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Possible Therapeutic Role of *Jasonia Candicans* and *Jasonia Montana* Extracts in the Regression of Alzheimer's Disease in Experimental Model

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ABSTRACT

The present article aimed to investigate the potential role of the ethanolic extracts of the aerial parts of *Jasonia candicans* and *Jasonia montana* in management of Alzheimer's Disease (AD) in experimental model. Supplementation of drinking water $AlCl_3$ (0.3%) for 16 weeks induced AD in male rats with siginifcant increase in brain Acetylcholinesterase (AchE) activity, Tumour Necrosis Factor (TNF-α), Transforming Growth Factor β (TGF-β) and 8 hydroxydeoxyguanosine (8-OHdG) levels. $AlCl_3$ supplementation produced significant decrease in Brain insulin Like Growth Factor (IGF-1) and Derived Neurotrophic Factor (BDNF) levels as compared to the control values. Also, $AlCl_3$ supplementation caused significant decline in the expression levels of nucleoporin P^{62} (P^{62}) and a disintegrin and metalloproteinase 17 (ADAM 17) genes accompanied with significant elevation in the expression levels of brain cyclooxygenase (Cox-2) gene. Brain histopathological examination of AD-induced rats showed formation of amyloid plaques in hippocampus and cerebrum. Oral administration of each of selected extract (150 mg/kg b.wt) in AD-induced rats daily for 6 weeks resulted in significant decrease in brain AchE activity, TNF-α, TGF-β and 8-OHdG levels. The treatment produced significant increase in brain IGF-1 and BDNF levels as compared to AD–induced rats. The treatment with these extracts could significantly increase the gene expression levels of brain P^{62} and ADAM17 accompanied with significant decrease in the expression levels of Cox-2 gene in the brain. Histopathological examination of brain tissue of the treated rats showed marked improvement in the morphological structure of the brain especially in the hippocampus and cerebrum areas. High content of terpenes, sesquiterpenes and flavonoids in the ethanolic extract of the selected plants may responsible for the anticholinesterase activity, anti-inflammatory action, antioxidant capacity and neurotrophic effect as well as anti-amyloidogenic potential of these extracts. These results suggest that these extracts may effectively ameliorate the inflammation and neurodegeneration characterizing AD. Thus, these extracts may have a therapeutic application in the treatment of Alzheimer's disease.

Keywords: Alzheimer's Disease (AD), Inflammation, Oxidative Stress, Gene Expression, *Jasonia Candicans*, Alzheimer's Disease (AD), Tumour Necrosis Factor (TNF-α)

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1. INTRODUCTION

Alzheimer's Disease (AD) is a heterogeneous progressive age-related neurodegenerative disease of the brain that affects memory, thought, reasoning and language. It is characterized by a progressive memory decline as well as serious cognitive disability due to the progressive dysfunction and death of nerve cells that are responsible for the storage and processing of information (Takeda *et al*., 2008).

In 1970s, it was discovered that the brains of patients with AD were deficient in Acetylcholine (ACh) "cholinergic hypothesis" and sice then the impairment of cholinergic neurotransmission became a well-established fact in AD (Zivin and Pregelj, 2008).

Several mechanisms have been postulated to explain AD pathogenesis. Such mechanisms include Aβ toxicity, cholinergic dysfunction, tau protein hyperphosphorylation, oxidative damage, synaptic dysfunction and inflammation secondary to senile plaques (Bernhardi, 2007).

Oxidative modifications of both nuclear DNA and mitochondrial DNA have been proposed as one biochemical change that could lead to the neuropathology, neuronal dysfunction and death in AD (Polidori *et al*., 2007). Indeed, there are strong indications that oxidative stress occurs prior to the onset of symptoms in AD and oxidative damage is found mainly in the vulnerable regions of the brain affected by this disease (Casadesus *et al*., 2007).

Cerebral inflammation and systemic immunological alterations have been reported in the pathogenesis of AD (Salminen *et al*., 2009). The inflammatory changes include activation of microglia and astrocytes and infiltrating inflammatory cells in the cerebral inflammation with increased levels of proinflammatory cytokines (Galasko and Montine, 2010).

Evidence for the contribution of Al to Alzheimer's Disease (AD) remains contradictory (Gupta *et al*., 2005), however, epidemiological studies have indicated a link between Al in drinking water and AD. Also, variety of human and animal studies has implicated learning and memory deficits after Al exposure (Exley, 2005). Kawahara *et al*. (2001) investigated the changes induced by chronic neurotoxicity of Al and found that long-term exposure to $AlCl_3$ for more than 3 months caused morphological changes involving depositions immunopositive to tau proteins, neurofilaments and β-Amyloid (Aβ) in neurons. This suggests that Al-intoxicated brain neurons exhibit some similarities to the neuropathological hallmarks of AD.

Rivastigmine hydrogen tartrate (S)-N-ethyl-3-[(1-dimethyl amino) ethyl]-N-methyl-phenylcarbamate hydrogen tartrate is AChI of the carbamate type approved for the treatment of AD (Pommier and Frigola, 2003). It is licensed for use in the UK (Foye *et al*., 1995) and US Food and Drug Administration for the symptomatic treatment of mild-to-moderately severe AD (Joshi *et al*., 2010). Rivastigmine is absorbed rapidly and completely after oral administration; reaching peak plasma concentration in about 1 h. Inhibition of AchE in the CSF is maximal at 2.4 h after drug intake in healthy volunteers (Kennedy *et al*., 1999).

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The use of traditional medicine during last decade have expanded globally and are gaining popularity. The genus *Jasonia*, of the family Asteraceae, tribe Iinuleae, subtribe Inulinea is a small genus with about five species and mainly distributed in the Mediterranean region (Bermejo *et al*., 2002). Earlier work on the chemistry of four species, *J. glutinosa*, *J. mberosa*, *J. montana* and *J. candicans*, revealed the presence of several sesquiterpenes and sesquiterpene lactone derivatives (De Pascuai *et al*., 1980) eudesmanoic acids, eudesmanolides, (Ahmed *et al*., 1994) guaianolides and pseudoguaianolides, (Ahmed *et al*., 1993) together with several polymethoxylated flavonoids and some coumarins (Ahmed *et al*., 1994).

Plant sesquiterpenes are known to show diverse biological and pharmacological actions, including anti-inflammatory activity (Recio *et al*., 2000). Polyphenols are abundant in *Jasonia montana* and are used as antioxidants (Al-Howiriny *et al*., 2005).

The current study was constructed to investigate the potent role of *Jasonia candicans* and *Jasonia montana* ethanolic extracts in the regression of the neurodegeneration characteristics of Alzheimer's disease in experimental animal model in an attempt to understand their mechanism of action which may pave the way for possible therapeutic applications.

2. MATERIALS AND METHODS

2.1. Chemicals

- Aluminum Chloride ($AlCl_3$) was purchased from Sigma Co. USA with molecular weight 133.34

- Rivastigmine (Exelon 1.5 mg) was purchased from Novartis Co. Germany

2.2. *Jasonia Extracts Preparation*

The aerial parts of *Jasonia candicans* and *Jasonia montana* were collected from west of Alexandria and hilly areas from El-Arbaeen valley, Saint Catherine, South Sinai, Egypt respectively in November 2009. The taxonomical features of the plants were kindly confirmed by Prof. M.N.El-Hadidi, Prof. of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University. Voucher specimens were kept in the museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Two kgs of each of *Jasonia candicans* and *Jasonia montana* air dried plant materials were separately grounded and were exhaustively extracted by percolation in 70% aqueous ethanol at room temperature. The filterates were concentrated under *vacuo* at 40°C to yield 170 g dried extract from *Jasonia candicans* and 200 g dried extract from *Jasonia montana*. Stock solutions were prepared by dissolving 60 g of each extract in 400 mL water (Soliman *et al*., 2009).

2.3. *Rat AD Model*

Seventy seven adult male *Sprague Dawley* rats (140-160 g), 4 months old were enrolled in the current study. The rats were purchased from the Animal House Breeding Colony of the National Research Centre, Cairo, Egypt. The rats were housed in polypropylene cages in an environmentally controlled clean air room with a temperature of 24±1°C, a 12 h light/ 12 h dark cycle, a relative humidity of 60±5% and free access to tap water and food. Rats were allowed to adapt to these conditions for two weeks before begining the experimental protocol. All animals received human care and use according to the guidelines for Animal Experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt. Alzheimer's Disease (AD) was induced in rats by supplementing the drinking water with Aluminum Chloride ($AlCl_3$) (0.3%) for 16 weeks (Erazi *et al*., 2010). The animals used in the current study were classified into seven groups: (1) Healthy control group (negative control), (2) *Jasonia candicans*-treated group in which the animals were orally administered with *Jasonia candicans* extract in a dose of 150 mg/kg b.wt. (Hussein, 2008) daily for 6 weeks, (3) *Jasonia Montana*-treated group in which the animals were orally administered with *Jasonia montana* extract in a dose of 150 mg/kg b.wt daily for 6 weeks, (4) AD- induced group, (5) AD- induced group treated orally with *Jasonia candicans* extract (150 mg/kg b.wt) daily for 6 weeks , (6) AD- induced group treated orally with *Jasonia montana* extract (150 mg/kg b.wt.) daily for 6 weeks and (7) AD-induced group treated orally with rivastigmine in a dose of 0.3 mg/kg b.wt. (Carageorgious *et al*., 2008) daily for 6 weeks for comparison.

At the end of the experimental period, the animals were fasted overnight and subjected to anaesthesia using diethylether. Then, the animals were rapidly killed and the brain tissues were dissected, thoroughly washed with isotonic saline, dried and then weighed. The whole brain of a certain number of animals in each group was fixed in formaline buffer (10%) for histological examination and the whole brain of the rest number of animals in each group was mid-sagitally divided into two portions. The first portion was immediately stored in liquid nitrogen for gene expression analysis and HPLC analysis of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 2-deoxyguanosine (2-dG). While, the second portion of the brain was weighed and immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl (PH 7.4) and 300 mM sucrose (Tsakiris *et al*., 2004). The homogenate was centrifuged at 1800 xg for 10 min. at 4°C. The supernatant (10%) was used for the determination of the different biochemical analyses.

