



Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2018 15(3): 1931-1942.

OPEN ACCESS

Amyloid beta-peptide (1-42) induced neurotoxicity in experimental rats: Effect of Donepezil

Yasser M. Moustafa¹, Dalia Medhat^{2*}, Sawsan A. Zaitone^{1,4}, Zakaria El-Khayat², Omar M. E. Abdel-Salam³ and Alhammali A.M. Abdalla¹

¹Pharmacology and Toxicology Department, Faculty of Pharmacy, Suez Canal University, **Egypt**.

²Medical Biochemistry Department, National Research Center, 33 El Behouth St., 12622, Dokki, Cairo, **Egypt**.

³Toxicology and Narcotics Department, National Research Centre, Tahrir St., Dokki, Cairo, **Egypt**.

⁴Pharmacology and toxicology Department, Faculty of Pharmacy, University of Tabuk, **Saudi Arabia**.

*Correspondence: dalia_8383@hotmail.com Accepted: 18 Mar. 2018 Published online: 13 Aug. 2018

Neurodegenerative disorders results in inflammatory processes, including inflammatory cytokine secretion and concomitant superoxide production. Acetylcholinesterase inhibitors (AChEIs) influence neuro degeneration through anti-inflammatory effects. Donepezil as an AChEIs also provide neuro protection. This study aimed to evaluate the effect of donepezil (DZ) as a potent acetyl-cholinesterase (AChE) inhibitor, L-N^G-Nitroarginine methyl ester (L-NAME) as non-selective nitric oxide synthase inhibitor and 7-nitroindazole (7-NI) as a selective neuronal nitric oxide synthase (NOS) inhibitor against Amyloid beta-peptide (1-42) ($A\beta_{(1-42)}$) induced neurological disorder. Rats were divided into six groups including control, $A\beta_{(1-42)}$, $A\beta_{(1-42)}$ +L-arginine, $A\beta_{(1-42)}$ +L-NAME, $A\beta_{(1-42)}$ +7-NI, and $A\beta_{(1-42)}$ +DZ. Brain AChE, malondialdehyde, nitric oxide, super oxidizedismutase, catalase, reduced glutathione, interleukin 1-beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) were measured. Also, brain fatty acids fractions were estimated by high performance liquid chromatography (HPLC). $A\beta_{(1-42)}$ significantly alter levels of brain antioxidant, inflammatory markers and fatty acids content compared to the control group. Treatment with L-NAME, 7-NI and especially DZ improve these parameters. Administration of acetylcholinesterase inhibitors such as DZ attenuates neurodegenerative disorders through decreasing oxidation and inflammation.

Keywords: Neurotoxicity, $A\beta_{(1-42)}$, Donepezil, Nitric oxide, 7-nitroindazole, Fatty acids, HPLC

INTRODUCTION

Alzheimer's disease (AD) is characterized clinically by a progressive and gradual decline in cognitive function and neuropathologically by the presence of neuropil threads, specific neuron loss, and synapse loss in addition to the hallmark findings of neurofibrillary tangles and senile plaques (Murphy and LeVine, 2010).

AD is associated with initial memory loss, followed by language impairment (aphasia), motor function is intact (apraxia), inability to recognize object, sensory function is intact (agnosia), and impairment in judgment, executive functioning, and visuospatial functioning (Sharma and Wong,

2016).

Swerdlow et al., (2014) suggested a disease mechanism that has emerged from the observations linking mitochondrial dysfunction and increased production of reactive oxygen species (ROS) to the development of AD. The mitochondrial cascade hypothesis states that in sporadic, late-onset AD, loss of mitochondrial function associated with age affects the expression and processing of $A\beta$ PP initiating $A\beta$ accumulation. Mitochondrial dysfunction has been well documented in AD (Beal, 2005). Abnormal mitochondrial axonal trafficking is already observed in embryonic neurons from multiple transgenic mouse models of familial AD with

additional abnormalities in fission, fusion, and function detected prior to the development of amyloid plaques or memory impairment.

Amyloid beta-peptide (1-42) ($A\beta_{(1-42)}$) plays a central role in the cause and progression of AD, because it is a major component of the extracellular plaque found in AD brains. Increased $A\beta$ levels correlate with the cognitive decline observed in AD. Sporadic AD cases are linked with lack of $A\beta$ clearance from the brain, unlike familial AD which shows increased $A\beta$ production. $A\beta$ can inhibit long-term potentiation (LTP), a model system for synaptic strengthening and memory (Bharadwaj et al., 2009).

Soluble $A\beta$ is exchanged across the BBB by two principle mechanisms, the low-density lipoprotein receptor-related protein (LRP) on the abluminal (brain) side, and the receptor for advanced glycation end products (RAGE) on the luminal (blood) side. The distribution of $A\beta$ plaques changes with time and reflects the spread of $A\beta$ deposition in the diseased brain (Mufson et al., 2016).

Acetylcholinesterase inhibitors (AChE-inhibitors) are used for the treatment of Alzheimer's disease. Recently, the AChE-inhibitor donepezil was found to have neuroprotective effects. Donepezil (R,S -1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl] methylpiperidine hydrochloride), a potent acetylcholinesterase (AChE) inhibitor has been prescribed for the treatment of cognitive dysfunction in AD. It prevents the hydrolysis of acetylcholine (ACh) and elevates ACh concentrations in the synaptic cleft, which results in increased cholinergic transmission (Noh et al., 2009).

