



Zingiber officinale and Alzheimer's Disease: Evidences and Mechanisms

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ABSTRACT

This study was planned to assess the efficacy of ginger rhizomes extract and ginger rhizomes oil in the regression of Alzheimer's disease (AD) induced in rat model in attempt to explore their mode of action against this neurodegenerative disease. Seventy male Wistar rats were divided into seven groups; G(1): Negative control group (con), G(2): Ginger extract control group orally administered 100 mg/kg of ginger rhizomes extract (GE), G(3): Ginger oil control group orally administered 100 mg/kg of ginger rhizomes oil (GO), G(4): Positive control group orally received aluminum chloride in a dose of 17 mg/kg to induce AD (AD), G(5): Ginger extract treated group orally administered 100 mg/kg of ginger rhizomes extract after induction of AD (AD+GE), G(6): Ginger oil treated group orally administered 100 mg/kg of ginger rhizomes oil after induction of AD (AD+GO) and G(7): Memantine treated group orally administered 10 mg/kg of memantine after induction of AD (AD+M). Brain acetylcholinesterase (AChE) activity was estimated colorimetrically. Brain acetylcholine (ACh), nuclear factor kappa B (NF- κ B), caspase3 and p53 levels were determined using ELISA technique. Immunohistochemical procedure was used for detection of brain acetylcholinesterase (AChT) activity. Additionally, histopathological investigation of brain tissue sections was carried out. In comparison with the negative control group, AD group recorded significant increase in the brain values of AChE, NF- κ B, caspase3 and p53 in concomitant with significant decrease in the brain level of ACh. Moreover, immunohistochemical finding revealed negative reaction concerning AChT activity in AD group. Furthermore, histopathological investigation of brain tissue sections of rats in AD group showed the formation of amyloid plaques. In contrast, the treatment of AD group with GE or GO resulted in an improvement in the most studied biochemical parameters as indicated by the decreased brain values of AChE, NF- κ B, caspase3 and p53 accompanied with significant increase in the brain level of ACh as compared to untreated AD group. Immunohistochemical results showed positive reaction regarding AChT activity in the AD groups treated with either GE or GO. Moreover, AD groups treated with GE or GO showed great improvement in the brain morphological structure with the disappearance of the most amyloid plaques. This current study indicated that GE and GO significantly ameliorates the neuroinflammation and apoptosis characterizing Alzheimer's disease in the experimental model due to their anticholinesterase activity and antiapoptotic potential besides the anti-inflammatory effect.

Keywords: Alzheimer's disease, ginger extract, ginger oil, anticholinesterase, neuroinflammation, apoptosis.

INTRODUCTION

Alzheimer's disease (AD) is heterogeneous progressive age-related neurodegenerative disease of the brain that effects memory, thought, reasoning and language. Very little is known about the causes of AD, except that its end stages involve extensive neuronal loss and the appearance of distinctive neuropathological features. Substantial evidence indicates that the amyloid β -protein (A β) is a seminal factor in disease causation and it may be considered as an attractable therapeutic target. The ability of A β to self-associate to form oligomeric assemblies appears to underlie the early toxic events that lead to memory impairment and subsequent neurodegeneration.¹ The main risk factors for AD are age, age related diseases such as cardiovascular disease, diabetes, obesity and cancer, low educational levels, head trauma and exposure to heavy metals such as aluminum, copper, iron and zinc.²⁻⁴

Aluminum (Al), a neurotoxin metal that accumulates in human brain, has been implicated as an etiopathogenic factor in AD.⁵ Different aluminum containing compounds, specially those in industrial waste water and in medical preparations such as antacids, phosphate binders,

buffered aspirins, vaccines and allergen injections, expose people to higher than normal levels of Al.⁶ Excessive Al intake has been found to result in memory impairments,⁷ deposition of amyloid protein in central nerve cells and overexpression of β -amyloid precursor protein (APP).^{8,9}

AD has been found to be associated with cholinergic deficit in the brain especially in the cerebral cortex. Acetylcholine (ACh) deficiency is one of the major features seen in sufferers of Alzheimer's and this is due to the high activity of acetylcholinesterase (AChE), an enzyme that converts acetylcholine into inactive choline and acetate. Aluminum has been found to increase AChE activity and accelerate the assembly of β -amyloid into fibrils.¹⁰

Inflammatory components related to AD neuroinflammation include brain cells such as microglia and astrocytes, the classic and alternate pathways of the complement system, the pentraxin acute-phase proteins, neuronal-type nicotinic acetylcholine receptors (AChRs), peroxisomal proliferators-activated receptors (PPARs), as well as cytokines and chemokines. β -amyloid protein has been shown to act as a proinflammatory agent causing



the activation of many of the inflammatory components.¹¹

Apoptosis has also been associated with the pathophysiology of AD and AI is thought to induce apoptosis in the hippocampus cells *via* downregulation of the expression of antiapoptotic mediators and upregulation of the expression of apoptotic factors.¹²

Spices such as ginger (*Zingiber officinale*) are the common dietary adjuncts that contribute to the taste and flavour of foods. Apart from its flavor, ginger is known to contain a number of potentially bioactive phytochemicals, mainly gingerols and their related dehydrating products, shagols as well as volatile oils. Ginger also contains sesquiterpenes, such as β -bisabolene, (-)-zingiberene, and monoterpenes, mainly geranial and neral.¹³ Although *in vitro* data has shown that ginger's active principles protect nerve cells and may have potential in the treatment of Alzheimer's disease,¹⁴ limited information is available on the possible mechanism by which ginger renders its anti-Alzheimer's disease properties.

