POTENTIAL ROLE OF *Jasonia montanta* AND *Jasonia candicans* AGAINST ALZHEIMER'S DISEASE: ASSESSMENT OF OXIDATIVE STRESS AND GENE EXPRESSION CHANGES IN AD-INDUCED RATS

SABAH A. A. LINJAWI[1], EKRAM S. AHMED[2], W. K. B. KHALIL[2] AND HODA F. BOOLES[2]

1. *Biology Department, College of Science, King AbdulAziz University, Saudi Arabia*
2. *Cell Biology Department, National Research Centre, 12622 Dokki, Giza, Egypt*

Oxidative stress-induced damage was shown to be one of the important mechanisms to indicate that aluminum has an association with the etiology of Alzheimer's disease (Kumar *et al.*, 2009). Increased generation of reactive oxygen species (ROS) and lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown etiology and in the toxic actions of many compounds (Andallu and Varadacharyulu, 2003). Aluminium is a non-redox active metal which is capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties of transition metals such as iron and copper (Bjertness *et al.*, 1996). An unusual aspect of the biochemistry of this non-redox active metal is its pro-oxidant activity, which might be explained by the formation of an Al superoxide semi-reduced radical ion (AlO_2^{2+}) (Exley, 2005). It has been shown that chronic aluminium exposure is involved in the impairment of mitochondrial electron transport chain (ETC) and increased production of ROS (Kumar *et al.*, 2008). The formation of excessive ROS and reactive nitrogen species (RNS) can lead to oxidative injury. Reactive oxygen species (ROS) interact with all biological macromolecules, including lipids, proteins, nucleic acids, and carbohydrates. The resulting stress increases neuronal death, which contributes to the neuropathology associated with several diseases (Baydas *et al.*, 2003).

Gene expression changes in the forebrain occur during normal and pathological aging. Altered gene expression is thought to contribute to the balance between normal aging and age-related memory disorders, including Alzheimer's disease (AD) (Berchtold *et al.*, 2008). Synaptic dysfunction in AD is apparent before synapse and neuron loss and caused likely by accumulation of β-amyloid (Aβ) peptides (Selkoe, 2002). The cellular mechanisms underlying synaptic and memory dysfunction caused by altered activity-dependent gene transcription in AD are largely unknown. Understanding the molecular pathways regulating gene expression profiles in memory disorders may allow the identification of new signaling pathways for drug discovery (Altar *et al.*, 2009). Several PCR techniques are used to determine the changes in the gene expression, in which the most aqurat one is Real-time-PCR (RT-PCR). It is became the most popular method of quantitating steady-state mRNA levels (Bustin, 2000). It is most often used for two reasons: either as a primary investigative tool to determine gene expression or as a secondary tool to validate the results of DNA microarrays. Because of the precision and sensitivity of real-time RT-PCR, even subtle changes in gene expression can be detected. Thus real-time PCR can be used to assess RNA levels with great sensitivity and precision.

There are several genes play main role in occurrence the AD diseases. The generation of amyloid β-peptide (Aβ) is widely held to play an early and critical role in the pathogenesis of Alzheimer's disease (Hardy and Selkoe, 2002). Aβ is generated from the large precursor protein amyloid precursor protein (APP) by the sequential action of two proteases, β and γ-secretase. β-secretase has been identified as β-site APP-cleaving enzyme 1 (BACE1) which, together with its homolog BACE2, forms a novel subfamily of transmembrane aspartic proteases within the pepsin family (Vassar and Citron, 2000).

The formation of Alzheimer's Aβ peptide is initiated when the amyloid precursor protein (APP) is cleaved by the enzyme β-secretase (BACE1); inhibition of this cleavage has been proposed as a means of treating Alzheimer's disease.

Cyclooxygenase (*COX-2*) is continuously expressed within a distinct population of neurons in the brain (Breder *et al.*, 1995), which is a common attribute in enzymes involved in physiological functions of the central nervous system such as memory, sensory integration, and autonomic regulation (Kaufmann *et al.*, 1997). On the other hand, in various neuropathological conditions accompanied by inflammatory reaction, such as stroke (Tomimoto *et al.*, 2000) and amyotrophic lateral sclerosis (ALS) (Yasojima *et al.*, 2001), *COX-2* up-regulation is thought to mediate neuronal damage presumably by producing excessive amounts of harmful prostanoids and free radicals. In AD brains, it has been reported that the expression of *COX-2* mRNA and protein was elevated (Yasojima *et al.*, 1999). However, it is not yet completely delineated how and in what type of cells *COX-2* is increased in the AD brain.