2.4. Biochemical Analyses

Brain cholinesterase activity was determined kinetically using kit purchased from Biostc Co., Europe, according to the method of Shaw *et al*. (1983). Brain TNF-α level was assayed by Enzyme Linked Immunosorbent Assay (ELISA) technique using kit purchased from AviBion Co., USA, according to the method described by Intiso *et al*. (2004). Brain IGF-1 and TGF-β levels were determined by ELISA technique using kit purchased from DRG instrument GmbH, Germany, according to the methods described by Lewitt *et al*. (1993) and Kropf *et al*. (1997) respectively. Brain BDNF level was detected by ELISA technique using kit purchased from RayBiotech, Inc., USA, according to the method described by Barde (1990). Colorimeteric estimation of total protein level in the brain homogenate was carried out according to the method of Lowry *et al*. (1951) to express the concentration of different brain parameters per mg protein (Karthikeyan *et al*., 2007).

2.5. High-performance Liquid Chromatography

DNA was extracted from rat brain by homogenization in buffer containing 1% sodium dodecyl

sulphate, 10 mM Tris, 1mM EDTA (pH 7.4) and an overnight incubation in 0.5 mg mL^{-1} proteinase K at 55°C. Homogenates were incubated with RNase (0.1 mg mL^{-1}) at 50°C for 10 min and extracted with chloroform/isoamyl alcohol. The extracts were mixed with 3M sodium acetate and two volumes of 100% ethanol to precipitate DNA at -20°C. The samples were washed twice with 70% ethanol, air-dried for 15 min and dissolved in 100 µL of 10mM Tris/1mM EDTA (pH 7.4) (Khalil *et al*., 2011). DNA was then digested and the adduct 8-Hydroxy-2-deoxyguanosine (8-OhdG) was measured with High-Performance Liquid Chromatography (HPLC) equipped with a CoulArray system (Model 5600). Analytes were detected on two coulometric array modules, each containing four electrochemical sensors attached in series, which allows identification targets based on reduction potential. The UV detection was set to 260 nm. The HPLC was controlled and the data acquired and analyzed using CoulArray software. The mobile phase was composed of 50mM sodium acetate 5% methanol at pH 5.2. Electrochemical detector potentials for 8- OHdG and 2-deoxy guanesine (2-dG) were 120/230/280/420/600/750/840/900mV and the flow rate was 1 mL min^{-1}.

2.6. *Quantitative Real Time-Polymerase Chain Reaction*

Brain tissues of rats within each group were used to extract total RNA using TRIzol® Reagent (cat#15596-026, Invitrogen, USA). Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, USA) to digest DNA residues, re-suspended in DEPC-treated water and photospectrometrically quantified at A_{260}. Purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for Reverse Transcription (RT), otherwise stored at -80°C.

The complete $Poly(A)^+$ RNA isolated from rat brain tissue was reverse transcribed into cDNA in a total volume of 20 µL using RevertAid^{TM} First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5 µg) was used with a reaction mixture, termed as Master Mix (MM). The MM consisted of 50 mM $MgCl_2$, 5x reverse transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3), 10 mM of each dNTP, 50 µM oligo-dT primer, 20 U ribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNAse activity) and 50 U M-MuLV reverse transcriptase. The mixture of each sample was centrifuged for 30 sec at 1000 xg and transferred to the thermocycler (Biometra GmbH, Gottingen, Germany). The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C and finished with a denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through Real Time-Polymerase Chain Reaction (RT-PCR).

An iQ5-BIO-RAD Cycler (Cepheid. USA) was used to determine the quail cDNA copy number. PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer, 6.5±l distilled water and 5 µL of cDNA template.

The reaction program consisted of three steps. The first step was at 95.0°C for 3 min. The second step consisted of 40 cycles in which each cycle was divided into three sub-steps: (a) at 95.0°C for 15 sec.; (b) at 55.0°C for 30 sec and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec. up to 95.0°C. At the end of each sqRT-PCR a melting curve analysis was performed at 95.0°C to check for the quality of the used primers. Each experiment included a distilled water control.

The quantitative values of qRT-PCR of p^{62}, full-length coding region, forward 5'- TTG AAT TCG CCA TGG TGA ACA AGC TTT ACA TCG GGA ACC-3'; reverse 5'- TTT ATG TCG ACG GTG TTG GAA GGG CTA CAT T- 3' (Lu *et al*., 2001); Cox-2 (forward: 5′- TGA TCG AAG ACT ACG TGC AAC A -3′, R: 5′- GCG GAT GCC AGT GAT AGA GTG -3′ (Oyama *et al*., 2005); a disintegrin and metalloproteinase 17 *(*ADAM17; forward 5'- CAG CAG CAC TCC ATA AGG AAA -3'; reverse 5'- TTT GTA AAA GCG TTC GGT A -3' (Franzke *et al*., 2009) genes were normalized on the bases of β-actin (β-actin-F: 5′- CCC AGA GCA AGA GAG GTA TC -3′, β-actin-R: 5′- AGA GCA TAG CCC TCG TAG AT -3′) expression.

At the end of each qRT-PCR, a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

First the amplification Efficiency (Ef) was calculated from the slope of the standard curve using the following formulae (BRLI, 2006):

$$Ef = 10^{-1/slope}$$

$$Efficiency\ (\%) = (Ef-1)\times 100$$

The relative quantification of the target to the reference was determined by using the ΔC_T method if E

for the target (p^{62}, Cox-2 and ADAM17) and the reference primers (β-Actin) are the same (BRLI, 2006):

$$Ratio_{(reference/\ target\ gene)} = Ef^{C_T\ (reference)} \_ ^{C_T\ (target)}$$

The amplification Efficiency (E) for p^{62}, Cox-2 and ADAM17 were 2.002 (%E = 100.081), 1.995 (%E = 99.251) and 2.003 (%E = 99.664) respectively. Whereas, the PCR conditions indicated that the slopes of p^{62}, Coc-2 and ADAM17 were -3.318, -3.333 and -3.313, respectively.

Further, to ensure that the PCR efficiency (E = 10-1/s - 1) was similar between the sample and the standard which was close to 2, we analyzed whether the addition of RT products to the reaction mixture for the standard curve which was prepared for purified RNA affected the PCR efficiency.

2.7. Histolopathological Examination

After twenty four hours of brain tissue fixation, washing was done in tap water, then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin stains (Banchroft, 2008) for histopathological examination through the light microscope, Nikon, Japan, with objectives 5x.

2.8. Statistical Analysis

The results were expressed as Mean ± S.E of the mean. Data were Analyzed by one Way Analysis of Variance (ANOVA) and was performed using the Statistical Package for the Social Science (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when P value was <0.05.

3. RESULTS

3.1. Biochemical Results

The data in **Table (1)** represented the effect of *Jasonia candicans* and *Jasonia montana* extracts on brain cholinesterase activity and TNF-α level in AD model induced in male rats by receiving $AlCl_3$ in drinking water for 4 months. Administration of *Jasonia candicans* or *Jasonia montana* extract produced insignificant decrease ($p>0.05$) in brain cholinesterase activity and TNF-α level except *Jasonia candicans* extract which caused significant decrease ($p<0.05$) in brain TNF-α level in comparison with the negative control group. On the other hand, AD-induced group showed significant increase ($p<0.05$) in brain cholinesterase activity and TNF-α level in comparison with the negative control group. While, treatment of AD-induced group with *Jasonia candicans* or *Jasonia montana* extract or rivastigmine produced significant decrease ($p<0.05$) in brain cholinesterase activity and TNF-α level in comparison with the untreated AD-induced group. Meanwhile, in comparison with AD-induced group treated with rivastigmine, treatment of AD-induced group with *Jasonia candicans* or *Jasonia montana* extract caused significant decrease ($p<0.05$) in brain cholinesterase activity. Treatment of AD-induced group with *Jasonia candicans* extract caused significant decrease ($p<0.05$) in brain TNF-α level as compared with that in AD-induced group treated with rivastigmine.

The data in **Table (2)** indicated the effect of *Jasonia candicans* and *Jasonia montana* extracts on brain IGF-1, TGF-β and BDNF levels in AD-induced rats.

The groups of rats administered with *Jasonia candicans* or *Jasonia montana* extract showed insignificant change ($p>0.05$) in brain IGF-1 level in comparison with the negative control group. While, each of the administered extract showed significant decrease ($p<0.05$) in TGF-β level associated with significant increase ($p<0.05$) in BDNF level as compared with the negative control group. On the other side, the AD-induced group showed significant decrease ($p<0.05$) in brain IGF-1 and BDNF levels in concomitant with significant increase ($p<0.05$) in TGF-β level in comparison with the negative control group. While, treatment of AD-induced group with *Jasonia candicans* or *Jasonia montana* extract or rivastigmine produced insignificant increase ($p>0.05$) in brain IGF-1 level and significant increase ($p<0.05$) in BDNF level in comparison with the AD-induced group. In contrast, the plant extracts and even rivastigmine administration in AD-induced group caused significant decrease ($p<0.05$) in TGF-β level in comparison with the untreated AD group.

In comparison with AD-induced group treated with rivastigmine, the treatment of AD-induced group with *Jasonia candicans* or *Jasonia montana* extract caused insignificant change ($p>0.05$) in brain IGF-1 level. While, AD-induced group treated with *Jasonia candicans* extract showed significant decrease ($p<0.05$) in brain TGF-β and insignificant decrease ($p>0.05$) in brain BDNF levels as compared with AD-induced group treated with rivastigmine.

Table 1. Effect of *Jasonia candicans* and *Jasonia montana* extracts on brain cholinesterase activity and TNF-α level in AD-induced rats. Data are represented as Mean ± S.E of 8 determinations /group

Parameters Groups	Cholinestrase (U/mg protein)	TNF-α (pg/mg protein)
Negative control	561±3.2	336±2.7
Jasonia candicans-treated group	560±8.6	309±9.4[a]
Jasonia montana-treated group	552±2.8	325±4.4
AD-induced group	780±8.6[a]	542±7.0[a]
AD + *Jasonia candicans*	666±7.6[bc]	457±5.8[bc]
AD + *Jasonia montana*	672±5.3[bc]	480±4.4[b]
AD + Rivastigmine	692±2.6[b]	484±3.7[b]

a: $p<0.05$ vs negative control; b: $p<0.05$ vs AD group; c: $p<0.05$ vs AD+rivastigmine group

Table 2. Effect of *Jasonia candicans* and *Jasonia montana* extracts on brain IGF-1, TGF-β and BDNF levels in AD-induced rats. Data are represented as Mean ± S.E of 8 determinations/group

Parameters groups	IGF-1 (ng/mg protein)	TGF-β (pg/mg protein)	BDNF (ng/mg protein)
Negative control	12.5±0.8	666±2.6	0.24±0.009
Jasonia candicans-treated group	12.6±0.5	517±4.3[a]	0.28±0.003[a]
Jasonia montana- treated group	11.8±0.4	544±5.5[a]	0.29±0.01[a]
AD-induced group	9.2±0.2[a]	1049±4.5[a]	0.18±0.006[a]
AD + *Jasonia candicans*	9.9±0.1	761±6.2[bc]	0.25±0.007[b]
AD + *Jasonia montana*	9.5±0.3	790±5.1[b]	0.23±0.007[bc]
AD + Rivastigmine	9.6±0.5	789±3.2[b]	0.26±0.007[b]

a: $p<0.05$ vs negative control; b: $p<0.05$ vs AD group; c: $p<0.05$ vs AD+rivastigmine group

The group of AD treated with *Jasonia montana* extract showed insignificant increase ($p>0.05$) in brain TGF-β level associated with significant decrease ($p<0.05$) in brain BDNF level as compared with AD-induced group treated with rivastigmine.