Therefore, this study was undertaken to explore the mechanisms by which ginger rhizomes extract and ginger rhizomes oil could activate the cholinergic system, repress neuroninflammation, neuronal apoptosis and consequently ameliorate Alzheimer's disease in the experimental model.

MATERIALS AND METHODS

Chemicals and Drugs

Aluminum chloride, sodium chloride, Tris-HCl, sucrose and ginger rhizomes oil were purchased from Sigma Chemical Co., USA. Ginger rhizomes extract in form of capsules with 400mg concentration was purchased from Pharaonia Pharmaceuticals Co. in Burg Al Arab, Egypt. Memantine hydrochloride was purchased from ADWIA Co. S.A.E. 10th of Ramadan City, Egypt.

Experimental Animals

Adult male Wistar rats (150 \pm 10 g), 16 weeks old were obtained from the Animal House Colony of the National Research Centre. They were kept in plastic cages at controlled room temperature (25 \pm 2 °C) and humidity (55%) under a 12 h dark-light cycle. All animals were accommodated with laboratory conditions for at least two weeks before the experiment and maintained under the same conditions all over the experiment. Diet and water were allowed *ad libitum*. All animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research of the National Research Centre, Cairo, Egypt.

Experimental Design

Animals were randomly divided into seven groups (10 rats/group). The first group received saline solution orally and served as negative control group (Con group). The second group was orally administered with 100mg/kg ginger rhizomes extract (GE)¹⁵ daily for two months

(Ginger extract control group) (GE group). The third group was orally administered with 100mg/kg of ginger rhizomes oil (GO)¹⁶ daily for two months (Ginger oil control group) (GO group). The fourth group was orally administered with aluminum chloride (AlCl₃) in a dose of 17 mg/kg¹⁷ daily for two months to induce AD (Positive control group) (AD group). The fifth group was orally administered with AlCl₃ for two months and then treated orally with 100 mg/kg of ginger rhizomes extract daily for other two months (Ginger extract treated group) (AD+GE). The sixth group was orally administered with AlCl₃ for two months and then treated orally with ginger rhizomes oil in a dose of 100 mg/kg for other two months (Ginger oil treated group) (AD+GO). The seventh group was orally administered with AlCl₃ for two months and then treated orally with 10 mg/kg memantine drug (M) daily for other two months (Memantine treated group) (AD+M). The dose of memantine drug is equivalent to the recommended human dose according to Barnes and Paget.¹⁸

Sample Collection

At the end of the experimental period, animals were sacrificed and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried on filter paper and then weighed. Each brain was sagittally divided into two halves. One half of each brain was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl (pH 7.4) and 300 mM sucrose.¹⁹ The homogenate was centrifuged at 1800 xg for 10 min at 4 °C. The supernatant (10%) was separated and stored at -70 °C for biochemical analyses. The second half of each brain was fixed in 10% buffered formalin for immunohistochemical and histopathological investigations.

Biochemical Analyses

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of Lowry et al.²⁰ Brain acetylcholinesterase (AChE) was determined by colorimetric method using acetylcholinesterase assay kit purchased from Centronic GmbH Am Kleinfeld 11,85456 Wartenberg, Germany, according to the method of Henry.²¹ Brain acetylcholine (ACh) was measured by ELISA method using choline/acetylcholine assay kit purchased from Biovision Research Product Co., Linda Vista Avenue, USA, according to the method of Oswald et al.²² Nuclear factor kappa B (NF- κ B) level in the brain was determined by ELISA technique using rat nuclear factor kappa B ELISA kit purchased from Glory Science Co., Ltd, USA, according to manufacturer's instruction. Brain caspase3 was estimated by ELISA technique according to the manufacturer's instruction of ELISA kit for rat caspase 3 purchased from Glory Science Co., Ltd, USA. Brain P53 was assayed by ELISA technique according to the manufacturer's instruction of ELISA kit of rat p53 purchased from Glory Science Co., Ltd, USA.



Immunohistochemical Investigation

After 24 hours of fixation, the fixed brain tissue of each rat in the different studied groups was washed in tap water then, subjected to serial dilutions of alcohol (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μ m by slide microtome. The obtained tissue sections were collected on glass positive slides and fixed in a 65°C oven for 1 hr. Then, the slides were placed in a coplin jar filled with 200 ml of triology working solution (Cell Marque, CA-USA. Cat# 920p-06) which is a product that combines the three pretreatment steps: deparaffinization, rehydration and antigen unmasking. After that, the jar is securely positioned in the autoclave. The autoclave was adjusted so that the temperature reached 120°C and maintained stable for 15 min after which pressure is released and the coplin jar is removed to allow slides to cool for 30 min. Slides were then washed and immersed in Tris buffer saline (TBS) to adjust the pH, this is repeated between each step of the immunohistochemical procedure. Quenching endogenous peroxidase activity was performed by immersing slides in 3% hydrogen peroxide for 10 min. Power stain TM 1.0 Poly HRP DAB Kit Cat# 54-0017 (Genemed Biotechnologies, CA-USA) was used to visualize any antigen-antibody reaction in the tissues. Two to three drops of the rabbit polyclonal primary antibody (acetylcholine transferase Cat#RB-9072-R7, ThermoScientific, CA-USA) was applied, then the slides were incubated in the humidity chamber for overnight at 4°C. Henceforward, polyhorse reddish peroxidase (HRP) enzyme conjugate was applied to each slide for 20 min. 3, 3'- Diaminobenzidine (DAB) chromogen was prepared and 2-3 drops were applied on each slide for 2 min. Then, DAB was rinsed, after which counterstaining with mayerhematoxylin was performed as the final step prior examination of the slides under the light microscope. Then, Image J software (NIH, version v1.45e, USA) was calibrated and the image was opened on the computer screen for image analysis.²³