Aim of the study

This study aimed to evaluate the effect of DZ as a potent acetylcholinesterase (AChE) inhibitor, L-NAME as non-selective nitric oxide synthase (NOS) inhibitor and 7-NI as a selective neuronal NOS inhibitor against $A\beta_{(1-42)}$ induced neurological disorder.

MATERIALS AND METHODS

Materials

Animals

Male albino rats weighting 140-180g were used for induction of brain injury. Animal obtained from the Animal house of National Research Center, Giza, Egypt. Animals were housed in individual suspended stainless cages in controlled

environment (22-25°C) and 12 hours light, 12 hours dark with food and water *ad libitum* freely available.

Drugs and chemicals

Amyloid beta-peptide (1-42) $A\beta_{(1-42)}$, L-N^G-Nitroarginine methyl ester (L-NAME), L-arginine (L.arg.), 7-nitroindazole (7-NI) and fatty acids standards were purchased from Merck, Darmstadt, Germany. Acetonitrile, methanol, ethanol, N-hexane, 2-propanol and phosphoric acid HPLC grade were purchased from purchased Merck, Darmstadt, Germany. Donepezil tablets (10mg) were purchased from Pfizer Egypt.

Methods

Induction of $A\beta_{(1-42)}$

Rats were anesthetized with ethyl ether. An incision was made in scalp and hole was drilled in the skull over the injection site. The 30-gauge-needle was lowered into the dorsal hippocampus. Coordinates for the anterior-posterior (from bregma), medial-lateral (from midline), and dorsal-ventral (from surface of the skull) axes were -2.3, ±2.5, and -1.5 mm, respectively. Bilateral intrahippocampal infusion was administered via a 10.0 μ l Hamilton microsyringe with a 30-gauge needle fitted to the arm of the stereotaxic instrument. A 0.6 μ l volume of oligomer $A\beta_{1-42}$, freshly made $A\beta_{1-42}$ peptide solution, or double-distilled water alone (as a vehicle control) was slowly infused at a rate of 0.2 μ l/min. After an additional 5 min, to assure adequate diffusion, the needle was slowly retracted (Huang et al., 2007).

Experimental design

36 rats were divided to six groups (6 rats in each). Group I: healthy rats received normal saline. Group II: $A\beta_{(1-42)}$ group. Group III, IV, V and VI: rats injected by $A\beta_{(1-42)}$ then treated with L.arg. (750mg/kg), L. NAME (50 mg/kg), 7-NI (25mg/kg) and DZ (10 mg/Kg) subcutaneously daily for 15 days.

Preparation of tissue homogenate

Brain homogenate was done according to Manna et al., (2005). Briefly, brain was removed quickly and placed in iced normal saline, perfused with the same solution to remove blood cells, blotted on filter paper and frozen at -80°C. The frozen tissue was cut into small pieces and homogenized in 5 ml cold buffer (0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500 ml

deionized water (pH 7.4) then centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was removed for biochemical parameters estimation.

Biochemical assays

Determination of brain malondialdehyde (MDA)

MDA was determined according to Uchiyamara et al., (1978). The kit was supplied from Biodiagnostic, Egypt.

Determination of brain nitric oxide (NO)

Brain nitric oxide was determined using colorimetric kit purchased from biodiagnostics, Egypt according to Montgomery et al., (1995).

Determination of brain superoxide dismutase (SOD)

Brain superoxide dismutase (SOD) activity was measured using kinetic kit purchased from biodiagnostics, Egypt according to Nishikimi et al., (1972).

Determination of brain catalase activity (CAT)

Brain catalase activity was determined according to the methods described by Johansson and Borg (1988).

Determination of brain reduced glutathione (GSH)

Reduced glutathione was determined using kinetic kit (from Biodiagnostic, Egypt) according to the method of Beutler et al., (1963).

Determination of brain acetylcholinesterase activity (AChE)

acetylcholinesterase activity was determined using quantitative colorimetric kinetic assay (BEN Biochemical Enterprise, Milano, Italy) according to Young, (2000).

Determination of brain Interleukin -1 β (IL- 1 β) and tumor necrosis factor- α (TNF- α)

Quantitative determination of brain IL- 1 β and TNF- α were done by ELISA kits purchased from Assaypro according to the method described by Barland et al., (2004) and Aukrust (1994) respectively.

Determination of brain fatty acids

Brain α -linolenic acid (ALA), arachidonic acid (AA), and linolenic acid (LA) were fractionated and

analyzed according to (El-khayat et al., 2013). Briefly, brain was homogenized in 2% acetic acid/ethyl ether mixture (2:1 volume ratio). The solution was then vortex mixed and centrifuged at 3000rpm, the organic phase was evaporated (under nitrogen gas stream) to dryness; the residue was dissolved in 500 μ l acetonitrile and filtered by PVDF 0.45 μ m filter before injection onto HPLC.

HPLC Conditions

Fatty acids were determined using reversed phase HPLC ODS column (250x4.6, particle size 5 μ m). Mobile phase used was acetonitrile/ water mixture (70:30 v/v) by isocratic elution with flow rate 1ml/min. Wavelength of the UV detector was adjusted at 200nm. Serial dilutions of each standard were injected onto HPLC and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentrations in samples were obtained from the standard curve.

Statistical Analysis

All data was expressed as mean \pm SE. Distribution of the data was verified to be normal using Tests of Normality (SPSS package). Statistical significance was tested by one way analysis of variance (ANOVA).

RESULTS

Brain oxidant /antioxidant levels in different studied groups.

