (0.2 mg/kg/day by i.p injection for 4weeks) showing loss of cells and vacuolization (↓) shrunken cytoplasm (↑) with pycnotic nuclei (→) (c) GBE treated group (300 mg/kg/day orally) showing normal histological picture (d) FA+GBE treated rats in the above mentioned doses showing decreased degenerated cells. (Hx and E X400)

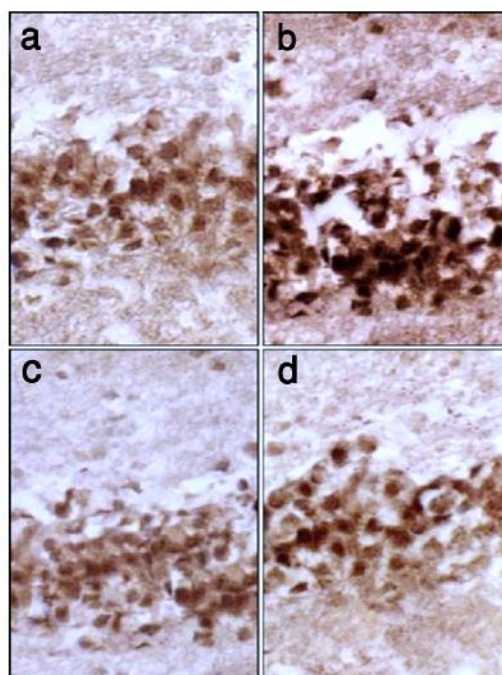


Figure 2: A photomicrograph of immunohistochemical staining of GFAP -stained cells in hippocampus tissue of rat brain (a) control group showing faint staining (b) FA treated group (0.2 mg/kg/day by i.p injection for 4weeks) showing dark staining (c) GBE treated group (300 mg/kg/day orally for 4weeks) showing faint staining (d) FA +GBE treated rats with the above mentioned doses showing decreased number of GFAP positive cells. (streptavidin biotin complex X400)

Discussion

The central nervous system (CNS) is one of the most important systems affected by FA (Tang et al., 2012). The hippocampus is among sites in the brain that coordinates spatial learning and memory. It is also found to be the first brain region affected in age

associated impairments of the brain like Alzheimer's disease. Moreover, hippocampus is also known to respond to sound stimulation and seems to play a role in auditory sensory gating (Abousetta et al., 2014).

Considering its serious threats to human health, FA has raised concern about public safety. To prevent against FA-induced neurotoxic effects, the mechanisms on the noxious effects of FA should be elucidated.

The body has a defense system to protect against damage induced by oxidative stress. It consists of antioxidant enzymes such as GPx, SOD and CAT. Antioxidant enzymes react directly with oxygen free radicals to yield non-radical products (Gizi et al., 2011).

Whereas SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, CAT converts hydrogen peroxide into water. Therefore, SOD–CAT system provides the first defense system against oxidative stress and these enzymes work together to eliminate active oxygen species (Wafa et al., 2011).

Glutathione peroxidases are antioxidant selenoenzymes that are present in the cytosol of cells. The major function of these enzymes, which use glutathione (GSH) as a substrate, is to reduce soluble hydrogen peroxide and alkyl peroxidases (Demir et al., 2011).

Malondialdehyde (MDA) has been widely studied as a product of polyunsaturated fatty acid peroxidation (Lee et al., 2014). Under conditions of oxidative stress, free radical-mediated oxidative damage occurs at various sites within the cell such as peroxidation of cellular membrane lipids resulting in the generation of toxic products including MDA (Dias et al., 2013).

In the present study, the hippocampus brain tissue of rats that was exposed to FA had significantly decreased SOD, CAT and GSH-Px activities, which indicates that FA had disrupted the antioxidant defense mechanism in these tissues, causing oxidative damage. Also the MDA levels of FA administered group were significantly higher than those of the controls. This showed that FA had caused lipid peroxidation of the hippocampus tissue; thus, leading to oxidative damage. These results are in agreement with previous study of, Zararsiz et al. (2006 and 2007) who found that exposure to FA led to an increase in the MDA level and a decrease in the activity of SOD and GSH-Px in the rat prefrontal cortex. Tang et al. (2012) demonstrated that oxidative stress is one of the most critical effects of FA exposure on rats hippocampus.