Histopathological Examination

The paraffin bees wax tissue blocks prepared for immunohistochemical investigation were also used for histopathological examination after sectioning by slide microtome into 5 μ m thick. The obtained tissue sections were collected on glass slides and stained with hematoxylin-eosin (H&E). Then, the slides were examined under the light microscope.²⁴

Statistical analysis

In the present study, all results were expressed as mean \pm S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 14.0 was used to compare significance between each two groups. Difference was considered significant when P <0.05.

RESULTS

The results in Table (1) showed the effect of treatment with ginger rhizomes extract and ginger rhizomes oil on brain AchE activity and Ach level in AD model. In comparison with the negative control group, there was significant increase in AchE activity (112.36%) associated with significant reduction in Ach level (-31.5%) in AD group. On the other hand, the treatment of AD group with either GE, GO or M resulted in significant decrease in the activity of brain AchE (-41.85%), (-51.73%) and (-63.64%) respectively. Moreover, significant elevation in brain Ach level in AD group treated with GE (23.8%), GO (11.35%) or M (29.9%) was recorded compared with untreated AD group. In comparison with the group treated with M (AD+M), there was significant increase in the brain activity of AchE in AD group treated with GE (59.92%) and AD group treated with GO (32.95%). Meanwhile, significant decrease in the brain level of Ach was detected in the group of AD treated with GE (-4.71%) and in the group AD treated with GO (-14.3%) when compared with AD group treated with M (AD+M).

Table 1: Effect of treatment with ginger rhizomes extract and ginger rhizomes oil on brain AchE activity and Ach level in AD model.

Groups	Acetylcholinesterase (AchE) (U/mg protein)	Acetylcholine (Ach) (nmol/mg protein)
Con group	25.98 \pm 0.91	8.1 \times 10 ⁻² \pm 0.11 \times 10 ⁻²
GE group	24.12 \pm 1.02 a (-7.16%)	7.41 \times 10 ⁻² \pm 0.14 \times 10 ⁻² a (-8.5%)
GO group	26.41 \pm 1.13 a (1.66%)	7.34 \times 10 ⁻² \pm 0.10 \times 10 ⁻² a (-9.4%)
AD group	55.17 \pm 2.23 ^a a (112.36%)	5.55 \times 10 ⁻² \pm 0.11 \times 10 ^{-2a} a (-31.5%)
AD+GE group	32.08 \pm 1.42 ^{bc} b (-41.85%) c (59.92%)	6.87 \times 10 ⁻² \pm 0.43 \times 10 ^{-2bc} b (23.8%) c (-4.71%)
AD+GO group	26.63 \pm 1.61 ^{bc} b (-51.73%) c (32.95%)	6.18 \times 10 ⁻² \pm 0.24 \times 10 ^{-2bc} b (11.35%) c (-14.3%)
AD+M group	20.06 \pm 0.61 ^b b (-63.64%)	7.21 \times 10 ⁻² \pm 0.21 \times 10 ^{-2b} b (29.9%)

a: P<0.05 vs the negative control group; b: P<0.05 vs the AD group; c: P<0.05 vs M treated group

The data in Table (2) illustrated the effect of treatment with ginger rhizomes extract and ginger rhizomes oil on brain NF-KB, caspase3 and P53 levels in AD model. Alzheimer's disease (AD) group showed significant elevation in the brain levels of NF-KB (46.75%), caspase3 (23.1%) and P53 (54.37%) as compared to the negative control group. In contrast, treatment of AD group with GE, GO or M ped to significant reduction in the brain levels of NF-KB (-22.81%, -24.57% and -28.62% respectively) as compared to the untreated AD group.



Moreover, the treatment with either GE, GO or M in AD group reduced the brain level of apoptotic marker (caspase3) significantly (-13.3%, 12.9% and -16.3% respectively) as compared to the untreated AD group. Furthermore, AD group treated with either GE, GO or M displayed significant reduction in the level of P53 (-14.05%, -16.35% and -26.83% respectively) when compared with untreated AD group. In comparison with