Aluminum-induced depletion of antioxidants such as glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and catalase (CAT) (Mahieu *et al.*, 2005). Antioxidants thus play key roles in the protection against damage caused by reactive oxygen species (Baynes, 1991). Many plant extracts and plant products have been shown to have significant antioxidant activity (Anjali and Manoj, 1995), which may be an important property of medicinal plants associated with the treatment of several diseases including neurodegenerative diseases. Thus, herbal plants are considered useful means to prevent and/or ameliorate certain disorders, such as Alzheimer's disease. Among these herbal resources, the plant *Jasonia montana* and *Jasonia candi-*

cans occur in the Mediterranean and surrounding areas (Merxmuller *et al.*, 1977), including the Sinai Peninsula (Tackholm, 1974). The herb has a strong aromatic odor and is used in traditional medicine for diarrhea, stomach ache, and chest diseases (Tackholm, 1969). A literature survey indicated that some mono- and sesquiterpenes (Ahmed and Jakupovic, 1990; Ahmed, 1991), flavonoids (Ahmed *et al.*, 1989) and essential oils (Hammerschmidt *et al.*, 1993) have been reported from the plant.

Rivastigmine is a carbamate derivative pseudo-irreversible cholinesterase inhibitor which can both inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE1). This drug is licensed for use in the UK for the symptomatic treatment of mild-to-moderately severe AD due to its inhibitory action on AchE activity (Foye *et al.*, 1995). Rivastigmine has been reported to protect mice against cognitive impairment caused by oxygen deficit, improve learning in rats, and antagonize scopolamine induced impairment of cognitive function in rats (Desai and Grossberg, 2001; Howes *et al.*, 2003). It has been demonstrated that Rivastigmine supplementation increased the concentration of acetylcholine and inhibited acetylcholine esterase activity (Liang and Tang, 2004).

The present study was designed to investigate the potential role of *J. montanta* and *J. candicans* total extracts against $AlCl_3$- induced oxidative stress and gene expression alteration of AD-related enzymes characterizing Alzheimer's disease in male rats.

MATERIALS AND METHODS

Chemicals

- Aluminium Chloride ($AlCl_3$): was purchased from Sigma. Its M.W. 133.34.
- Rivastigmine: Exelon 1.5 mg, was purchased from Novartis Co.

Medicinal plants

Fresh aerial parts of *J. montana* and *J. candicans* were collected from the Sinai Peninsula. Authentication of the plant was carried out by Prof. Dr. Samir M. Othman, Department of Pharmacognosy, Faculty of Pharmacy, October 6th University, October 6th City, Egypt.

Preparation of medicinal plant extracts

According to López *et al.* (2008), plant material was air-dried in the dark at room temperature. The dried-powder (180 meshes) was successively extracted 3-times with 200 ml dichloromethane, ethyl acetate, methanol, and water after maceration at 4°C for 24h. The dichloromethane, ethyl acetate, and methanol extracts were dried under reduced pressure at 30°C in a rotary evaporator and the aqueous extracts were lyophilized. The dry extracts were stored in glass vials at −40°C until tested and analyzed.

Experimental animals

Male aged Sprague Dawley rats (14-16 months) weighing 250-300 g were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt and acclimated in a specific pathogen free barrier area at 25±1°C. Rats were kept constantly at a 12h light/dark cycle. They were individually housed with *adlibitum* access to standard balanced diet. Animals received human care in compliance with the guidelines of the Ethical Committee of Medical Research of National Research Centre, Egypt.

Experimental Design

Rats were randomly assigned into seven groups, ten rats each. The first group served as normal control. The second group was provided with $AlCl_3$ in drinking water in a dose of 0.3% for forty five days (Erazi *et al.*, 2010), and served as Al intoxicated group. The third and fourth groups rats were given $AlCl_3$ in drinking water daily for forty five days then they were orally treated with 150 mg/kg b.wt./day of *J. montanta* and *J. candicans* extract, respectively, for another forty five days (Hussein, 2008). The fifth group rats were given $AlCl_3$ in drinking water daily for forty five days then they were orally treated with Rivastigmine in a dose of 0.3 mg/kg b. wt. (Carageorgious *et al.*, 2008) as a reference drug daily for another forty five days. Rats in the sixth and seventh groups were administered orally with *Jasonia montanta* and *Janosia candicans* extracts, respectively, for forty five days.

At the end of experimental period, fasting blood samples were collected from retro-orbital venous plexus under anesthesia, in heparinezed tubes, and then centrifuged at 3000 rpm for 15 min. Plasma was separated and stored at -20°C until analysis.

After blood collection, brains were rapidly dissected, thoroughly washed with isotonic saline, and dried. Each brain was mid-saggitally divided into two portions. The first portion was stored in liquid nitrogen for gene expression analysis. While the second portion was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 50 mM tris HCl and 300 mM sucrose. The homogenate was centrifuged under cooling at 3000 rpm for 10 min. The supernatant (10%) was used for biochemical analyses.