Assessment of 8-OHdG generation in the brain of rats in the different studied groups is summarized in **Fig. 1**. Insignificant change ($p>0.05$) in brain 8-OHdG level was detected in *J. Montana* and *J. candicans*-treated groups as compared with the negative control group. Brain 8-OHdG level in AD-induced rats was significantly higher ($p<0.05$) (12.6±0.4 8-OHdG per 10^5 dG) than that in the negative control group (4.1±0.2 8-OHdG per 10^5 dG). This result indicated that the ratio of 8-OHdG/2-dG generation increased to 3.07-fold following $AlCl_3$ administration (AD-induced) in comparison with the negative control group (**Fig. 1**). In contrary, treatment of AD-induced group with *J. montana* or *J. candicans* decreased the ratio of 8-OHdG/2-dG generation significantly ($p<0.05$) by 1.37 and 1.45-fold respectively in comparison with the untreated AD-induced group. In the same trend, the ratio of 8-OHdG/2-dG generation in AD-induced group was decreased significantly ($p<0.05$) by 1.49-fold following rivastigmine treatment in comparison with the untreated AD- induced group (**Fig. 1**).

3.2. Molecular Genetic Results

3.2.1. Expression of p^{62}, COX-2 and ADAM17 Genes

Treatment with *J. montana* or *J. candicans* resulted in insignificant changes ($p>0.05$) in the expression levels of P^{62}, Cox-2 and ADAM17 genes in the brain of the treated groups as compared with those in the negative control group (**Fig. 2-4**).

Significant decrease in the expression levels of P^{62} and ADAM17 genes in the brain of AD-induced group compared with those in the negative control group (**Fig. 2-4**). Our findings showed that *J. montana* and *J. candicans* or rivastigmine were able to increase the expression levels of P^{62} and ADAM17 genes significantly in the brain of the treated AD-induced groups compared with untrated AD-induced group (**Fig. 2-4**).

On the other hand, the expression level of Cox-2 gene in the brain of rats treated with $AlCl_3$ (AD-induced) was significantly higher than that found in the negative control group (**Fig. 3**). The present results revealed that *J. montana* and *J. candicans* or rivastigmine were able to reduce brain expression level of Cox-2 gene significantly in the treated AD-induced groups compared with untreated AD-induced group (**Fig. 3**).

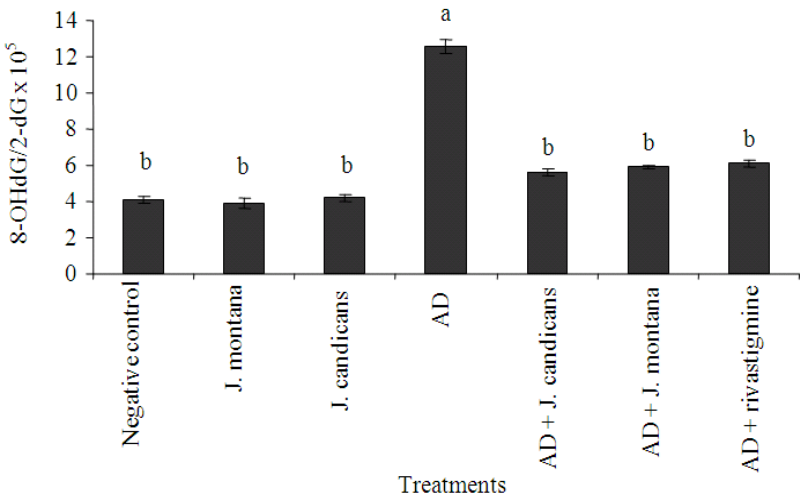

Fig. 1. Generation of 8-OHdG in AD-brain of rats following *J. montana* and *J. candicans* treatment. Brain tissue was harvested, DNA extracted and HPLC performed. DNA damage was expressed as the ratio of oxidized DNA base (8-OHdG) to non-oxidized base (2-dG) in brain DNA. Results are expressed as mean±SE of data from at least eight samples. Mean values with unlike superscript letters were significantly different ($p<0.05$). Mean values with similar superscript letters were not significantly different ($p>0.05$)

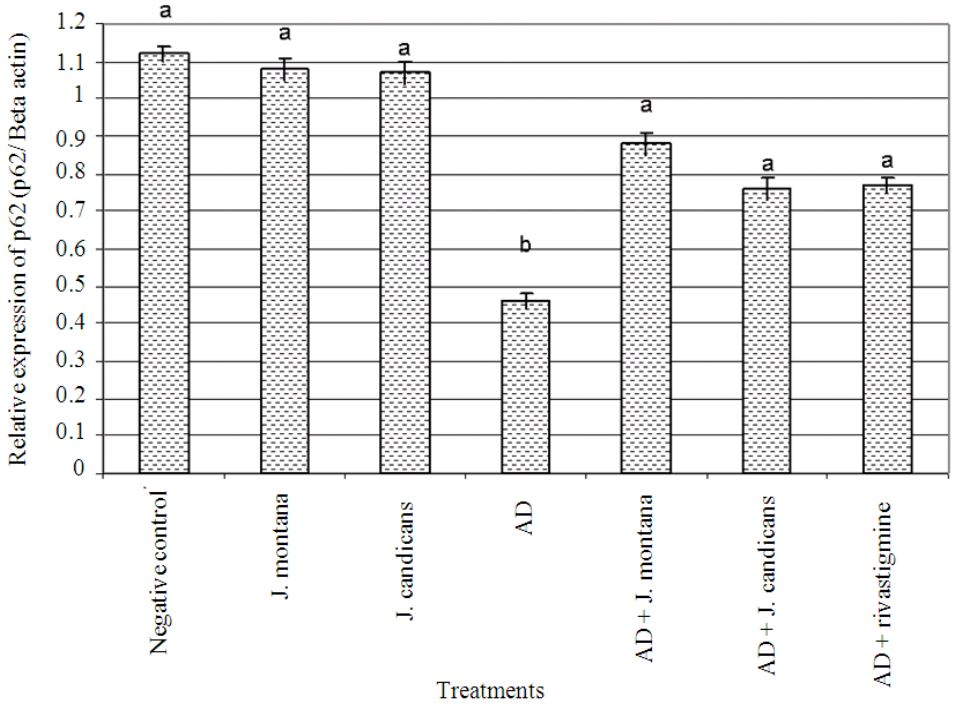

Fig. 2. Semi-quantitative Real Time-PCR analysis of p^{62}-mRNAs in brain tissues collected from male rats (n = 8) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, differ significantly ($p<0.05$).

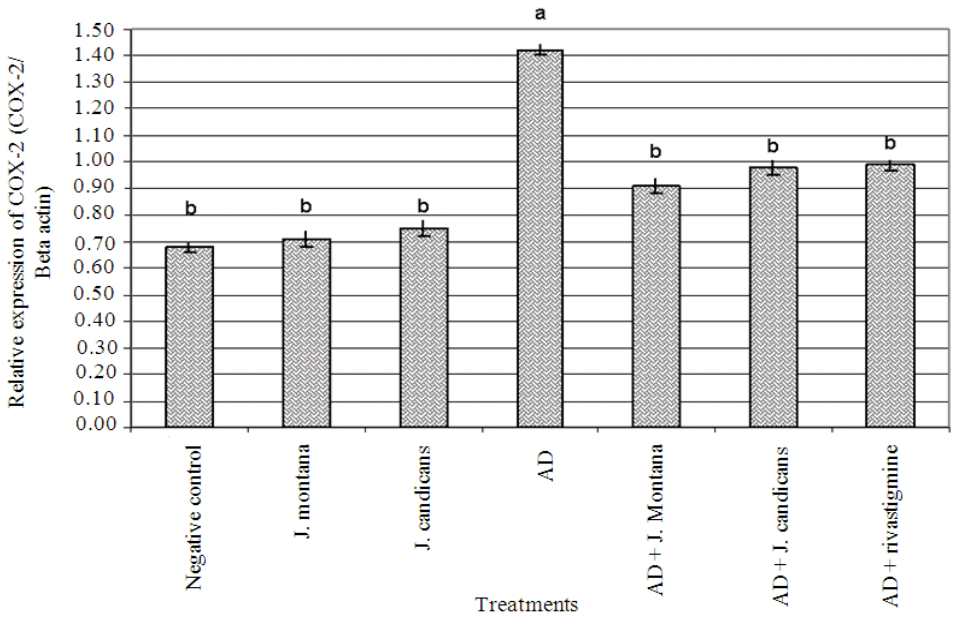

Fig. 3. Semi-quantitative Real Time-PCR analysis of Cyclooxygenase (COX-2)-mRNAs in brain tissues collected from male rats (n = 8) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, differ significantly ($p<0.05$)