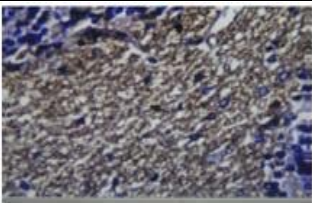
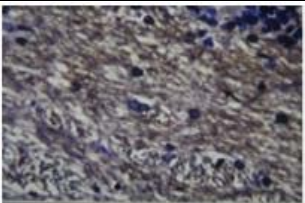
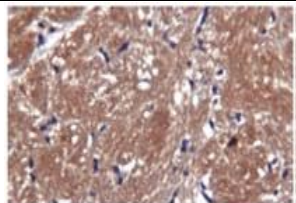
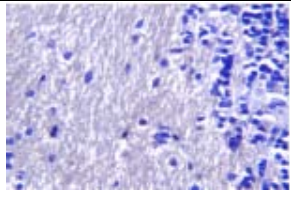
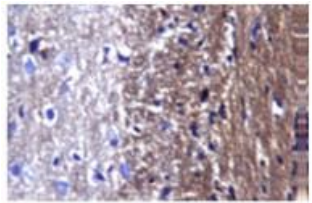
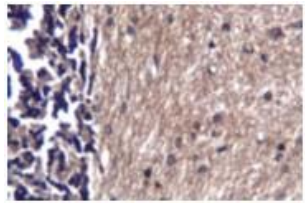
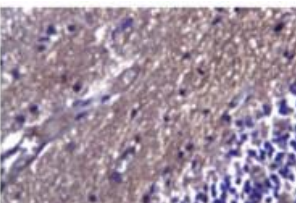
AD group treated with M (AD+M), there was significant increase in the brain levels of NF-κB (8.15% in AD group treated with GE and 5.68% in AD group treated with GO), caspase3 (3.5% in AD group treated with GE and 4.0% in AD group treated with GO) and P53 (17.48% in AD group treated with GE and 14.32% in AD group treated with GO).

Table 2: Effect of treatment with ginger rhizomes extract and ginger rhizomes oil on brain NF-κB, caspase3 and P53 levels in AD model.

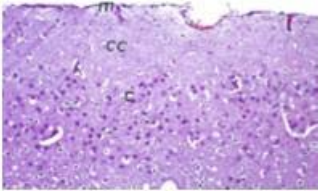
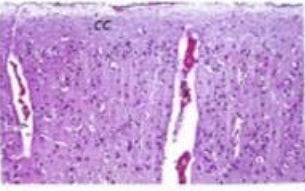
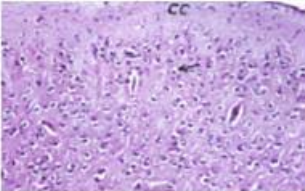
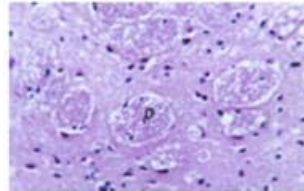
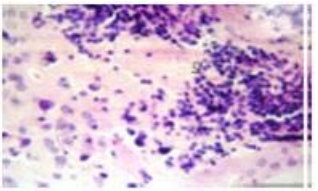
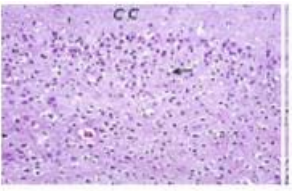
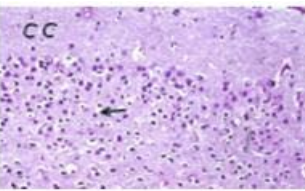
Groups	NF-κB (ng/mg protein)	Caspase 3 (ng/mg protein)	P53 (pg/mg protein)
Con group	71.93±2.13	0.780±0.0035	61.80±1.77
GE group	65.92±2.31 a (-8.36%)	0.758±0.007 a (-2.8%)	59.25±1.05 a (-4.12%)
GO group	63.18±2.01 a (-12.16%)	0.762±0.004 a (-2.3%)	55.80±0.95 a (-9.70%)
AD group	105.56±3.55 ^a a (46.75%)	0.960±0.0055 ^a a (23.1%)	95.40±1.50 ^a a (54.37%)
AD+GE group	81.48±2.47 ^{bc} b (-22.81%) c (8.15%)	0.832±0.0037 ^{bc} b (-13.3%) c (3.5%)	82.00±0.71 ^{bc} b (-14.05%) c (17.48%)
AD+GO group	79.62±3.62 ^{bc} b (-24.57%) c (5.68%)	0.836±0.0051 ^{bc} b (-12.9%) c (4.0%)	79.80±1.36 ^{bc} b (-16.35%) c (14.32%)
AD+M group	75.34±1.05 ^b b (-28.62%)	0.804±0.022 ^b b (-16.3%)	69.80±1.20 ^b b (-26.83%)

a: P<0.05 vs the negative control group; b: P<0.05 vs the AD group; c: P<0.05 vs M treated group

Immunohistochemical Investigation

			
Fig. (1): Photomicrograph of brain tissue section using antibody against AchT showed positive reaction in the negative control group.	Fig. (2): Photomicrograph of brain tissue section using antibody against AchT showed positive reaction in GE control group.	Fig. (3): Photomicrographs of brain tissue section using antibody against AchT showed positive reaction in GO control group.	Fig. (4): Photomicrographs of brain tissue section using antibody against AchT showed negative reaction in AD group.
			
Fig. (5): Photomicrographs of brain tissue section using antibody against AchT showed positive reaction in AD group treated with GE.	Fig. (6): Photomicrographs of brain tissue section using antibody against AchT showed positive reaction in AD group treated with GO.	Fig. (7): Photomicrographs of brain tissue section using antibody against AchT showed positive reaction in AD group treated with M.	