Induction of A β ₍₁₋₄₂₎ causes significant increase in brain MDA levels and nitric oxide compared to the control group, while induction of A β ₍₁₋₄₂₎ causes significant decrease in brain SOD, CAT, and GSH levels. Treatment with L.arg., L.NAME, 7- NI and DZ attenuate these values compared to A β ₍₁₋₄₂₎ group as shown in table (1).

Brain acetylcholinesterase activity

Results of this study indicated that there was a significant increase in ChE activity in A β ₍₁₋₄₂₎ group compared to the control group, while this value significantly decreased by treatment with L.arg., L.NAME, 7- NI and DZ compared to A β ₍₁₋₄₂₎ group fig. (1).

Table (1): Brain oxidant /antioxidant levels in different studied groups.

Groups Parameters	Control	A β ₍₁₋₄₂₎	A β ₍₁₋₄₂₎ + L.arg.	A β ₍₁₋₄₂₎ + L. NAME	A β ₍₁₋₄₂₎ + 7-NI	A β ₍₁₋₄₂₎ + DZ
MDA(nmol/g tissue)	45.73 \pm 3.81	185.11 ^a \pm 7.42	120.83 ^{a,b} \pm 6.26	86.56 ^{a,b} \pm 3.84	102.81 ^{a,b} \pm 8.12	65.67 ^b \pm 2.33
NO (U/g tissue)	9.82 \pm 1.14	26.07 ^a \pm 2.21	23.13 ^a \pm 2.96	17.31 ^{a,b} \pm 2.62	19.16 ^{a,b} \pm 1.13	11.25 ^b \pm 1.64
SOD (U/g tissue)	50.94 \pm 3.46	26.84 ^a \pm 2.38	25.86 ^a \pm 6.42	39.13 ^{a,b} \pm 5.99	37.82 ^{a,b} \pm 2.94	49.19 ^b \pm 3.53
CAT(U/g tissue)	3.93 \pm 0.24	1.53 ^a \pm 0.06	4.96 ^{a,b} \pm 0.32	4.15 ^b \pm 0.29	3.53 ^{a,b} \pm 0.21	4.48 ^b \pm 1.32
GSH (mol/g/min)	15.44 \pm 1.29	6.92 ^a \pm 1.96	7.23 ^a \pm 1.06	9.77 ^{a,b} \pm 1.30	11.16 ^{a,b} \pm 1.91	17.23 ^b \pm 2.42

P^a: Significant difference from the control group (p< 0.05). P^b: Significant difference from the A β ₍₁₋₄₂₎ group (p< 0.05).

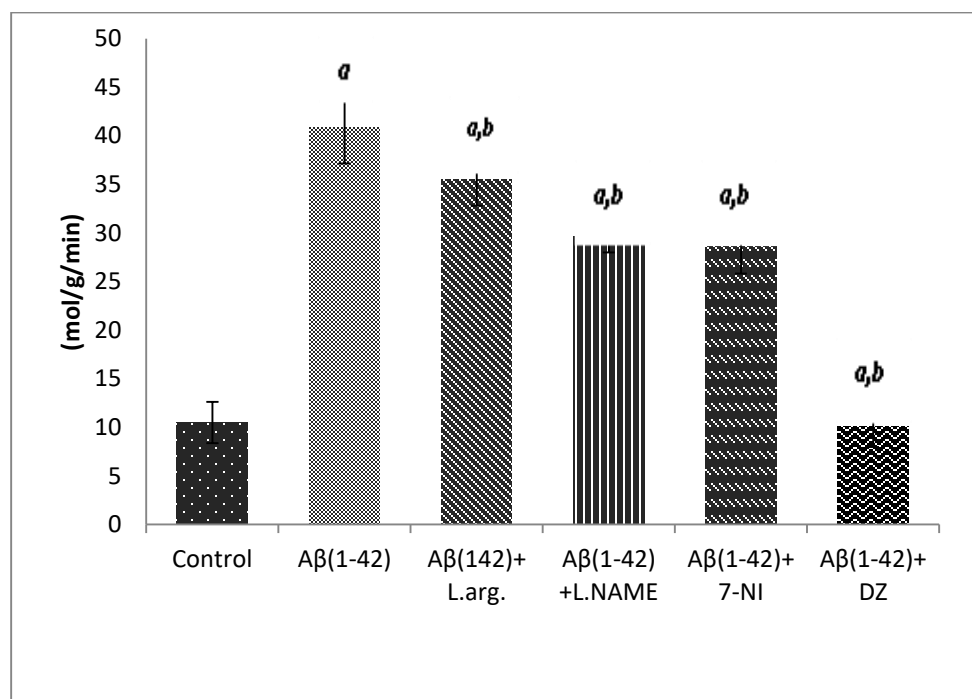


Figure. (1): Brain AChE. activity in different studied groups.

P^a: Significant difference from the control group ($p < 0.05$). P^b: Significant difference from the A β (1-42) group ($p < 0.05$).