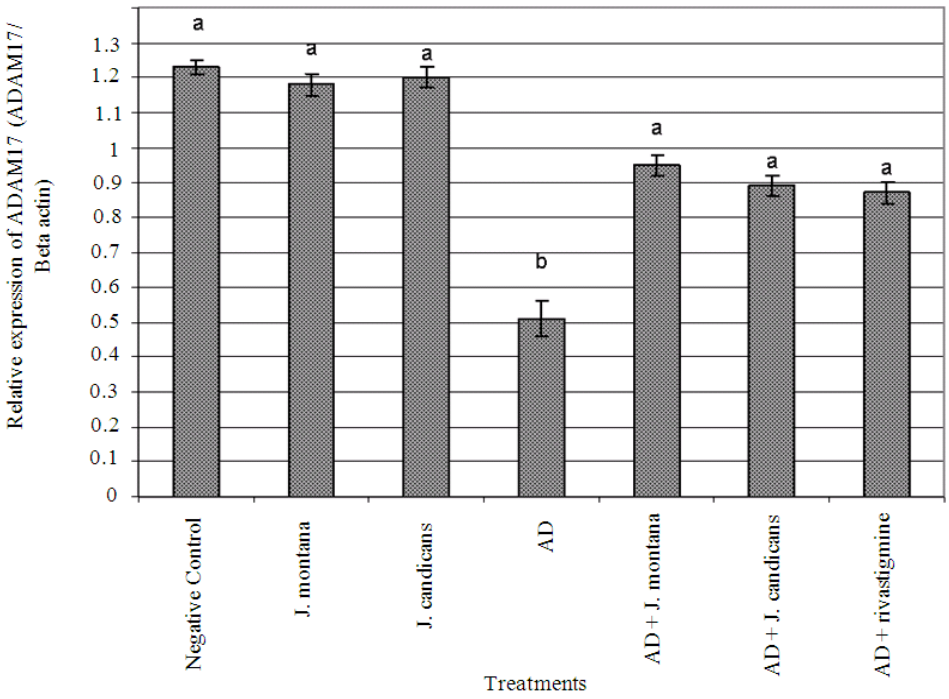

Fig. 4. Semi-quantitative Real Time-PCR analysis of ADAM17-mRNAs in brain tissues collected from male rats (n = 8) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters differ significantly ($p<0.05$)

3.3. *Histopathological* Results

Microscopic examination of brain sections of rats in the negative control group showed intact histological structure of menings, cereberal cortex (**Fig. 5**) hippocampus (**Fig. 6**) and cerebellum (**Fig. 7**).

Histological investigation of brain sections of *Jasonia candicans*-treated group showed perivascular oedema in the cerebrum (**Fig. 8**). Microscopic examination of brain sections of *Jasonia montana*-treated group showed no recorded histpathological findings in the cerebrum and hippocampus (**Fig. 9**).

Microscopic investigation of brain sections of AD-induced group revealed amyloid plaque formation with diffuse gliosis in the hippocampus and cerebrum (**Fig. 10 and 11**).

Histological examination of brain sections of AD-induced group treated with *Jasonia candicans* showed the presence of some focal gliosis associated with small sized plaques in the cerebrum as compared with AD-induced group (**Fig. 12**).

Microscopic investigation of brain sections of AD-induced group treated with *Jasonia montana* revealed congestion in the cerebral blood vessels associated with diffuse gliosis (**Fig. 13**).

Histological investigation of brain sections of AD-induced group treated with rivastigmine showed diffuse gliosis in the cerebrum (**Fig. 14**).

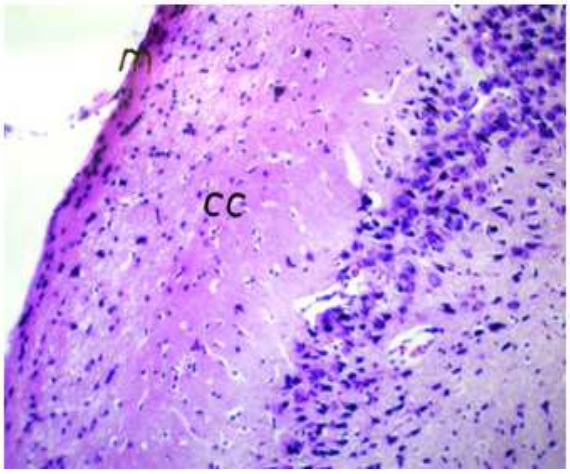

Fig. 5. Microscopic examination of brain sections of rats in the negative control group showed intact histological structure of menings (m), cereberal cortex (cc) (H&E 80)

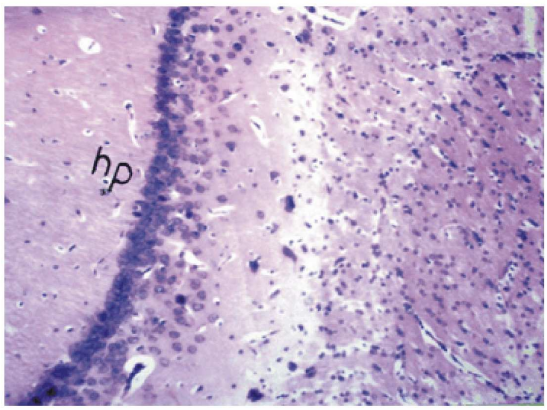

Fig. 6. Microscopic examination of brain sections of rats in the negative control group showed intact histological structure of hippocampus (hp) (H&E 80)

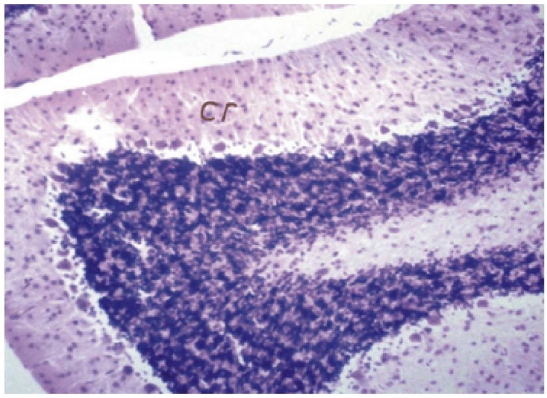

Fig. 7. Microscopic examination of brain sections of rats in the negative control group showed intact histological structure of cerebellum (cr) (H&E 80)

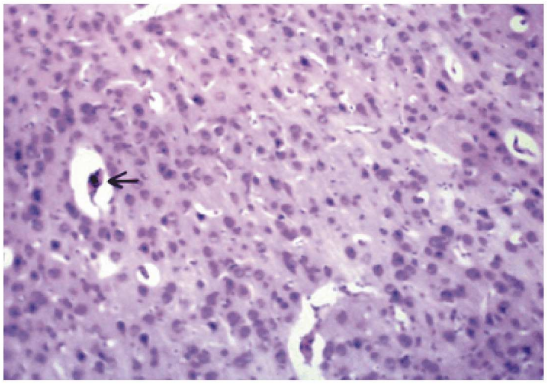

Fig. 8. Microscopic examination of brain sections of *Jasonia candicans*-treated group showed perivascular oedema (arrow) in the cerebrum. (H&E 40)

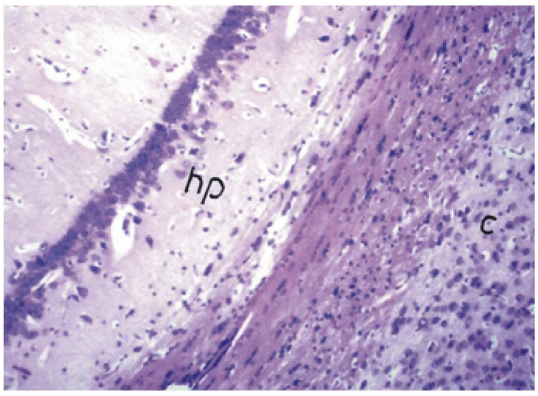

Fig. 9. Microscopic examination of brain sections of *Jasonia montana*-treated group showed no recorded histopathological findings in the cerebrum (c) and hippocampus (hp) (H&E 40)

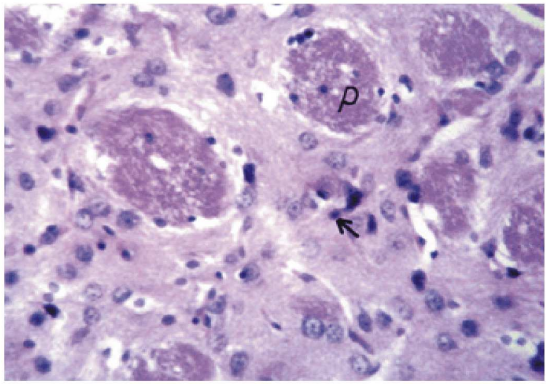

Fig. 10. Microscopic investigation of brain sections of AD-induced group revealed amyloid plaque formation with diffuse gliosis in the hippocampus and cerebrum (H&E 40)

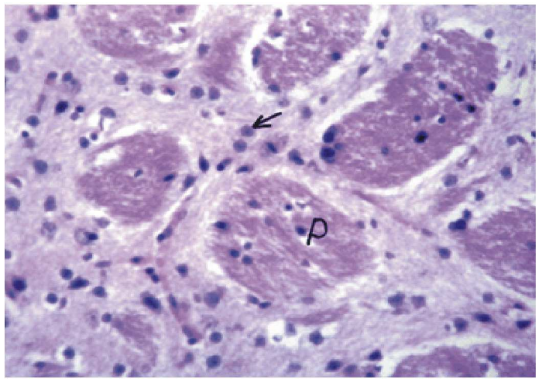

Fig. 11. Microscopic investigation of brain sections of AD-induced group revealed amyloid plaque formation with diffuse gliosis in the hippocampus and cerebrum (H&E 40)

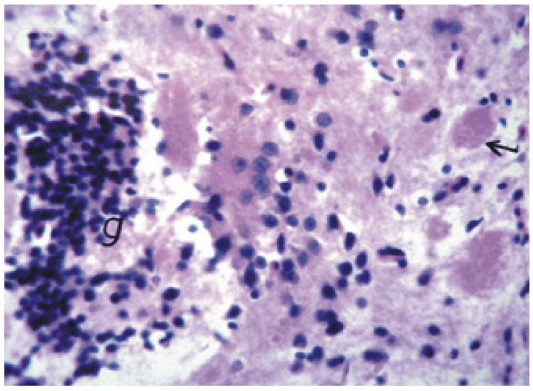

Fig. 12. Microscopic investigation of brain sections of AD-induced group treated with *Jasonia candicans* showed the presence of some focal gliosis associated with small sized plaques in the cerebrum (H&E 80)