Histopathological Examination

			
Fig. (8): Photomicrograph of brain tissue section of negative control group rat showing normal histological structure of the meninges (m), cerebral cortex (cc) and cerebrum (c) (H&E×40).	Fig. (9): Photomicrograph of brain tissue section of rat in GE control group showing congestion in cerebral blood vessels (v) (H&E×40).	Fig. (10): Photomicrograph of brain tissue section of rat in GO control group showing neuronal cellular oedema (arrow) in cerebrum (H&E×40).	Fig. (11): Photomicrograph of brain tissue section of rat in AD group showing various sizes of amyloid plaques formation (P) in the cerebral cortex (H&E ×40).
			
Fig. (12): Photomicrograph of brain tissue section of rat in AD group treated with GE showing focal gliosis (g) in the cerebral cortex associated with disappearance of the most of amyloid plaques (H&E X40).	Fig. (13): Photomicrograph of brain tissue section of rat in AD group treated with GO showing neuronal cellular oedema (arrow) in cerebrum (H&E×40).	Fig. (14): Photomicrograph of brain tissue section of rat in AD group treated with M showing neuronal cellular oedema in the cerebrum (H&E×40).	

Immunohistochemical Investigation

Immunohistochemical examination of brain tissue section using antibody against AchT showed positive reaction in negative control group (Fig. 1). Figs (2 and 3) represented immunohistochemical investigation of brain tissue sections using antibody against AchT showed positive reaction in GE control group and GO control group respectively. While, Fig. (4) illustrated the immunohistochemical examination of brain tissue section using antibody against AchT of AD group revealed negative reaction. Figs (5 and 6) represented immunohistochemical investigation of brain tissue sections using antibody against AchT of AD group treated with GE and AD group treated with GO respectively, showed positive reaction. Immunohistochemical investigation of brain tissue section using antibody against AchT of AD group treated with M showed positive reaction (Fig. 7).

Histopathological Examination

Fig. (8) illustrated the photomicrograph of brain tissue section of rats in the negative control group showing normal morphological structure of the meninges (m), cerebral cortex (cc) and cerebrum (c) (H&E×40). Fig. (9) represented the photomicrograph of brain tissue section of rats in the GE control group showing congestion in cerebral blood vessels (v) (H&E×40). The photomicrograph of brain tissue section of rats in the GO control group showing neuronal oedema (arrow) in cerebrum (H&E×40) (Fig. 10). The photomicrograph of

brain tissue section of rats in AD group showing various sizes of amyloid plaques formation (P) in the cerebral cortex (H&E ×40) (Fig. 11). Fig. (12) represented the photomicrograph of brain tissue section of rats in AD group treated with GE showing focal gliosis (g) in the cerebral cortex associated with disappearance of the most of amyloid plaques (H&E X40). Fig. (13) illustrated the photomicrograph of brain tissue section of rats in AD group treated with GO showing neuronal cellular oedema (arrow) in cerebrum (H&E×40). The photomicrograph of brain tissue section of rats in AD group treated with M showing neuronal cellular oedema in the cerebrum (H&E×40) (Fig. 14).

DISCUSSION

The neurotransmitter acetylcholine (Ach) is essential for the central and peripheral control of movement, autonomic nervous system function, regulation of sleep, and multiple cognitive processes including timing, attention, learning, and memory. Cholinergic neurons express three factors with combined activities that ensure efficient synthesis, storage, and release of Ach: (1) the uptake of choline from the extracellular space to the neuronal cytoplasm; (2) the synthesis of Ach by cholineacetyltransferase (AChT), using choline and acetyl coenzyme A (Acetyl-CoA) as substrates; and (3) the release of Ach from synaptic vesicles and its hydrolysis by acetylcholinesterase (AChE) in the postsynaptic neurons. Acetyl-CoA is an important molecule in metabolism, used in many biochemical reactions. Its main use is to convey the carbon atoms within the acetyl group to the citric acid

cycle to be oxidized for energy production. Acetyl-CoA is also an important component in the biogenic synthesis of the neurotransmitter Ach. In order to synthesize Ach; the nerve endings need the presence of three substances AchT, choline, and acetyl-CoA. Only one of these substances, acetyl-CoA, is produced directly in the nerve ending. Choline is probably obtained from the extracellular fluid and AchT is supplied from the cell bodies of cholinergic neurons by the mechanism of axonal transport.²⁵

The present study demonstrated an increase in the brain AchE activity in AD group induced by oral administration of $AlCl_3$. This result coincides with that in the study of Ahmed et al.²⁶. The possible mechanism underlying the increased AchE activity in the brain of rats administered $AlCl_3$ is related to the neurotoxic effects of Al compounds. Aluminum could promote the formation and accumulation of insoluble beta amyloid peptide (Abeta) and induce the hyperphosphorylation of tau protein.²⁷ This finding is documented by the current histopathological results which showed the formation of amyloid plaques in the cerebral cortex of rats administered $AlCl_3$ for two months Fig. (11). Altered isoforms of AchE have been found to be increased in AD brain and co-localized with amyloid plaques.^{28,29} Also, Fodero et al.³⁰ reported that the increased activity of AchE is associated with amyloid plaques in human brain. Hu et al.³¹ stated that $A\beta$ increases AchE *via* reducing the enzyme degradation process. While, Fodero et al.³² observed that $A\beta$ -induction of AchE in cultured neurons is associated with an agonist effect of $A\beta$ at $\alpha 7$ -nicotinic receptors which can be antagonized by inhibitors of $\alpha 7$ nicotinic receptors. Melo et al.³³ suggested that $A\beta$ can induce an increase in AchE activity *via* the production of lipid peroxidation in neuronal membranes as a result of the generation of hydrogen peroxide (H_2O_2). H_2O_2 acts as a factor of damage inducing oxidative stress and as modulator (may be allosteric) of the activity of functionally important proteins, receptors and enzymes. Thus, H_2O_2 may have a direct influence on AchE activity.