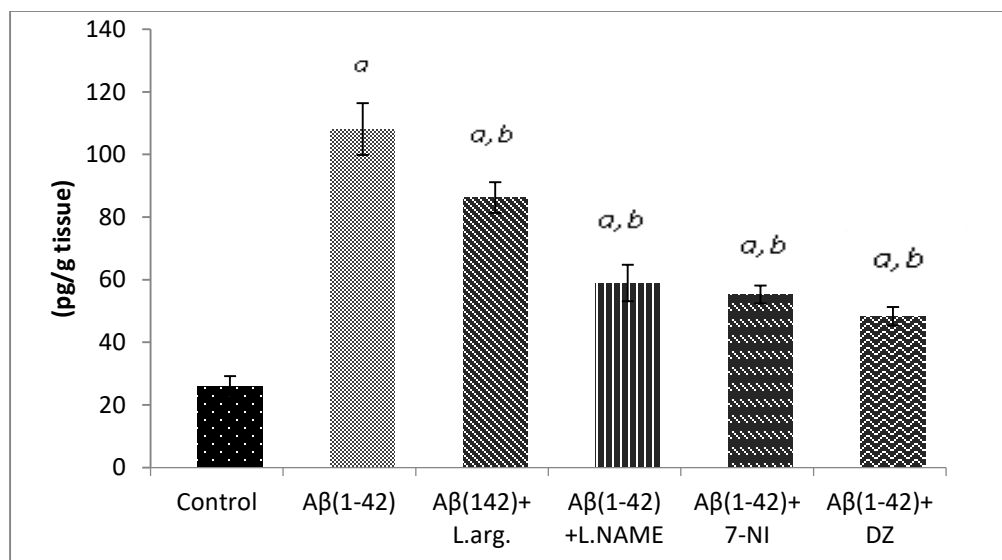


Figure. (2): Brain IL-1β in different studied groups.

P^a: Significant difference from the control group (p< 0.05). P^b: Significant difference from the Aβ₍₁₋₄₂₎ group (p< 0.05).

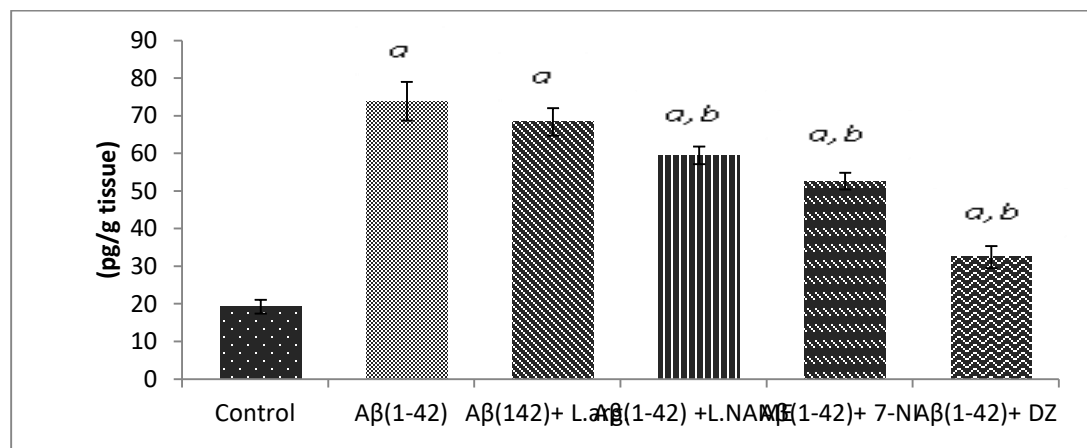


Figure. (3): Brain TNF-α in different studied groups.

P^a : Significant difference from the control group ($p < 0.05$). P^b : Significant difference from the $A\beta_{(1-42)}$ group ($p < 0.05$).

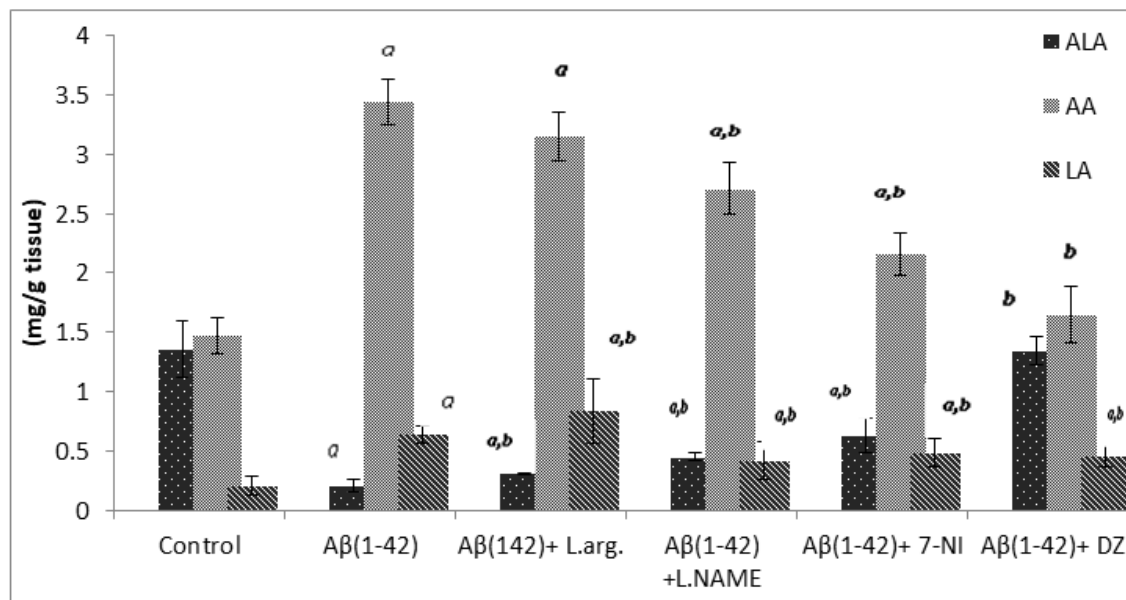


Fig. (4): Brain fatty acids in different studied groups.