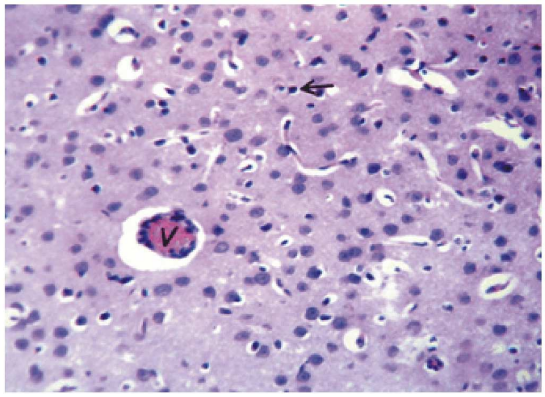

Fig. 13. Microscopic investigation of brain sections of AD-induced group treated with *Jasonia montana* revealed congestion in the cerebral blood vessels associated with diffuse gliosis (H&E 64)

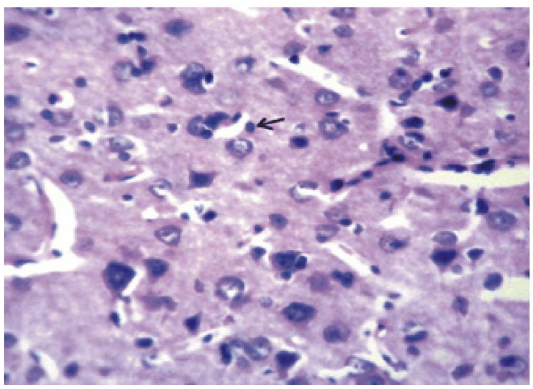

Fig. 14. Microscopic investigation of brain sections of AD-induced group treated with rivastigmine showed diffuse gliosis in the cerebrum (H&E 80)

4. DISCUSSION

The data in the current study showed that there is significant increase in brain AchE activity in AD model. This finding is in agreement with that of Kaizer *et al*. (2008) and Kumar *et al*. (2009a). The suggested mechanism for Al-induced promotion of AchE activity in the brain depends on the neurotoxic effects of Al-induced promotion and accumulation of insoluble Aβ protein (Nayak, 2002). This finding is well documented by our histological results which showed the formation of plaques in the hippocampus and cerebrum of Al administered rats (**Fig. 10 and 11**). Aβ-induced elevation in AchE activity through induction of lipid peroxidation in neuronal membranes due to the production of hydrogen peroxide (H_2O_2) (Melo *et al*., 2003). H_2O_2 may have a direct action on the enzyme as it is well to bear in mind that there are several aspects of H_2O_2 action: as a factor of damage inducing oxidative stress and as modulator (may be allosteric) of the activity of functionally important proteins, receptors and enzymes (Kamsler and Segal, 2004).

Treatment of AD-induced group with either *Jasonia candicans* or *Jasonia montana* ethanolic extract resulted in significant inhibition on brain AchE activity. This finding could be attributed to 1,8-cineole component in these extracts (Hammerschmidt *et al*., 1993) which showed actylcholinesterase inhibiting activity suggesting its utility in the clinical treatment Of AD (Perry *et al*., 2000). Moreover, flavonoids ingredients in these extract (Ahmed and Mahmoud, 1998) that exhibit anti-lipidperoxidative effect (Hussein, 2008) may participate in amelioration of H_2O_2 action on the activity of AchE.

Treatment of AD-induced group with rivastigmine produced significant decrease in brain AchE activity. This result is in agreement with Liang and Tang (2004). Rivastigmine is a carbamate derivative pseudo-irreversible cholinesterase inhibitor which can inhibit AChE in the cortex and hippocampus (Auriacombe *et al*., 2002). Rivastigimine exerts its inhibtory effects on AchE activity by interacting with the esteratic site in AchE molecules (Bar-On *et al*., 2002).

In the current study, $AlCl_3$ supplementation significantly increased Tumor Necrosis Factor-α (TNF-α) brain level. This result concides with that of Campbell *et al*. (2004). Lukiw *et al*. (2005) attributed the elevation of this pro-inflammatory cytokine to the induction of inflammatory genes by Al. Furthermore, Aβ deposition due to Al-intoxication has been found to be Aβ plaques are surrounded by activated astrocytes and microglia which contribute to Al neurotoxicity through the induction of inflammatory cytokines such as IL-1β and TNF-α (Thatcher *et al*., 2005).

The reducing influence of both *Jasonia candicans* and *Jasonia montana* extracts on brain TNF-α in the present work could be attributed to the flavonoids content in these extracts. These compounds showed an inhibitory effect on serum TNF-α level in iron overload treated rats (Hussein and Farghaly, 2010). Also, Hussein and Abdel-Gawad (2010) reported that the flavonoids (or bioflavonoids) have the ability to suppress many types of transcription factors including TNF-α and H_2O_2 as these natural compounds possess anti-inflammatory and antioxidant activities (Carlo *et al*., 1991; Rathee *et al*., 2006).

The findings of the current study revealed that the treatment of AD-induced group with rivastigmine resulted in significant depletion in TNF-α brain level. This result is in agreement with Nizri *et al*. (2008). Rivastigmine reduced demyelination, microglia activation and axonal damage. It could decrease the reactivity of encephalitogenic T-cells and the production of pro-inflammatory cytokines (TNF-α, IFN-γ and IL-17) (Park *et al*., 2005; Steinman, 2007).

The present data indicated that brain level of IGF-1 was significantly decreased in AD-induced group. Carro and Torres-Aleman (2004) stated that serum IGF-I level is altered in AD patients and probably in close association to this change, cell sensitivity towards IGF-I is decreased in these patients. Carro *et al*. (2002) demonstrated that serum IGF-1 levels correlate strikingly with cerebral levels of Aβ in rats and mice. These authors provided evidence for the effect of IGF-1 on Aβ clearance which is mediated by enhancing the transport of Aβ carrier proteins albumin and transthyretin into the brain through the choroid plexus to transport Aβ out of the brain. TNF-α which is involoved in neurodegeneration associated brain impairment (Pratico and Delanty, 2000) has been found to block the effect of IGF-1 on Aβ clearance and the decreased IGF-1 levels could elevate brain Aβ burden and indirectly increase tau pathology in AD (Gasparini and Xu, 2003). Therefore, we could propose that the induction Aβ accumaltion and the stimulation of TNF-α production due to $AlCl_3$ supplementation contributed in the decreasing IGF-1 brain level in the present study.

The stimulation of inflammatory cascade with the consequent production of pro-inflammatory cytokines including TNF-α contributes in the decreasing brain IGF-1 level. Therefore, the above mentioned anti-inflammatory effect with consequent inhibition of brain TNF-α due to treatment of AD-induced rats with Jasonia extracts in the current study seem to play an important

role in elevating brain level of IGF-1 in the treated rats as shown in our results. The elevated brain level of IGF-1 helps in Aβ clearance out of the brain (Carro *et al*., 2002) as indicated in our histological results in this concern (**Fig. 12 and 13**).

The results of the present study showed that the treatment with of AD-induced rats with rivastigmine led to insignificant elevation in brain IGF-1 level. It has been demonstrated that oral administration of rivastigmine reversed considerably the age-related down-regulation of the GH/IGF-1 axis in the elderly male (Obermayr *et al*., 2005). This effect rivastigmine is clearly evidenced by our histological results which showed the disappearance of amyloid plaques from the brain (**Fig. 14**) that may be due to the up-regulation of IGF-1.

Treatment of healthy rats with *Jasonia candicans* or *Jasonia montana* extract showed significant decrease in the brain TGF-β level associated with significant increase in BDNF level as compared to negative control rats as shown in **Table (2)** in the present study. These effects could be attributed to the anti-inflammatory influence of the bioactive constituents of Jasonia extracts (sesquiterpenes and/or flavonoids) (Carlo *et al*., 1991; Sobota *et al*., 2000).

The AD-induced rats in the current study showed significant increase in TGF-β brain level. Some studies reported an increased TGF-β1 level in the brain and cerebrospinal fluid and reduced TGF-β1 level in the serum of AD patients (Wyss-Coray *et al*., 1997; Zetterberg *et al*., 2004; Mocali *et al*., 2004). Although miRNAs and the TGF-β signaling pathway are involved in the pathogenesis of AD, it remains unclear whether miRNAs implicated in the AD pathogenesis by regulating TGF-β signaling pathway (Wang *et al*., 2010). As mentioned above, Al induces Aβ deposition in the brain and the experimental studies on microglial cells stimulated by 1-42 peptide of Aβ showed high transcription level of the pro-inflammatory cytokine genes such as TNF-α, MCP-1 (Walzer *et al*., 2001; Wang *et al*., 2003).

Sesquiterpenes and/ or flavonoids present in *Jasonia candicans* and *Jasonia montana* extracts may be responsible for the anti-inflammatory property of these extracts (Carlo *et al*., 1991; Sobota *et al*., 2000). These bioactive compounds could inhibit the production of pro-inflammatory cytokines including TGF-β and other cytokines. This is the possible mechanism by which both Jasonia extracts could decrease brain TGF-β level in AD- induced rats as detected in our work.

Treatment of AD-induced rats with rivastigmine produced significant decrease in TGF-β brain level. Within the CNS, TGF-β is produced by both glial and neuronal cells (Pratt and McPherson, 1998). The production of TGF- β is regulated by other cytokines including TNF-α (Sjogren *et al*., 2004). Therefore, the inhibiting effect of rivastigimine on TNF-α brain level as shown in the present study may be contributed in the depeletion of TGF-β brain level in rivatigmine treated group.

The data in the present study revealed that the brain level of Brain Derived Neurotrophic Factor (BDNF) is significantly reduced in AD-induced rats. It has been proposed that there is a cytokine/neurotrophin balance in the brain and the disruption in this balance leads to detrimental change in CNS (Aloe *et al*., 1999). Aluminum has been found to cause a shift in this balance towards increased pro-inflammatory cytokine production. In addition to the increase in Tumor Necrosis Factor-α (TNF-α) and Macrophage Inflammatory Protein-1α (MIP-1α), a dramatic decrease in Nerve Growth Factor (NGF) and BDNF was seen after Al intoxication (Viviani *et al*., 2000). Another possible mechanism for Al-induced BDNF decrement in the brain is related to formation of Aβ. It has been demonstrated that subtoxic concentration of Aβ reduces BDNF content and function (Cotman, 2005). Furthermore, Aβ oligomers impair BDNF retrograde transport, so that Aβ reduces BDNF signaling by impairing axonal transport and this may underlie the synaptic dysfunction observed in AD patients (Poon *et al*., 2011).