In the present study, the brain level of Ach was significantly decreased in AD induced in rats by $AlCl_3$ supplementation. In accordance with our results, Williams et al.³⁴ demonstrated that the severe memory loss observed in AD is related to a loss of the neurotransmitter acetylcholine (Ach). A correlation between amyloid plaques levels and cholinergic dystrophic neuritis has been established in AD patients as $A\beta$ peptides are selectively toxic to the cholinergic neurons and in turn they attenuate cholinergic signaling. Tissue culture experiments have demonstrated that cholinergic signaling promotes the production of soluble APP (sAPP) and decreases the production of the toxic $A\beta$ form by altering the activity of cleavage enzymes.³⁴ Therefore, it has been suggested that a loss of cholinergic function may promote the production of the toxic form of amyloid.

The reduction in AchT expression in the brain of AD group as indicated by the negative reaction in the immunohistochemical finding of AchT in the current study attributed to the direct anticholinergic effect of $A\beta$. A growing of evidence indicated that $A\beta$ causes selective degeneration of cholinergic neurons. A suppressive influence of $A\beta$ on the synthesis of Ach in the cholinergic neurons, via its inhibitory effect on AchT activity, is the most relevant issue in the early stages of AD before significant plaque formation and the apparent toxicity that accompanies $A\beta$ deposition.³⁵

The group treated with ginger extract (AD+GE) and that treated with ginger oil (AD+GO) showed significant decrease in the activity of AchE in the brain. This could be attributed to the antioxidant and free radical scavenging properties of the ginger extract and ginger oil³⁶ against Al-induced lipid peroxidation.³⁷ It has been demonstrated that the active ingredients of these extracts such as tannins, terpenoids, alkaloids and flavonoids have the ability to scavenge free radicals including H_2O_2 ³⁸ with consequent inhibition of lipid peroxidation. Thus, the anti-lipidperoxidative effect of these extracts may participate in amelioration of H_2O_2 action on AchE activity.

Treatment of AD groups with either ginger extract (AD+GE) or ginger oil (AD+GO) produced significant improvement in brain Ach level. This finding might be due to the free radical scavenging activity of the active constituents of ginger mainly phenolic compounds. Natural polyphenols are capable to remove free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals and inhibit oxidases.³⁹ Such activities enable these extracts to counteract $A\beta$ deposition and in turn inhibit AchE activity in the brain. Many studies have suggested that inhibiting neuronal AchE activity would increase the level of Ach and improve the cognitive function effectively.^{40, 41}

Immunohistochemical investigation of cholineacetyltransferase (AchT) expression in the brain of AD groups treated with either ginger extract (AD+GE) or ginger oil (AD+GO) revealed positive reaction indicating the increased of AchT in these groups. This could be explained by the ability of ginger extract and ginger oil to activate the cholinergic neural transmission by stimulating AchT activity. This could be explained by the free radical scavenging activity of the active constituents of ginger represented by flavonoids. The influence of these compounds upon cellular pathways *via* inhibiting the peroxidation of liposomes and reducing malondialdehyde levels, indicates the usefulness of these compounds in the treatment of the degenerative disorders.^{36,42} As these compounds could maintain the structure and function of the cholinergic neurons.

Our data revealed that, treatment of AD group with memantine (AD+M) results in significant inhibition of brain AchE activity. Gupta et al.⁴³ stated that memantine exerts various pharmacological effects by multiple mechanisms. The primary mechanism appears to be



blockade of NMDA receptor channels, and thereby preventing excessive Ca²⁺ influx, excessive formation of free radicals, depletion of high-energy phosphates, and mitochondrial/neuronal damage. These authors provided evidence for antioxidant activity of memantine *via* its ability to prevent the formation of excessive free radicals. Thus, memantine may have a suppressive effect on H₂O₂ generation and hence it could attenuate the influence of H₂O₂ on AchE activity.

The current results revealed that the treatment of AD group with memantine results in significant increase in brain Ach level. Treatment with a therapeutically relevant dose of memantine significantly attenuated Aβ₄₂-induced loss of cholinergic neurons and microglia activation in the neocortex and magnocellular basal nucleus (MBN). Memantine also reversed the attention and learning deficits in the Aβ₄₂-treated rats.⁴⁴ These data indicate the ability of memantine to rescue brain cells from the neurotoxic effect of Aβ₄₂ oligomers *in vivo*. Memantine improves learning and memory in entorhinal cortex lesioned in aged rats and improves spatial learning in APP/PS1 transgenic and triple-transgenic AD mice overexpressing Aβ. Memantine also increases the maintenance of long-term potentiation in the hippocampus of old rats and of transgenic mice overexpressing Aβ. In addition it prevents Aβ-evoked decrease in hippocampal somatostatin and substance P level, two neuropeptides that otherwise support synaptic plasticity in the hippocampus. These data indicate the ability of memantine to preserve Ach level in the brain of AD group as shown in the present study.