P^a : Significant difference from the control group ($p < 0.05$). P^b : Significant difference from the $A\beta_{(1-42)}$ group ($p < 0.05$).

Groups treated with L.arg., L.NAME, 7- NI and DZ decreased ChE activity when compared to A β 1-42 group by -20%, -27%, -30%, and -56% respectively the recorded mean values are represented in fig. (1).

3. Brain IL- 1 β and TNF- α

Brain injury induced by A β (1-42) results in significant increase in IL-1 β and TNF- α levels by +315% and +284% respectively in A β (1-42) group compared to the control group. These levels were significantly decreased by treatment especially treatment by DZ compared to A β (1-42) group. The percent of change of IL-1 β in groups treated by L.arg., L. NAME, 7- NI and DZ were -20%, -45%, -49% and -55% respectively compared to A β 1-42 group. In the same line The percent of change of TNF- α in groups treated by L.arg., L. NAME, 7-NI and DZ were -7%, -19%, -29% and -56% respectively compared to A β (1-42) group. Fig.(2,3) summarize the mean values in different studied groups.

4. Brain fatty acids in different studied groups.

In this study, A β (1-42) induction results in significant increase in brain ALA and AA accompanied by a significant decrease in brain LA. The recorded mean values were 1.36 ± 0.24 and 0.21 ± 0.05 mg/g tissue in control and A β (1-42) groups respectively. A β (1-42) significantly decrease ALA level by 85% when compared with control group. Treated groups with L.arg. , L.NAME, 7-NI and DZ showed significant increase compared to A β (1-42) group with no significant difference between DZ and control. The recorded mean values \pm SE of AA were 1.47 ± 0.15 and 3.44 ± 0.50 mg/g tissue in control and A β (1-42) groups respectively. A β (1-42) significantly increase AA level by 134% when compared with control group. Groups treated with DZ. DZ significantly decreased AA level when compared with A β (1-42) by 52%. Compared with control group there is no significant difference with DZ. Mean values \pm SE of LA were 0.21 ± 0.08 and 0.64 ± 0.07 mg/g tissue in control and A β (1-42) groups respectively. A β (1-42) significantly increase LA level by 205% when compared with control group, treatment with DZ showed a significant decrease in LA level (0.46 ± 0.10 mg/g tissue). Percent of change of LA level compared to the control group was 119%, while treatment with donepezil significantly decreased the per cent of change of LA level by 28% compared to A β (1-42) group fig.(4)

summarized the recorded mean values.

DISCUSSION

The key in the formation and development of the neuropathological process of AD is A β (Qiliang et al., 2016). Also, Boncristiano et al., (2002) reported that Neuronal cell dysfunction and oxidative cell death caused by A β contribute to the pathogenesis of AD.

Acetylcholinesterase (AChE) is responsible for acetylcholine (ACh) metabolism whose alterations caused neurobehavioral changes especially memory, learning and cognitive functions (Platt *et al.*, 2001). Kar *et al.* (2004) and Yamaguchi *et al.* (2006) have shown that A β can lead to cholinergic dysfunction and cognitive impairment. Enhance the levels of ACh in the brain is very important in disease prevention, it could help prevent neuronal degeneration by compensate the loss of cholinergic neuron. AChE is important for maintaining a stable level of ACh in brain (Tian-Xia *et al.*, 2015). These finding were in agreement with our results in which induction of A β (1-42) caused significant increase in ChE activity. In addition, acetylcholinesterase (AChE) has been found to co-localize with A β deposits and promotes the assembly of A β into amyloid fibrils forming A β -AChE complex that is more toxic than amyloid fibrils (Holmquist *et al.*, 2007).

Oxidative stress, which is a key protagonist in AD, also plays a role in the enhancement of AChE activity induced by A β peptide (Melo *et al.*, 2003). In addition, Crockett *et al.* (2008) reported that oxidative stress and inflammation cause deficiency of several major neurotransmitters, including ACh. Since neurons have the lowest level of anti-oxidants in addition to brain's high lipid content, thus it affects by free radicals leading to tissue damage (Kumar *et al.*, 2014).

Brain arachidonic acid (AA) and docosohexanoic acids (DHA) are highly oxidizable polysaturated fatty acids (Hardas *et al.*, 2013) and (Smith *et al.*, 2007). DHA derived from α -linolenic acid (ALA) as well as arachidonic acid (AA) derived from linoleic acid (LA) (Sinclair, 1975).

AA is the most abundant fatty acid in the brain its release by phospholipases A, and then converted it into eicosanoids that participate in neuroinflammation (Vasilache *et al.*, 2015). Among the various enzymes which produced these eicosanoids, cyclooxygenases-1 and -2 (COX-1/COX-2) convert AA into PGH₂. The arachidonic acid metabolite prostaglandin E₂ (PGE₂) is involved in the inflammatory responses

following brain injury, bacterial or viral infections (Matsumura and Kobayashi, 2004) and (Vasilache *et al.*, 2015).

In this study, there was a positive correlation between increasing levels of oxidant and increasing levels of AA and LA in A β ₍₁₋₄₂₎ group, these observations were accompanied by decrease in ALA. These findings were in accordance with Moustafa *et al.*, (2018) and Rapoport, (2008) who reported that AA is overexpressed and released in neurodegenerative disorders such as AD.