The current finding revealed that the treatment of AD-induced rats with either *Jasonia candicans* or *Jasonia montana* ethanolic extract produced significant elevation in brain BDNF level. This result could be attributed to the anti-inflammatory influence of the bioactive constituents of Jasonia extracts (sesquiterpenes and/or flavonoids) as mentioned before. Previous studies have demonstrated a reciprocal relationship between pro-inflammatory cytokines and neutrotrophic factors in the CNS (Nagatsu and Sawada, 2005). Therefore, the inhibition of inflammatory cascade and in turn the production of pro-inflammatory cytokines due to the treatment with Jasonia extracts leads to stimulation of neurotrophic factors release and consequently BDNF production as demonstrated in the present work. Moreover, the effect of 1,8-cineole present in Jasonia extracts which acts as AchE inhibitor contributes in the elevating brain BDNF level in AD-induced rats treated with Jasonia. The inhibition of AchE leads to restoring the level of Ach to upregulate the expression of neurotrophic factors including BDNF in the brain (Craig *et al*., 2011).

Rivastigmine produced significant increase in brain BDNF level in AD-treated group. Rivastigmine supplementation increased the concentration of acetylcholine and inhibited acetylcholineesterase activity (Liang and Tang 2004) allowing Acetylcholine (Ach) to do its function. Acetylcholine modulates the expression of neurotrophic factors, such as Brain Derived Neurotrophic Factor (BDNF) (Craig *et al*., 2011).

Numerous studies revealed that, 8-hydroxy 2-deoxy-guanosine (8-OHdG) levels were increased in mice and human tissues of several neurodegenerative diseases (Chen *et al*., 2007). An increased production of superoxide radicals inside the mitochondria may oxidize DNA resulting in the formation of 8-OHdG that is the most commonly analyzed biomarker of DNA damage and the predominant adduct among oxidized bases (Dizdaroglu *et al*., 2000). Al has been shown to induce oxidative stress *via* production of ROS and this considered as one of the important mechanisms indicating that aluminum has an association with the etiology of Alzheimer's disease (Kumar *et al*., 2009b). Our results are in consistent with the previous findings, where 8-OHdG generation was significantly higher in rat administered $AlCl_3$ than that in the control one (Kumar *et al*., 2009b).

The observed significant reduction in 8-OhdG generation in the brain of AD-induced rats treated with either *Jasonia candicans* or *Jasonia montana* extract could be attributed to the flavonoid ingredients in these extracts which have the ability to supress the production of ROS (Hussein and Abdel-Gawad, 2010) that induce 8-OHdG generation.

Rivastigmine protects behavioral changes, restores antioxidant defense enzyme in brain and improves mitochondrial enzyme activity (Kumar and Kumar, 2009). Rivastigmine might act through the glutameric mechanism to decrease the oxidative stress and to restore the capacity of the antioxidant defense system (Shah and Reichman, 2006; Porsteinsson *et al*., 2008). Thus, it could reduce the level of 8-OHdG in the brain as shown in the present study.

It has been reported that p^{62} activates the Antioxidant Response Element (ARE) and protects cells from oxidative stress (Liu *et al*., 2007). It can stimulate NF-E2-related Factor 2 (Nrf2) nuclear translocation to activate the expression of many antioxidant enzymes. Also it has been found that the decreased p^{62} expression is correlated with increased oxidative damage to p^{62} promoter in AD brain (Du *et al*., 2009). In agreement with this finding, our results revealed significant decrease in the expression levels of p^{62} gene in $AlCl_3$ administered rats. Al has been shown to cause impairment of mitochondrial functions *in vitro* (Niu *et al*., 2007) and *in vivo* (Kumar *et al*., 2008). Al accelerates mitochondrial DNA (mt DNA) damage *via* decreasing glutathione (GSH) content and mitochondrial superoxide dismutase (Mn SOD) activity in different brain regions (Kumar *et al*., 2009b).

Treatment of AD- induced rats with either *J. montana* or *Jasonia candicans* resulted in significant increase in the expression level of p^{62} gene in the brain. This means that p^{62} plays a central role in the regulation of neurodegeneration *via* its ability to activate survival signaling (Moscat *et al*., 2007). The active consituent of *Jasonia candicans* and *Jasonia montana* represented by flavonoids possess a powerful antioxidant capacity (Hussein and Abdel-Gawad, 2010) to reduce the oxidative damage in the brain caused by Al. Thus, the treatment of AD- induced rats by these extracts could restore the expression level of p^{62} gene in the brain due to the decreased oxidative stress.

Rivastigmine treatment in AD-induced rats produced significant elevation in the expression level of p^{62} gene in the brain. This finding also indicates the antioxidant activity of rivastigmine as reported in the previous study of Kumar and Kumar (2009). This suggests that the elevation of p^{62} levels in brain might serve as a potential therapeutic target for treatment of various neurodegenerative diseases.

The current results revealed a significant increase in the expression levels of Cox-2 gene in the brain of rats supplemented with $AlCl_3$. In consistent with our observations, it has been reported that in AD brains, the expression of Cox-2 mRNA and protein were elevated (Yasojima *et al*., 1999). Cox-2 expression increased in various neuropathological conditions accompanied by inflammatory reaction, such as stroke and AD (Tomimoto *et al*., 2000; Salminen *et al*., 2009). Cox-2 up-regulation is thought to mediate neuronal damage presumably by producing excessive amounts of harmful prostanoids and free radicals. Al has been found to stimulate the inflammatory cascade and increased the level of Cox-2 enzyme level in the brain (Hoozemans *et al*., 2008). This is another further confirmation for the implication of Al in AD pathgogensis.

The treatment of AD-induced rats with the extract of *Jasonia candicans* or *Jasonia montana* led to significant reduction in the expreassion level of Cox-2 gene in the brain. This result could be attributed to the anti-inflammatory effect of the active principals in these extracts particularly sesquiterpenes (Bermejo *et al*., 2002) which

could block the inflammatory process and in turn reduce the Cox-2 expression as well as activity in the brain.

Rivastigmine treatment in AD-induced rats revealed significant reduction in Cox-2 gene expression in the current study. Rivastigmine has been shown to have anti-inflammatory activity (Nizri *et al*., 2007). This property of rivastigmine enables it to stop the inflammatory reactions and consequently inhibit the activity of Cox-2 enzyme activity as well as its expression in the brain.

Alzheimer's Disease (AD) is characterized by excessive deposition of Amyloid β-peptides (Aβ peptides) in the brain. In the non-amyloidogenic pathway, the Amyloid Precursor Protein (APP) is cleaved by the α-secretase within the Aβ peptide sequence. Proteinases of the ADAM family are the main candidates as physiologically relevant α-secretases (Postina *et al*., 2004). In the present study, significant decrease in gene expression level of ADAM17 was detected in $AlCl_3$ administered rats which further confirmed the implication of Al in the pathogenesis of AD. This result indicates that Al could promote the amyloidogenic pathway in the brain and this explains Aβ deposition (amyloid plaques) due to $AlCl_3$ supplementation as shown in our histological results (**Fig. 10 and 11**).

The ability of *Jasonia candicans* and *Jasonia montana* to significantly increase gene expression levels of ADAM17 in the brain of AD-treated rats indicates that the active ingredients in these extracts could reverse the amyloidogenic pathway to the non-amyloidogenic one *via* motivating the activity of α- secretase like action. This explaination was further confirmed by our histological results in **Fig. 12 and 13** which showed a marked reduction in Aβ deposition in the brain of AD-induced rats treated with either one of Jasonia extract.

Treatment of AD-induced rats with rivastigmine resulted in significant elevation in the gene expression level of ADAM17 in the brain indicating the effectiveness of rivastigmine to stimulate α- secretase like action. This finding shedlights for the first time on the activity of rivastigmine as anti-amyloidogenic drug beside its well established action as anti-cholinesterase agent. This finding was documented by our histological data as shown in **Fig. 14**.

5. CONCLUSION

In conclusion, the potent effects of *Jasonia candicans* and *Jasonia montana* extracts in management of AD have not been reported earlier to our knowledge and this study is perhaps the first observation in this concern. Jasonia extracts displayed this potent influence due to their anti-cholinesterase activity, anti-inflammatory action, antioxidant capacity in addition to anti-amyloidogenic potential and neurotrophic effect. Therefore, the traditional plants may represent new sources for treatment of AD with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine.

6. REFERENCES

Ahmed, A.A. and A.A. Mahmoud, 1998. Jasonol, a rare tricyclic eudesmane sesquiterpene and six other new sesquiterpenoids from *jasonia candicans*. Tetrahedron, 54: 8141-8152. DOI: 10.1016/S0040-4020(98)00453-0

Ahmed, A.A., A.A. Mahmoud, H.J. Williams, A.I. Scott and J.H. Reibenspies *et al*., 1993. New sesquiterpene alpha-methylene lactones from the Egyptian plant *Jasonia candicans*. J. Nat. Prod., 56: 1276-1280. PMID: 8229012

Ahmed, A.A., A.A. Mahmoud, T. Tanaka and M. Iinuma, 1994. Two methylated flavonols from *jasonia candicans*. Phytochemistry, 35: 241-243. DOI: 10.1016/S0031-9422(00)90542-9

Al-Howiriny, T.A., A.J. Al-Rehaily, J.R. Pols, J.R. Porter and J.S. Mossa *et al*., 2005. Three new diterpenes and the biological activity of different extracts of *jasonia montana*. Nat. Prod. Res., 19: 253-265. PMID: 15702639

Aloe, L., M. Fiore, L. Probert, P. Turrini and P.Tirassa, 1999. Overexpression of tumour necrosis factor alpha in the brain of transgenic mice differentially alters nerve growth factor levels and choline acetyltransferase activity. Cytokine, 11: 45-54. PMID: 10080878

Auriacombe, S., J.J. Pere, Y. Loria-Kanza and B. Vellas, 2002. Efficacy and safety of rivastigmine in patients with Alzheimer's disease who failed to benefit from treatment with donepezil. Curr. Med. Res. Opin., 18: 129-38. PMID: 12094822

Banchroft, J.D., 2008. Theory and Practice of Histological Techniques. 6nd Edn., Elsevier Health Sciences, Edinburgh, ISBN-10: 0443102791, pp: 725.