The current result of immunohistochemical examination of AchT expression in the brain of AD group treated with memantine revealed positive reaction indicating the increased expression of AchT in this group. Nyakas et al.⁴⁴ suggested that memantine may improve cognition by protecting cholinergic neurons from Aβ toxicity as Aβ is known to exert a number of cellular and molecular pathologies leading to cognitive deficits, LTP disruption (disruption of synaptic plasticity), oxidative stress and apoptosis. Memantine can interfere with all these pathological processes. It improves learning and memory in entorhinal cortex lesioned⁴⁵ as well as in aged brain⁴⁶ rats and improves spatial learning in APP/PS1 transgenic⁴⁷ and triple-transgenic AD mice⁴⁴ overexpressing Aβ. Memantine increases the maintenance of long-term potentiation in the hippocampus of old rats⁴⁶ and of transgenic mice overexpressing Aβ.⁴⁴ In addition it prevents Aβ evoked decrease in hippocampal somatostatin that otherwise support LTP in the hippocampus.⁴⁸

There is increasing evidence that neurotoxicity in AD is mediated by inflammatory processes⁴⁹ and one contributing factor in AD is the presence of glial cells that are activated by Aβ to produce pro-inflammatory cytokines like IL-1, IL-6, and TNF-α. It is also worth noting that all synapses are encapsulated by cytokine generating

astrocytes and those cytokines are co-localized with senile plaques in AD.^{49,50}

The present study demonstrated significant increase in the brain level of NF-κB in AD group induced by AlCl₃ administration. Accumulating evidence indicates that the activation of NF-κB is one of the intracellular signaling pathways by which Aβ exerts its neurotoxic effects.⁵¹ In unstimulated cells, NF-κB is retained in the cytoplasm by binding to inhibitors IKB (IKBα, IKBβ, and IKBγ). Upon the activation by stimuli, such as Aβ, IKB is sequentially phosphorylated by IKB kinases (IKK1 or IKK2) and then degraded by the proteasome. This process makes free NF-κB available in the cytoplasm and allows the translocation of NF-κB protein from the cytoplasm to the nuclei.⁵²

Treatment of AD groups with either ginger extract (AD+GE) or ginger oil (AD+GO) caused significant reduction in the brain level of NF-κB. Ginger extract and ginger oil possess antineuroinflammatory capacity as they could inhibit the activation of the convergent upstream transcription factor, NF-κB. It has been demonstrated that ginger suppresses iNOS, COX-2, IL-1β and TNF-α expression through downregulation of p38 mitogen activated protein kinase (MAPK) signalling and NF-κB activation in microglial cells.⁵³ Ho et al.⁵⁴ demonstrated that gingerols and shogaols (active compounds in ginger) exert their antineuroinflammatory effect through the inhibition of the upstream NF-κB activation and subsequent proinflammatory gene expression.

Our data recorded significant depletion in the brain level of NF-κB in the AD group treated with memantine (AD+M). Memantine could significantly reduce neuroinflammation in AD context as indicated by the diminished IL-1β and TNF-α mRNA levels compared to untreated AD animals. This suggests that memantine may prevent Aβ-induced cytokines transcription.⁵⁵

The current study revealed significant increase in caspase 3 level in the brain of AD group induced by AlCl₃ supplementation. Caspase 3 has been reported to be associated with neuritic degeneration in AD brain and participated in Aβ-induced neuronal death, but its precise role in the disease remained controversial.^{56,57} Aβ was found to induce caspase 3 expression in a JNK3-dependent manner.^{57,58} Kuranaga,⁵⁷ provided evidence that Aβ- induces the activation of c-Jun N-terminal kinase in a JNK-dependent manner. JNK3 appears to promote apoptosis by phosphorylating and activating the transcription factor c-Jun. Therefore, it is clear that JNK activity is important for Aβ induction of neuronal death, in as much as the disruption of JNK3 function leads to a marked decrease in the percentage of neurons undergoing Aβ-induced neuronal apoptosis. From other point of view, Aβ and its role in the production of neuronal oxidative stress have a major role in the pathology of AD. The relationship between oxidative stress and neuronal death has been extensively investigated. Oxidative stress causes damages to the



nucleic acids, proteins and lipids and potentially opens the mitochondrial permeability transition pore, which in turn can further stimulate ROS production, worsen energy failure and release proapoptotic factors such as cytochrome c into the cytoplasm *via* activation of the JNK pathway or by activation of NF- κ B transcription factors. Generation of high levels of ROS and downregulation of antioxidant mechanisms result in neuronal cell death in neurodegenerative diseases.⁵⁹

The present results recorded significant decrease in the brain level of caspase 3 in AD groups treated with ginger extract (AD+GE) or ginger oil (AD+GO). Shogaols, and zingerone in ginger have shown various pharmacological activities including antioxidant activity⁶⁰ so that, they can scavenge free radicals, inhibit the secretion of cytokines and suppress the cell death. The ability to control ROS is thus critical in neurodegenerative diseases, because neuronal damage occurs when the "oxidant– antioxidant" balance is disturbed in favor of excess oxidative stress. A growing body of evidence suggested that ROS-scavengers effectively protect neurons against both necrotic and apoptotic cell death.⁵⁹ The inhibitory effect ginger hydrolysed phenolic fractions on the apoptotic cell death indicating by inhibition of caspase-3 activity may result from the relieve of the oxidative stress and local inflammation.⁶¹