Microglia, astrocytes are involved in the inflammatory process in AD. A β can activate microglia which leads to an increase in cell surface expression of major histocompatibility complex II (MHC II) along with increased secretion of the pro-inflammatory cytokines interleukin1 β (IL1 β), interleukin6 (IL6), and tumor necrosis factor α (TNF- α) as well as the chemokines interleukin8 (IL8), macrophage inflammatory protein1 α (MIP1 α), and monocyte chemoattractant protein1 (Mohandas *et al.*, 2009).

A β also induces a phagocytic response in microglia and expression of nitric oxide synthase (NOS) resulting in neuronal damage (Ballotore *et al.*, 2007). Microglia may also play a role in the degradation of A β by the release of insulin degrading enzyme. Astrocytes also cluster at sites of A β deposits and secrete interleukins, prostaglandins, leukotrienes, thromboxanes, coagulation factors, and protease inhibitors. Neurons themselves are able to express significantly higher levels of classical pathway complement and proinflammatory products that trigger inflammatory processes. Further, the complement system, cytokines, chemokines, and acute phase proteins (especially pentraxins) contribute to the inflammatory response in AD. The neuroinflammation as a primary cause or secondary effect in AD (Tuppo & Arias, 2005). These finding were in accordance to our results in which A β ₍₁₋₄₂₎ results in increasing brain levels of IL-1 β and TNF α compared to the control group.

In this study, treatment with L-NAME, 7-NI and DZ showed significant decrease in levels of oxidant and inflammatory markers with increased levels of antioxidant compared to A β ₍₁₋₄₂₎ group with no significant difference between DZ and control.

The NO-synthesizing enzyme (NOS) is present in the mammalian brain in 3 different isoforms: 2 constitutive enzymes (neuronal nNOS and endothelial eNOS) and 1 inducible enzyme (iNOS). All 3 isoforms are aberrantly expressed

during AI intoxication. This gives rise to elevated levels of NO that are apparently involved in neurodegeneration by different mechanisms, including oxidative stress and activation of intracellular signaling mechanisms (Lüth *et al.*, 2001) L-NAME suppressed nitrite production and decreased neuron impairment via N-methyl-D-aspartic acid (NMDA) receptors. Neuropharmacological data indicate that Abeta toxicity is mediated by an excitotoxic cascade involving blockade of astroglial glutamate uptake, sustained activation of NMDA receptors and an overt intracellular Ca²⁺ influx. These changes are associated with increased NOS activity in cortical target areas that may directly lead to the generation of free radicals.

A sustained overproduction of NO via NOS expression may be responsible, at least in part, for some of the neurodegenerative changes caused by stress and support a possible neuroprotective role for NOS inhibitors in this context (Jellinger *et al.*, 2003). In addition 7-NI is a heterocyclic compound, which inhibits nNOS by competing with both L-arginine and tetrahydrobiopterin and has been used extensively as a selective inhibitor of nNOS. There are a number of studies showing the beneficial effect of inhibiting nNOS activity as a means of reducing NO (Vitcheva, *et al.*, 2015). Hashimoto *et al.* (2005) reported that DZ has neuroprotective effects aside from its AChE-inhibiting action through up-regulation of the anti-apoptotic protein Bcl-2, stimulation of nicotinic acetylcholine receptors (nAChRs), and activation of the phosphoinositide 3 kinase (PI3K)/Akt pathway, and inhibition of AChE in the cortex and hippocampus of the brain (Akasofu *et al.*, 2006).

Cell death and changes in neurite morphology were partly reduced when the NO concentration was inhibited by NOS inhibitors (Münch *et al.*, 2003). Our results indicated positive effects of treatment with NOS inhibitors on the development of neurotoxicity.

The present study showed that, DZ attenuates the increased activity of ChE in A β ₍₁₋₄₂₎ intoxicated rats. In addition, DZ significantly decreased brain levels of AA, and LA level while significantly increasing the brain level of ALA and attenuates brain IL-1 β and TNF- α level when compared to A β ₍₁₋₄₂₎ treated rats. These results were in agreement with Medhat *et al.* (2017) who suggested that increasing levels of ALA decrease both production and releasing of pro-inflammatory cytokines such as IL-1 β .

Waetzig *et al.* (2005) suggested that JNK activation might be a primary inducer of tau

pathology and it could be suppressed by DZ. In addition, JNK is an essential mediator of microglial activation, as well as expression and release of pro-inflammatory cytokines, such as IL-1 β , IL-6, MCP-1, and TNF- α from microglia. Over-expressed tau protein mice could induce JNK activation, followed by phosphorylation of tau protein, as well as microglial activation that coincide with over-expression and secretion of pro-inflammatory cytokines. Subsequently, the inflammatory reaction could synergistically aggravate JNK activity, resulting in accelerated tau phosphorylation and increased aggregation, thus DZ regulates JNK activation by inhibiting inflammation.

Administration of AChE inhibitors (AChEIs), or AChE antisense oligonucleotides, significantly attenuates IL-1 β production in the hippocampus and blood, with concomitant reduced AChE activity (Pollak et al., 2005). In addition, AChEIs suppresses TNF production in the blood and spleen following LPS-induced systemic inflammation (Pavlov et al., 2009). Therefore, it was speculated that DZ suppressed tau pathology and neurodegeneration through anti-inflammatory mechanisms.

CONCLUSION

Nitric oxide synthase and cholinesterase inhibitors suppress neurodegeneration and reduces neuroinflammation induced by A β (1-42).