Barde, Y.A., 1990. The nerve growth factor family. Prog. Growth Factor Res., 2: 237-248. PMID: 2133291

Bar-On, P., C.B. Millard, M. Harel, H. Dvir and A. Enz *et al*., 2002. Kinetic and structural studies on the interaction of cholinesterases with the anti-Alzheimer drug rivastigmine. Biochemistry, 41: 3555-3564. PMID: 11888271

Bermejo, B.P, M.J. Abad, A.M. Diaz, L. Villaescusa and M.A. Gonzalez *et al*., 2002. Sesquiterpenes from Jasonia glutinosa: In vitro anti-inflammatory activity. Biol. Pharm. Bull., 25: 1-4. PMID: 11824534

Bernhardi, R.V., 2007. Glial cell dysregulation: A new perspective on Alzheimer disease. Neurotox Res., 12: 215-232. PMID: 18201950

BRLI, 2006. Real-Time PCR applications guide. Bio-Rad Laboratories, Inc.,

Campbell, A., A. Becaria, D.K. Lahiri, K. Sharman and S.C. Bondy, 2004. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. J. Neurosci. Res., 75: 565-572. PMID: 14743440

Carageorgious, H., A.C. Sideris, I. Messari, C.I. Liakou and S. Tsakiris, 2008. The effects of rivastigmine plus selegiline on brain acetylcholinesterase, (Na^+, K^+), Mg^{2+}-ATPase activities, antioxidant status and learning performance of aged rats. Neuropsychiatric Dis. Treat., 4: 687-699. PMCID: PMC2536534

Carlo, G., N. Mascolo, A. Izzo and F. Capasso, 1991. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. Life Sci., 65: 337-353.DOI: 10.1016/S0024-3205(99)00120-4,

Carro, E. and I. Torres-Aleman, 2004. Insulin-like growth factor I and alzheimer's disease: Therapeutic prospects? Expert Rev. Neurother, 4: 79-86. PMID: 15853618

Carro, E., J.L. Trejo, T. Gomez-Isla, D. LeRoith and I. Torres-Aleman, 2002. Serum insulin-like growth factor I regulates brain amyloid-beta levels. Nat. Med., 8: 1390-1397. PMID: 12415260

Casadesus, G., M.A. Smith, S. Basu, J. Hua and D.E. Capobianco *et al*., 2007. Increased isoprostane and prostaglandin are prominent in neurons in Alzheimer disease. Mol. Neurodegener, 22: 2-9. PMID: 17241462

Chen, C.M., Y.R. Wu, M.L. Cheng, J.L. Liu and Y.M. Lee *et al*., 2007. Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. Biochem. Biophys. Res. Commun., 359: 335-340. PMID: 17543886

Cotman, C.W., 2005. The role of neurotrophins in brain aging: A perspective in honor of regino perez-polo. Neurochem. Res., 30: 877-881. PMID: 16187222

Craig, L.A., N.S. Hong and R.J. McDonald, 2011. Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neurosci. Biobehav. Rev., 35: 1397-1409. PMID: 21392524

De Pascuai, T.J.D., A.F. Barrero, A.S. Feliciano, M. Grand and M. Medarde, 1980. Phytochemistry, 19: 2155-2157. DOI: 10.1016/S0031-9422(00)82213-X

Dizdaroglu, M., P. Jaruga, M. Birinciogev and H. Rodriguez, 2000. Free radical-induced damage to DNA: Mechanisms and measurement. Free Radic. Biol. Med., 32: 1102-1115. PMID: 12031895

Du, Y., M.C. Wooten, M. Gearing and M.W. Wooten, 2009. Age-associated oxidative damage to the p62 promoter: Implications for alzheimer disease. Free Radic Biol. Med., 46: 492-501. PMID: 19071211

Erazi, H., W. Sansar, S. Ahboucha and H. Gamrani, 2010. Aluminum affects glial system and behavior of rats. C R Biol., 333: 23-7. PMID: 20176332

Exley, C., 2005. The aluminium-amyloid cascade hypothesis and Alzheimer's disease. Subcell Biochem., 38: 225-234. PMID: 15709481

Foye, W.O., T.L. Lemke and D.A. Williams, 1995. Principles of Medicinal Chemistry. 4th Edn., Williams and Wilkins, Baltimore, ISBN-10: 0683033239, pp: 995.

Franzke, C.W., L. Bruckner-Tuderman and C.P. Blobel, 2009. Shedding of collagen XVII/BP180 in skin depends on both ADAM10 and ADAM9. J. Biol. Chem., 284: 23386-23396. PMID: 19574220

Galasko, D. and T.J. Montine, 2010. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. Biomark Med., 4: 27-36. PMID: 20383271

Gasparini, L. and H. Xu, 2003. Potential roles of insulin and IGF-1 in Alzheimer's disease. Trends Neurosci., 26: 404-406. PMID: 12900169

Gupta, V.B., S. Anithaa, M.L. Hegdea, L. Zeccab and R.M. Garrutoc *et al*., 2005. Aluminium in Alzheimer's disease: Are we still at a crossroad? Cell Mol. Life Sci., 62: 143-158. PMID: 15666086

Hammerschmidt, F.J., A.M. Clark, F.M. Soliman, E.S. El-Kashoury and M.M. Abd El-Kawy *et al*., 1993. Chemical composition and antimicrobial activity of essential oils of *Jasonia candicans* and *J. montana*. Planta Medica, 59: 68-70. PMID: 8441785

Hoozemans, J.J., J.M. Rozemuller, E.S.V. Haastert, R. Veerhuis and P. Eikelenboom, 2008. Cyclooxygenase-1 and -2 in the different stages of alzheimer's disease pathology. Curr. Pharm. Des., 14: 1419-1427. PMID: 18537664

Hussein, M.A. and H.S. Farghaly, 2010. Protective effects of *jasonia montana* against lipid peroxidation in liver and kidney of iron-overloaded rats. Australian J. Basic Applied Sci.

Hussein, M.A. and S.M. Abdel-Gawad, 2010. Protective effect of *Jasonia montana* against ethinylestradiol-induced cholestasis in rats. Saudi Pharmaceutical J., 18: 27-33. DOI: 10.1016/j.jsps.2009.12.002

Hussein, M.A., 2008. Antidiabetic and antioxidant activity of Jasonia Montana extract in Streptozotocin-induced diabetic rats. Saudi Pharmaceutical J., 16: 214-221.

Intiso, D., M.M. Zarrelli, G. Lagioia, F.D. Rienzo and C.C.D. Ambrosio *et al*., 2004. Tumor necrosis factor alpha serum levels and inflammatory response in acute ischemic stroke patients. Neurol. Sci., 24: 390-396. PMID: 14767684

Joshi, S.A., S.S., Chavhan and K.K., Sawant, 2010. Rivastigmine-loaded PLGA and PBCA nanoparticles: Preparation, optimization, characterization, in vitro and pharmacodynamic studies. Eur. J. Pharm. Biopharm., 76: 189-199. PMID: 20637869

Kaizer, R.R., M.C. Correa, L.R. Gris, C.S.D. Rosa and D. Bohrer *et al*., 2008. Effect of long-term exposure to aluminum on the acetylcholinesterase activity in the central nervous system and erythrocytes. Neurochem. Res., 33: 2294-2301. PMID: 18470612

Kamsler, A. and M. Segal, 2004. Hydrogen peroxide as a diffusible signal molecule in synaptic plasticity. Mol. Neurobiol., 29: 167-178. PMID: 15126684

Karthikeyan, K., B.R. Bai and S.N. Devaraj, 2007. Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. Int. J. Cardiol., 115: 326-333. PMID: 16828181

Kawahara, M., M. Kato and Y. Kuroda, 2001. Effects of aluminum on the neurotoxicity of primary cultured neurons and on the aggregation of beta-amyloid protein. Brain Res. Bull., 55: 211-217. PMID: 11470317

Kennedy, J.S., R.J., Polinsky, B., Johnson, P., Loosen and A., Enz *et al*., 1999. Preferential cerebrospinal fluid acetylcholinesterase inhibition by rivastigmine in humans. J. Clin. Psychopharmacol., 19: 513-521. PMID: 10587286

Khalil, W.K.B., W.S. Hasheesh, M.A.S. Marie, H.H. Abbas and E.A. Zahran, 2011. Assessment the impact of 17a-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; Oreochromis niloticus. Life Sci. J., 8: 329-342.

Kropf, J., J.O. Schurek, A.G. Wollner and M. Axel, 1997. Methodological aspects of the immunological measurement of transforming growth factor-beta 1 (TGF-β1) in blood. Assay development and comparison. Clinical Chemistry, 43: 1965-1974.

Kumar, A., S. Dogra and A. Prakash, 2009a. Protective effect of curcumin (Curcuma longa), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. Behav. Brain Res., 205: 384-390. PMID: 19616038

Kumar, P. and A. Kumar, 2009. Protective effect of rivastigmine against 3-nitropropionic acid-induced Huntington's disease like symptoms: Possible behavioural, biochemical and cellular alterations. Eur. J. Pharmacol., 615: 91-101. PMID: 19445928

Kumar, V., A. Bal and K.D. Gill, 2008. Impairment of mitochondrial energy metabolism in different regions of rat brain following chronic exposure to aluminium. Brain Res., 1232: 94-103. PMID: 18691561

Kumar, V., A. Bal and K.D. Gill, 2009b. Susceptibility of mitochondrial superoxide dismutase to aluminium induced oxidative damage. Toxicology, 255: 117-123. PMID: 19010380

Lewitt, M.S., H. Saunders and R.C. Baxter, 1993. Bioavailability of Insulin-like Growth Factors (IGFs) in rats determined by the molecular distribution of human IGF-binding protein-3. Endocrinology, 133: 1797-1802. PMID: 7691582

Liang, Y.Q. and X.C. Tang, 2004. Comparative effects of huperzine A, donepezil and rivastigmine on cortical acetylcholine level and acetylcholinesterase activity in rats. Neurosci. Lett., 361: 56-59. PMID: 15135892

Liu, Y., J.T. Kern, J.R. Walker, J.A. Johnson and P.G. Schultz *et al*., 2007. A genomic screen for activators of the antioxidant response element. Proc. Natl. Acad. Sci. USA., 104: 5205-5210.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol., 193: 265-275.