The present study demonstrated significant decline in the caspase 3 level in the brain of the memantine treated group (AD+M). In accordance with our results, Hemmati et al.⁵⁵ found that memantine prevents hippocampus neuronal death by inhibiting overexpression of JNK mRNA. c-Jun N-terminal kinase (JNK) signaling cascade plays a crucial role in A β -induced neuronal cell apoptosis and provides a molecular linkage between oxidative stress and neuronal apoptosis.⁶²

Our study recorded significant increase in the brain level of P53 in the AD group as a consequence of AlCl₃ administration. Recent study suggested the enhanced expression and activation of p53 is the main mechanism responsible for A β toxicity. Enhanced p53 levels were observed in the brains of the transgenic mice overexpressing A β 1-42. Oxidative stress due to A β accumulation induced p53 activation that leads to apoptotic cell death of neurons of the brain. Damaged neurons in the brain tissue of AD patients exhibit increased p53 immunoreactivity, suggesting the important role of p53 in the pathogenesis of AD.⁶³ It was previously demonstrated that activation of p53 signaling pathway in neurons occurred during unscheduled re entry into the cell cycle.^{64,65} Aberrant re expression of many cell cycle-related proteins, like cyclin/cyclin-dependent kinase (Cdk) complexes in vulnerable neuronal populations in AD was shown to be one of the earliest pathologic changes leading to neurodegeneration and cell death.⁶⁶ Cenini et al.⁶⁷ stated that oxidative stress which yields from the A β protein stimulates additional neuronal damage *via* the overexpression of inducible (i) and neuronal (n) specific

NO synthase (NOS: iNOS and nNOS) leading to increased levels of NO. NO and O₂⁻ react at diffusion controlled rates to produce peroxynitrite, an extremely strong oxidant that affects lipids, DNA, carbohydrates, and proteins with consequent increase of oxidative damage. These oxidative alterations activate the inflammatory response in AD brain. p53, the proapoptotic protein, has been found to be modified by covalent binding of the lipid peroxidation product and it is considered as a target for oxidative and nitrosative stress in the neurodegenerative condition.

Treatment of AD group with ginger extract (AD+GE) or ginger oil (AD+GO) resulted in significant decrease in the level of P53 in the brain. This may be due to the role of these ginger extracts with their active ingredients against A β -induced lipid peroxidation.⁶⁸ Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body.³⁹ The higher radical scavenging ability of the ginger could be attributed to the presence of some powerful antioxidant phytochemicals such as tannins, terpenoids and flavonoids that are potent radical scavengers.³⁶ This is a suggested mechanism by which ginger extracts could inhibit p53 level in the brain of AD induced in rats.

The treatment of AD group with memantine (AD+M) showed significant reduction in the level of P53 in the brain. This may be due to that memantine exerts various pharmacological effects by multiple mechanisms. The primary mechanism appears to be blockade of NMDA receptor channels, and thereby preventing excessive Ca²⁺ influx, excessive formation of free radicals, depletion of high-energy phosphates, and mitochondrial/neuronal damage.⁴³ Thus, the antiradical properties of memantine could be the cause of reducing p53 level in the brain of memantine treated group.

Microscopic investigation for the brain tissue section of rat in AD group revealed the presence of amyloid plaques formation in the cerebral cortex. In accordance of our results Abd El-Rahman,⁶⁹ demonstrated that aluminum administration causes the presence of neuritic plaques that appeared with dark center, neuronal damage and degeneration in the cerebral cortex and the hippocampus.

Microscopic investigation for brain tissue section of rat in AD group treated with ginger extract or ginger oil showed an improvement of the morphological feature of the brain tissue with the disappearance of most of amyloid plaques. This could be due to the antiinflammatory effect of the active constituents of these extracts particularly shogaols and zingerone. As a result the overexpression of proinflammatory cytokines and inflammatory markers such as NF- κ B is reduced.⁵³ Also, the role of both of ginger extract and ginger oil as antioxidants and free radical scavengers, due to their active ingredients, is responsible for the disappearance of most of A β from the brain of the treated rats.³⁶



Microscopic investigation for the brain tissue section of rat in AD group treated with memantine showed an improvement of the morphological feature of the brain tissue. This could be attributed to the ability of memantine to inhibit truncation of glycogen synthase kinase-3 triggered by activated calpain, which is believed to play a key role in the pathogenesis of Alzheimer's disease and notably the process of tau phosphorylation.⁴⁴ Calpain, a calcium dependent cysteine protease, is a downstream link of the NMDA receptor-induced neurodegeneration pathway.⁷⁰ Memantine increased production of glial cell line-derived neurotrophic factor (GDNF) in astroglia, also it displayed neuroprotective effects against LPS-induced neuronal damage through inhibition of microglia activation and reduction of pro-inflammatory factor production.

In conclusion, based on our results, we could conclude that GE and GO have a promising therapeutic role against AD induced in male rats as indicated by the observed improvement in biochemical and immunohistochemical markers which were confirmed by histological examination. This role was achieved through powerful anticholinesterase activity, anti-inflammatory properties and antiapoptotic effects of the active constituents of ginger rhizomes.

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