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

Copyrights: © 2018 @ author (s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

Akasofu S., Kimura M., Kosasa T., Ogura H. and Sawada K. (2006) Protective effect of donepezil in primary-cultured rat cortical neurons exposed to N-methyl-d-aspartate (NMDA) toxicity. *Eur. J. Pharmacol.* 530,

215–222.

- Aukrust P., Liabakk N.B., Muller F., Lien E., Espevik T., Froland S.S. (1994): Serum levels of tumor necrosis factor-alpha (TNF alpha) and soluble TNF receptors in human immunodeficiency virus type 1 infection—correlations to clinical, immunologic, and virologic parameters. *Infect J. Dis.*, 1994, 169, 420-424.
- Ballotore C, M-Y Lee V, Trojanowski JQ. (2007): Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature Reviews Neuroscience.* 8:663–72.
- Barland. C.O., Zettersten. E., Brown. B.S., Ye. J., Elias. P.M., and Ghadially. R., (2004); Imiquimod-induced interleukin-1 alpha stimulation improves barrier homeostasis in aged murine epidermis. *J Invest Dermatol;* 122(2):330-6.
- Beal M.F. (2005): Oxidative damage as an early marker of Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging* 26, 585–586.
- Beutler E, Duron O, Kelly BM. (1963): Improved method for the determination of blood glutathione. *J Lab Clin Med.* 61:882–888.
- Bharadwaj. P.R., Dubey. A.K., Masters. C.L., Martins. R.N., and Macreadie. I.G., (2009); A β aggregation and possible implications in Alzheimer's disease pathogenesis *J. Cell. Mol. Med.* Vol 13, No 3, pp. 412-421.
- Boncrisiano. S., Calhoun. M.E., Kelly. P.H., Pfeifer. M., Bondolfi. L., and Stalder. M., (2002); Cholinergic changes in the APP23 transgenic mouse model of cerebral amyloidosis. *J. Neurosci.* 22, 3234–3243.
- Crockett. M.J., Clark. L., Tabibnia. G., Lieberman. M.D., and Robbins. T.W., (2008); Serotonin modulates behavioral reactions to unfairness. *Science*, 320(5884);1739.
- El-khayat. Z., Abo el-matty. D., Rasheed. W., Hussein. J., Shaker. O. and Raafat. J., (2013); Role of cell membrane fatty acids in insulin sensitivity in diabetic rats treated with flaxseed oil. *international journal of pharmacy and pharmaceutical sciences.* 5 (2): 146-151.
- Hardas. S.S., Sultana. R., Clark. A.M., Beckett. T.L., Szewda. L.I., Murphy. M.P., (2013); Butterfield, D.A. Oxidative modification of lipoic acid by HNE in Alzheimer disease brain. *Redox Biol.* 1, 80–85.
- Hashimoto M., Kazui H., Matsumoto K., Nakano Y., Yasuda M. and Mori E. (2005) Does donepezil treatment slow the progression of hippocampal atrophy in patients with

- Alzheimer's disease? *Am. J. Psychiatry* 162, 676–682.
- Holmquist. L., Stuchbury. G., Berbaum. K., Muscat. S., Young. S., Hager. K., and Münch. G. (2007); Lipoic acid as a novel treatment for Alzheimer's disease and related dementias. *Pharmacology & Therapeutics*, 113(1), 154-164.
- Huang. H., Chie-PeinChen. C., MeiChen. C., and MeiHsieh-Li. H. (2007): Intrahippocampal administration of A β 1–42 impairs spatial learning and memory in hyperglycemic mice. *Neurobiology of Learning and Memory*. 87(4):483-494.
- Jellinger KA. General aspects of neurodegeneration. *J Neural Transm Suppl*. 2003;65:101–144.
- Johansson LH and Borg LA (1988): A spectrophotometric method for determination of catalase activity in small tissue samples. *Analytical Biochemistry*;174,33-36.
- Kar. S., and Quirion. R., (2004); Amyloid beta peptides and central cholinergic neurons: functional interrelationship and relevance to Alzheimer's disease pathology. *Prog Brain Res*. 145: 261-274.
- Kumar. V., and Gill. K.D., (2014); Oxidative stress and mitochondrial dysfunction in aluminium neurotoxicity and its amelioration: a review. *Neurotoxicology*. 41: 154-66.
- Lüth HJ, Holzer M, Gärtner U, Staufenbiel M, Arendt T. Expression of endothelial and inducible NOS-isoforms is increased in Alzheimer's disease, in APP23 transgenic mice and after experimental brain lesion in rat: evidence for an induction by amyloid pathology. *Brain Res*. 2001;913:57–67.
- Manna. F., Ahmed. H.H., Estefan. S.F., Sharaf. H.A., and Eskander. E.F., (2005); Saccharomyces cerevisiae intervention for relieving glutamate-induced hepatotoxicity in male rats. *Pharmazie*;60:689-695.
- Matsumura. K., and Kobayashi. S., (2004); Signaling the brain in inflammation: the role of endothelial cells. *Front Biosci* 9:2819–2826.
- Medhat D., El-Bana M.A., Ashour M.N., Badawy E., Diab Y., Hussein J. (2017): New approaches in protecting against atherosclerosis in experimental model of postmenopause; 7 (11), 090-096.
- Melo. J.B., Agostinho, P., and Oliveira. C.R., (2003); Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neuroscience Research*, 45(1), 117- 127.
- Moshage H., Kok B., Huizenga Jr. (1995): Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin. Chem.*, 41, 892-896.
- Moustafa Y.M., Medhat D., Zaitone S.A., El-Khayat Z., Abdel-Salam O.M.E. and Abdalla A.A.M. Role of Donepezil alone and in combination with L-arginine, L-NAME and 7-nitroindazole against AlCl₃ induced neurotoxicity. *Bioscience Research*; 15(2): 883-892.
- Mufson. E.J., Ikonomic. M.D., Counts. S.E., Perez. S.E., Malek-Ahmadi. M., Scheff. S.W., and Ginsberg. S.D., (2016); Molecular and Cellular Pathophysiology of Preclinical Alzheimer's Disease *Behav Brain Res*. September 15; 311: 54–69.
- Murphy M.P. and Le Vine H. (2010): Alzheimer's Disease and the β -Amyloid Peptide. *J Alzheimers Dis*; 19(1): 311. doi:10.3233/JAD-2010-1221.
- Nishikimi M., Roa N.A and Yogi. K. (1972): *Res. Commun.*, 1972, 46:849-854.
- Noh M.I., Koh S.H., Kim Y., Kim H.Y., Cho G.W., Kim S.H. (2009): Neuroprotective effects of donepezil through inhibiting of GSK-3 activity in amyloid β - induced neuronal cell death. *Journal of neurochemistry*, 108; 1116–1125.
- Pavlov VA, Parrish WR, Rosas-Ballina M, Ochani M, Puerta M, Ochani K, Chavan S, Al-Abed Y, Tracey KJ (2009): Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav Immun* 23 , 41-45.
- Platt. B., Fiddler. G., Riedel. G., and Henderson. Z., (2001). Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain research Bulletin*, 55(2), 257-267.
- Pollak Y, Gilboa A, Ben-Menachem O, Ben-Hur T, Soreq H, Yirmiya R (2005): Acetylcholinesterase inhibitors reduce brain and blood interleukin-1 β production. *Ann Neurol* 57, 741-745.
- Qiliang. Zhou., Siping. Nie., and Zhihuo. Liu., (2016); Effect of Donepezil Hydrochloride on Cognitive Function Recovery of Rats with Alzheimer's Disease *MED ONE*,1:e160023.
- Rapoport S. I. (2008); Arachidonic Acid and the Brain *J. Nutr.* 138: 2515–2520.
- Sharma K.V., Wong L.K.S. (2016): *Stroke* (Sixth Edition).
- Sinclair. A.J., (1975); Long chain polyunsaturated fatty acids in the mammalian brain. *Proc*