Tulpule et al. (2012) exposed oligodendroglial cells to FA which had severe consequences on the glutathione (GSH) metabolism of these cells. They were reported that FA induces a rapid GSH export from viable oligodendroglial cells. Such FA-induced GSH loss is likely to compromise the cellular antioxidative and detoxifying potential and may contribute to the known neurotoxicity of FA (Songur et al., 2010) and to the reported deficits in memory and learning after FA exposure (Tong et al., 2011).

Additionally, Songur et al. (2008) reported that the effect of FA on cerebellar oxidant/antioxidant systems increased in a dose-related manner and continued for a long time.

Zhang et al. (2013) mentioned that MDA can damage biomolecules, such as proteins and cellular DNA or RNA and can damage cells, such as hippocampal neurons. Levels of MDA in living organisms were found to be significantly modified in the pathological situations. Therefore, detection of MDA is critical to monitor the progression of these diseases and elucidate the underlying pathology mechanism.

The reduction in GSH-Px, CAT and SOD activities provide evidence that these enzymes have acted to protect cells from increased oxidative events.

Furthermore, these activities provide evidence for the involvement of glutathione, since FA is metabolized by FDH and this enzyme is dependent on glutathione. Therefore, it was postulated that FA causes oxidative damage as a general toxic effect. Also, FA neurotoxicity may be mediated by the activation of free radical producing enzymes and by the inhibition or expenditure of free radical scavenger systems, thereby enhancing the production of ROS (Songur et al., 2010).

Brain is particularly prone to damage by reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to five main reasons: the high oxygen required by this organ; the abundance of redox-active metals; the relative deficit in antioxidant systems; the presence of great amount of oxidizable polyunsaturated fatty acids and catecholamines; and the fact that neurons are post-mitotic cells with relatively restricted replacement by progenitor cells during lifetime (Kim et al., 2013 ; Pereir et al., 2013).

Regarding histopathological study, FA administration caused degenerative changes, shrunken cytoplasm and extensively dark pycnotic nuclei in neurons of the hippocampus tissues. These observations are in agreement with previous studies, which showed that FA treatment caused severe degenerative changes in neurons of frontal cortex and hippocampal tissues (Gurel et al., 2005). Also Lu et al. (2013) reported that FA is capable of causing cell stress responses including cell morphology changes, protein aberrant modifications and even cell death. Moreover exposure of rats to exogenous gaseous formaldehyde induces the accumulation of formaldehyde, decreases the number of hippocampal neurons, neurofilament protein changes and demyelization in hippocampal neurons (Tong et al., 2013). Matsuka et al. (2010) stated that FA has been shown to induce oxidative stress in mouse brain, lung and liver following exposure by inhalation. The increased levels of oxidative stress can induce inflammation and subsequently cell apoptosis

The results of Luo et al. (2012) have been demonstrated that FA has negative effects on central nervous system. Long-term exposure to FA may lead to irreversible neurotoxicity. The suggested mechanism is that FA actually induces Endoplasmic reticulum (ER) stress. Severe ER stress causes cell death.

Glial fibrillary acidic protein (GFAP), as a specific marker of differentiated astrocyte in the central nervous system (CNS), is used to detect the

morphological and functional alterations of astrocytes involved in brain damage (Wang et al., 2012 b).

The results of the present study showed that, as compared to the control group, GFAP immunoreactivity was markedly enhanced in the hippocampus of the FA group. In accordance with these results Dou et al (2012) who reported that GFAP immunoreactivity was markedly enhanced in formaldehyde exposed mice and GFAP-positive hippocampus cells increased with positive granules. Tong et al (2011) showed that excessive formaldehyde induces changes in neurofilament proteins in hippocampal neurons.