Lu, M., R.M. Nakamura, E.D. Dent, J.Y. Zhang and F.C. Nielsen *et al*., 2001. Aberrant expression of fetal RNA-binding protein p62 in liver cancer and liver cirrhosis. Am. J. Pathol., 159: 945-953. PMID: 11549587

Lukiw, W.J., M.E. Percy and T.P. Kruck, 2005. Nanomolar aluminum induces pro-inflammatory and pro-apoptotic gene expression in human brain cells in primary culture. J. Inorg. Biochem., 99: 1895-1898. PMID: 15961160

Melo, J.B., P. Agostinho and C.R. Oliveira, 2003. Involvement of oxidative stress in the enhancement of acetylcholinesterase activity-induced by amyloid-beta peptide. Neurosci. Res., 45: 117-127. PMID: 12507730

Mocali, A., S. Cedrola, N. Della Malva, M. Bontempelli and V.A. Mitidieri *et al*., 2004. Increased plasma levels of soluble CD40, together with the decrease of TGF beta 1, as possible differential markers of Alzheimer disease. Exp. Gerontol., 39: 1555-1561. PMID: 15501026

Moscat, J., M.T. Diaz-Meco and M.W.Wooten, 2007. Signal integration and diversification through the p62 scaffold protein. Trends Biochem. Sci., 32: 95-100. PMID: 17174552

Nagatsu, T. and M. Sawada, 2005. Inflammatory process in Parkinson's disease: Role for cytokines. Curr. Pharmacol. Des., 11: 999-1016. PMID: 15777250

Nayak, P., 2002. Aluminum impacts and disease. Env. Res., 89: 111-115. PMID: 12123643

Niu, P.Y., Q. Niu, Q.L. Zhang, L.P. Wang and S.E. He *et al*., 2007. Aluminum impairs rat neural cell mitochondria in vitro. Int. J. Immunopathol. Pharmacol., 18: 683-689. PMID: 16388716

Nizri, E., M, Irony-Tur-Sinai, N. Faranesh, I. Lavon and E. Lavi *et al*., 2008. Suppression of neuroinflammation and immunomodulation by the acetylcholinesterase inhibitor rivastigmine. J. Neuroimmunol., 203: 12-22. PMID: 18692909

Nizri, E., M. Irony-Tur-Sinai, I. Lavon, H. Meshulam and G. Amitai *et al*., 2007. IBU-Octyl-Cytisine, a novel bifunctional molecule eliciting anti-inflammatory and cholinergic activity, ameliorates CNS inflammation by inhibition of T-cell activity. Int. Immunopharmacol., 7: 1129-1139. PMID: 17630191

Obermayr, R.P., L. Mayerhofer, M. Knechtelsdorfer, N. Mersich and E.R. Huber *et al*., 2005. The age-related down-regulation of the growth hormone/insulin-like growth factor-1 axis in the elderly male is reversed considerably by donepezil, a drug for Alzheimer's disease. Exp. Gerontol., 40: 157-163. PMID: 15763392

Oyama, K., T. Fujimura, I. Ninomiya, T. Miyashita and S. Kinami *et al*., 2005. A COX-2 inhibitor prevents the esophageal inflammation-metaplasia-adenocarcinoma sequence in rats. Carcinogenesis, 26: 565-570.

Park, H., Z. Li, X.O. Yang, S.H. Chang and R. Nurieva *et al*., 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin. Nat. Immunol., 6: 1133-1141.

Perry, N.S.L., P.G. Houghton, A.E. Theolad, P. Jenner and E.K. Perry, 2000. In vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes. J. Pharm. Pharmacol., 52: 895-902. PMID: 10933142

Polidori, M.C., H.R. Griffiths, E. Mariani and P. Mecocci, 2007. Hallmarks of protein oxidative damage in neurodegenerative diseases: Focus on Alzheimer's disease. Amino Acids, 32: 553-559. PMID: 17273806

Pommier, F. and R. Frigola, 2003. Quantitative determination of rivastigmine and its major metabolite in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. J. Chromatogr. B, 784: 301-313. PMID: 12505778

Poon, W.W., M. Blurton-Jones, C.H. Tu, L.M. Feinberg and M.A. Chabrier *et al*., 2011. β-Amyloid impairs axonal BDNF retrograde trafficking. Neurobiol. Aging, 89: 1-13. PMID: 19540623

Porsteinsson, A.P., G.T. Grossberg, J. Mintzer and J.T. Olin, 2008. Memantine MEM-MD-12 Study Group. Memantine treatment in patients with mild to moderate Alzheimer's disease already receiving a cholinesterase inhibitor: A randomized, double-blind, placebo-controlled trial. Curr. Alzheimer Res., 5: 83-89. PMID: 18288936

Postina, R., A. Schroeder, I. Dewachter, J. Bohl and U. Schmitt *et al*., 2004. A disintegrin-metalloproteinase prevents amyloid plaque formation and hippocampal defects in an Alzheimer disease mouse model. J. Clin. Invest., 113: 1456-1464. PMID: 15146243

Pratico, D. and N. Delanty, 2000. Oxidative injury of the central nervous system: Focus on Alzheimer's disease. Am. J. Med., 109: 577-585. PMID: 11063960

Pratt, B.M. and J.M. McPherson, 1998. TGF-β in the central nervous system: Potential roles in ischemic injury and neurodegenerative diseases. Cytokine Growth Factor Rev., 8: 267-292. PMID: 9620642

Rathee, J.S., S.A. Hassarajani and S. Chattopadhyay, 2006. Antioxidant activity of Mammea longifolia bud extract. Food Chem., 99: 436-443. DOI: 10.1016/j.foodchem.2005.08.020

Recio, M.C., R.M. Giner, L. Uriburu, S. Maez and M. Cerda *et al*., 2000. In vivo activity of pseudoguaianolide sesquiterpene lactones in acute and chronic inflammation. Life Sci., 66: 2509-2519. PMID: 10883729

Salminen, A., J. Ojala, A. Kauppinen, K. Kaarniranta and T. Suuronen, 2009. Inflammation in Alzheimer's disease: Amyloid-β oligomers trigger innate immunity defence via pattern recognition receptors. Prog. Neurobiol., 87: 181-194. PMID: 19388207

Shah, S. and W.E. Reichman, 2006. Treatment of Alzheimer's disease across the spectrum of severity. Clin. Interv. Aging, 1: 131-142. PMID: 18044110

Shaw, LM., J.H. Stromme and J.L. London, 1983. Theodorsen International Federation of Clinical Chemistry, (IFCC), Scientific Committee, Analytical Section. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for gamma-glutamyltransferase [(gamma-glutamyl)-peptide: Amino acid gamma-glutamyltransferase, EC 2.3.2.2]. J. Clin. Chem. Clin. Biochem., 21: 633-646. PMID: 6139407.

Sjogren, M., S. Folkesson, K. Blennow and E. Tarkowski, 2004. Increased intrathecal inflammatory activity in frontotemporal dementia: Pathophysiological implications. J. Neurol. Neurosurg. Psychiatry, 75: 1107-1111. PMID: 15258209

Sobota, R., M. Szwed, A. Kasza, M. Begno and T. Kordula, 2000. Parthenolide inhibits activation of Signal Transducers and Activators of Transcription (STATs) induced by cytokines of the IL-6 family. Biochem. Biophys. Res. Commun., 267: 329-333. PMID: 10623619

Soliman, F.M., M.Y. Moussa, H.M. Abdallah and S.M. Othman, 2009. Cytotoxic activity of flavonoids of *Jasonia montana* Vahl. (Botsch) (Astraceae) growing in Egypt. Australian J. Basic Applied Sci., 3: 148-152.

Steinman, L., 2007. A brief history of T(H)17, the first major revision in the T (H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat. Med., 13: 139-145.

Takeda, S., N. Sato, T. Ogihara and R. Morishita, 2008. The renin-angiotensin system, hypertension and cognitive dysfunction in Alzheimer's disease: New therapeutic potential. Front Biosci., 13: 2253-2265. PMID: 17981707

Thatcher, G.R., B.M. Bennett and J.N. Reynolds, 2005. Nitric oxide mimetic molecules as therapeutic agents in Alzheimer's disease. Curr. Alzheimer Res., 2: 171-182. PMID: 15974915

Tomimoto, H., I. Akiguchi, H. Wakita, J.X. Lin and H. Budka, 2000. Cyclooxygenase-2 is induced in microglia during chronic cerebral ischemia in humans. Acta Neuropathol. (Berlin), 99: 26-30. PMID: 10651024

Tsakiris, S., K.H. Schulpis, K. Marinou and P. Behrakis, 2004. Protective effect of l-cysteine and glutathione on the modulated suckling rat brain Na^+, K^+–ATPase and Mg^{2+}-ATPase activities induced by the in vitro galactosaemia. Pharmacol. Res., 49: 475-479. PMID: 14998558

Viviani, B., E. Corsini, C.L. Galli, A. Padovani and E. Ciusani *et al*., 2000. Dying neural cells activate glia through the release of a protease product. Glia, 32: 84-90. PMID: 10975913

Walzer, D.G., L. Lih-Fen and T.G. Beach, 2001. Gene expression profiling of amyloid beta peptide stimulated human post-mortem brain microglia. Neurobiol. Aging, 22: 957-966. PMID: 11755004

Wang, G., Y. Zhang, B. Chen and J. Cheng, 2003. Preliminary studies on Alzheimer's disease using cDNA microarrays. Mech. Age. Dev., 124: 115-124. PMID: 12618014

Wang, Z.F., J. Yin, Y. Zhang, L.Q. Zhu and Q. Tian *et al*., 2010. Overexpression of tau proteins antagonizes amyloid-beta-potentiated apoptosis through mitochondria-caspase-3 pathway in N2a cells. J. Alzheimers Dis., 20: 145-157. PMID: 20164595

Wyss-Coray, T., E. Masliah, M. Mallory, L. McConlogue and K. Johnson-Wood *et al*., 1997. Amyloidogenic role of cytokine TGF-beta1 in transgenic mice and Alzheimer's disease. Nature, 389: 603-606. PMID: 9335500

Yasojima, K., C. Schwab, E.G. McGeer and P.L. McGeer, 1999. Distribution of cyclooxygenase-1 and cyclooxygenase- 2 mRNAs and proteins in human brain and peripheral organs. Brain Res., 830: 226-236. PMID: 10366679

Zetterberg, H., N. Andreasen and K. Blennow, 2004. Increased cerebrospinal fluid levels of transforming growth factor-beta1 in Alzheimer's disease. Neurosci. Lett., 367: 194-196. PMID: 15331151

Zivin, M. and P. Pregelj, 2008. Prolonged treatment with donepezil increases acetylcholinesterase expression in the central nervous system. Psychiatr. Danub., 20: 168-173. PMID: 18587286