- NutrSoc* 34, 287–291.
- Smith. D.G., Cappai. R., and Barnham. K.J., (2007); The redox chemistry of the Alzheimer's disease amyloid _ peptide. *Biochim. Biophys. Acta.* 1768, 1976–1990.
- Swerdlow R.H., Burns JM, Khan SM (2014): The Alzheimer's disease mitochondrial cascade hypothesis: Progress and perspectives. *Biochim Biophys Acta* 1842, 1219–1231.
- Taylor. P.C., (2001); Anti-tumor necrosis factor therapies. *Curr Opin Rheumatol*;13(3):164–169.
- Tuppo EE, Arias HR. (2005): The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol.* 37:289–305.
- Uchiyama M, Mihara M. (1978): Determination of malondialdehyde in tissue by thiobarbituric acid test. *Analytical Biochemistry.* 86:271–278.
- Vasilache. A.M., Qian. H., and Blomqvist. A. (2015); Immune challenge by intraperitoneal administration of lipopolysaccharide directs gene expression in distinct blood–brain barrier cells toward enhanced prostaglandin E2 signaling. *Brain Behav Immun*; S0889-1591(15)00021-5.
- Vitcheva V., Simeonova R., Kondeva-Burdina M., and Mitcheva M. (2015): Selective Nitric Oxide Synthase Inhibitor 7-Nitroindazole Protects against Cocaine-Induced Oxidative Stress in Rat Brain. *Oxid Med Cell Longev.* doi: 10.1155/2015/157876.
- Waetzig V, Czeloth K, Hidding U, Mielke K, Kanzow M, Brecht S, Goetz M, Lucius R, Herdegen T, Hanisch UK :(2005) c-Jun N-terminal kinases (JNKs) mediate pro-inflammatory actions of microglia. *Glia* 50 , 235-246.
- Xia T. Y., Zhang P., Guan Y., Wang M., and Zhen M. Q. (2015); Protective effects of luteolin against cognitive impairment induced by infusion of A β peptide in rats. *Int J Clin Exp Pathol.*8(6):6740-6747.
- Yamaguchi. Y., Miyashita. H., Tsunekawa. H., Mouri. A., Kim. H.C., Saito. K., Matsuno. T., Kawashima. S., and Nabeshima. T., (2006); Effects of a novel cognitive enhancer, spiro [imidazo-[1,2-a]pyridine-3,2-indan]-2 (3H)-one (ZSET1446), on learning impairments induced by amyloid-beta1-40 in the rat. *J PharmacolExpTher.* 317: 1079-1087.
- Yoshiyama Y, Kojima A, Ishikawa C and Arai K. (2010): Anti-Inflammatory Action of Donepezil Ameliorates Tau Pathology, Synaptic Loss, and Neurodegeneration in a Tauopathy Mouse Model. *Journal of Alzheimer's Disease* 22 ; 295–306
- Young. D.S., (2000); Effect of drugs on Clinical Lab. Test, 5th Ed. AACCC Press.