Astrocyte processes envelope neuronal synapses. There is ample evidence that astrocytes are actively involved in modulating synaptic transmission (Halassa and Haydon, 2010; Lee et al., 2012). In response to damage inflicted to the central nervous system, astrocytes can change into a so-called reactive state. This transition termed astrogliosis is characterized by an increase in the expression of their main intermediate filament GFAP by morphological alterations (hypertrophy) and by functional changes (Verkhatsky et al., 2010; Vincent et al., 2010). Intermediate filaments are dynamic structures that are involved in a range of cellular processes during homeostasis and stress (Feneberg et al., 2013).

The elevation of GFAP expression in astrocytes in FA-treated group usually is a response to sustained injury.

Neurotoxicity of FA is connected with overproduction of intracellular reactive oxygen species (ROS). As well as with many other types of intoxications associated with the overproduction of ROS, a promising area of search for potential bioprotectors against formaldehyde toxicity lies with antiradical/antioxidant action (Katsnelson et al., 2013).

Free radicals are well known for their dual role as beneficial and toxic components, higher levels of free radicals causing damage to cellular proteins, membrane lipids and nucleic acids leads to cell death. Antioxidants are effective against free radicals by donating their own electrons (Mazumder et al., 2012).

Ginkgo products have long been used in traditional medicine to treat blood disorders and improve memory. They are commercially available in leaves, standardized extracts, tablets, capsules, and teas (Liu et al., 2014).

In the current study the concomitant administration of GBE with Formaldehyde resulted in a significant increase in the activity of GPX, SOD and CAT enzymes and a significant decrease in MDA. These findings support the hypothesis that GBE could possess a neuroprotective effect against FA-induced neurotoxicity. These actions could be mediated through its antioxidant free radical scavenging and anti-liperoxidative properties (Kasper and Schubert, 2009). Kaur et al. (2013) showed that GBE caused significant decrease in oxidative stress as assessed by MDA levels and increase in the antioxidant enzymes (GPX, total glutathione, CAT and SOD) which were depressed by trimethyltin.

Mechanisms of action of GBE also include inhibiting beta-amyloid aggregation, attenuating beta-amyloid-induced neurotoxicity, scavenging free radicals, and promoting cerebral blood flow (Lau et al., 2013). Meanwhile Kaur et al. (2013) stated that although the mechanisms underlying the neuro-protective actions of GBE are unclear, there is some evidence showing that GBE can regulate the levels of neurotransmitters, such as serotonin, influence neurotransmitter receptors, regulate structural changes in hippocampal circuitry, affect neuronal excitability and trigger neurogenesis in the hippocampus.

The results of the present study also revealed that GBE lead to marked attenuation of FA induced histopathological changes. Additionally, the density of immunohistochemically-stained cells was minimal in this group. This could be attributed to its antioxidant effect that is expected to protect neural tissue from damage caused by reactive oxygen metabolites liberated during exposure to formaldehyde. These results are supported by previous reports of Yang et al. (2013) who revealed a highly neuroprotective effects of Ginkgo biloba extract against cerebral ischemia/reperfusion. Moreover, GBE also protected brain tissue against aluminum induced oxidative damage in rats (Abd-Elhady et al., 2013) and protected hippocampus against noise (Abousetta et al., 2014).

Kaur et al. (2013) reported that prophylactic treatment of Ginkgo biloba extract protected against the trimethyltin induced neurodegeneration

Diamond and Bailey (2013) stated that Ginkgo biloba special extract is used in most randomized control trials. Indications include cognition and memory in Alzheimer disease, age-associated dementia, cerebral insufficiency, intermittent claudication, schizophrenia, and multi-infarct dementia.

Conclusion and recommendations

In conclusion, this study has been shown that various biochemical and histological abnormalities are produced in the hippocampus tissues in response to the administration of Formaldehyde to adult male albino rats. These effects included oxidative damage and impairment of structure and function of neurons and appear to be mediated through the production of free radicals. Pre-treatment with GBE offered protection against such changes. Prevention of professional and environmental exposure to FA plays an important role for health care. The discovery of the protective effects of GBE on hippocampus cell injury might carry prophylactic effects against consequences of FA exposure. It should be kept in mind that results obtained from experimental animals should be interpreted cautiously when applied to humans.

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الملخص العربي

تأثير مستخلص أوراق الجنكة بيلوبا على بعض الآثار السمية المحدثة بالفورمالدهيد في حصين ذكور الجرذان البيضاء البالغة

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يعد مركب الفورمالدهيد من المركبات واسعة الانتشار في المستحضرات التي تستخدم في حياتنا اليومية، فهو يدخل في تركيب العديد من المواد الكيميائية و الطبية و مواد البناء و المفروشات. و للفورمالدهيد قدرة على إحداث آثار سمية على الجهاز العصبي المركزي. و يعتبر مستخلص أوراق الجنكة من مضادات الأكسدة ذات التأثير الفعال .

كان الهدف من هذا البحث هو دراسة فاعلية مستخلص أوراق الجنكة في تقليل الآثار السمية للفورمالدهيد على حصين ذكور الجرذان البيضاء البالغة وقد أجرى البحث على ثلاثين من هذه الجرذان تم تقسيمها إلى خمس مجموعات متساوية: المجموعة الأولى ضابطة سلبية و المجموعة الثانية ضابطة موجبة (أعطيت الماء المقطر عن طريق الحقن في الغشاء البريتوني)، المجموعة الثالثة أعطيت مستخلص أوراق الجنكة ٣٠٠ مجم/كجم من وزن الجسم يوميا عن طريق أنبوبة معدية. المجموعة الرابعة أعطيت ٠,٢ مجم/كجم من وزن الجسم من الفورمالدهيد يوميا عن طريق الحقن في الغشاء البريتوني، بينما أعطيت المجموعة الخامسة الفورمالدهيد و مستخلص أوراق الجنكة يوميا بنفس الجرعات السابقة.

وبعد انتهاء مدة التجربة (أربعة أسابيع) تم ذبح الفئران و قياس مستوى الإنزيمات المضادة للأكسدة (الجلوتاثيون بيروكسيداز والسوبر أكسيد ديسميوتيز و الكاتالاز) و نشاط المألونديالدهيد في الحصين كما تم فحص أنسجة الحصين هستوباثولوجيا و هستوكيميائيا. وقد أوضحت الدراسة أن الفورمالدهيد قد أدى إلى نقص ملحوظ في نسبة الإنزيمات المضادة للأكسدة و زيادة في مستوى المألونديالدهيد كما أدى إلى تغيرات هستوباثولوجية في الخلايا العصبية في الحصين شملت تحطم تلك الخلايا و تغيرات بالنواة و الستوبلازم مع زيادة ملحوظة في عدد الخلايا المناعية الإيجابية للبروتين الحامضي الليفى الدبقي. و قد كانت كل التغيرات السابقة ذات دلالة إحصائية.

و قد أدى استخدام مستخلص أوراق الجنكة مع الفورمالدهيد إلى زيادة ذات دلالة إحصائية في نسبة الإنزيمات المضادة للأكسدة و نقص ذو دلالة إحصائية في مستوى نشاط المألونديالدهيد مع تحسن ذي دلالة إحصائية في التغيرات الهستوباثولوجية في الخلايا العصبية و انخفاض عدد الخلايا الإيجابية مناعيا للبروتين الحامضي الليفى الدبقي مقارنة بالمجموعة التي أعطيت الفورمالدهيد منفردا.

مما تقدم يتضح أن للفورمالدهيد آثار سمية على حصين ذكور الجرذان البيضاء مصحوبة بزيادة في نواتج أكسدة الدهون و نقص في الإنزيمات المضادة للأكسدة. كما أوضحت الدراسة أيضا أن مستخلص أوراق الجنكة تأثير وقائي على تلك الآثار السمية التي يحدثها الفورمالدهيد في حصين ذكور الجرذان البيضاء لذا يوصى باستخدامه عند التعرض للفورمالدهيد لتقليل آثاره السمية.

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