Biochemical analyses

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of Lowry *et al.* (1951). Brain malondialdehyde (MDA) level was estimated by colorimetric method described by Ohkawa *et al.* (1979). Quantitative estimation of brain nitric oxide (BNO) level was assayed according to the method of Berkels *et al.* (2004) and brain total antioxidant capacity (TAC) level was colorimetrically determined according to the method of Koracevic *et al.* (2001).

Gene expression analysis

Extraction of total RNA

Brain tissues of rats within each group were used to extract total RNA using TRIzol® Reagent (cat#15596-026, Invitrogen, Germany). Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) 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.

Synthesis of the cDNA using reverse transcription (RT) reaction

The complete Poly$(A)^+$ RNA isolated from rat brain tissues was reverse transcribed into cDNA in a total volume of 20 µl using RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5µg) was used with a reaction mixture, termed as master mix (MM). The MM consisted of 50 mM $MgCl_2$, 5 x 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 g and transferred to the thermocycler (Biometra GmbH, Göttingen, 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).

Quantitative real time-polymerase chain reaction (qRT-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 µL distilled water, and 5 µL of cDNA template.

The reaction program consisted of three steps. The first step was at 95°C for 3 min. The second step consisted of 40 cycles in which each cycle was divided into three sub-steps: (a) at 95°C for 15 sec; (b) at 55°C for 30 sec; and (c) at 72°C for 30 sec. The third step consisted of 71 cycles which started at 60°C and then increased about 0.5°C every 10 sec up to 95°C. At the end of each sqRT-PCR a melting curve analysis was performed at 95°C to check for the quality of the used primers. Each experiment included a distilled water control.

The quantitative values of qRT-PCR of *Amyloid precursor protein (*APP; forward 5'-ACT GGC TGA AGA AAG TGA CAA T-3'; reverse 5'-AGA GGT GGT TCG AGT TCC TAC A-3'; Stein and Johnson, 2002); β-site APP cleaving enzyme 1*: BACE1* (forward 5'-GCG CTT GCC ATG TGC AC-3'; reverse 5'-TGC CGT AAC AAA CGG ACC TT- 3'; Luo *et al.*, 2003); β-site APP cleaving enzyme *2: BACE2* (BACE2 forward 5'-AAA TTT CTG GGC CCT TTT CC-3', Reverse 5'-GGG CTC ATT CAG AGC CTG TG-3', Luo *et al.*, 2003); and cyclooxygenase: *COX-2* (F: 5′- TGA TCG AAG ACT ACG TGC AAC A -3′, R: 5′- GCG GAT GCC AGT GAT AGA GTG -3′, Oyama *et al.*, 2005) genes were normalized on the bases of β-actin (β-actin-F: 5′- CCC AGA GCA AGA GAG GTA TC -3′, B-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°C to check the quality of the used primers.

Calculation of gene expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae (Bio-Rad 2006):

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

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

The relative quantification of the target to the reference was determined by using the ΔC_T method if E for the target (GH, IGF-1) and the reference primers (β-Actin) are the same (Bio-Rad 2006):

$$\text{Ratio}_{(\text{reference/ target gene})} = \text{Ef}^{C_T(\text{reference}) - C_T(\text{target})}$$

The amplification efficiency (E) for APP, BACE1, BACE2 and COX-2 were 1.996 (%E= 99.66), 2.00 (%E=100.08), 1.993 (%E=99.52) and 1.988 (%E=89.84) respectively. Whereas, the PCR conditions indicated that the slopes of APP, BACE1, BACE2 and COX-2 were -3.33, -3.32, -3.34 and -3.35, 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.

Statistical Analysis

All results were expressed as Mean±S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups (Armitage and Berry, 1987). Difference was considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Biochemical study

The data in Table (1) represents the effect of *J. candicans* and *J. montana* extracts on brain oxidant/antioxidant status in male rats received $AlCl_3$ in drinking water. Administration of *J. candicans* and

J. montana extracts showed significant decrease in brain malondialdehyde (MDA) and brain nitric oxide (BNO) levels except *J. montana* extract which showed insignificant decrease in brain nitric oxide level in comparison with the negative control group. Moreover, they showed insignificant increase in brain total antioxidant capacity (TAC) as compared to the negative control group. On the other hand, the untreated AD induced rats showed significant increase in brain MDA and BNO levels associated with significant decrease in brain TAC in comparison with the negative control group. While, treatment of AD induced rats with *J. candicans* or *J. montana* extracts or rivastigmine produced significant decrease in brain MDA and BNO levels accompanied with significant increase in brain TAC when compared with the untreated AD induced group. Noteworthy, the treatment of AD induced rats with *J. candicans* or *J. montana* extracts caused insignificant change in brain MDA, BNO and TAC levels as compared with the AD induced rats treated with rivastigmine.

In neurodegenerative disorders involving oxidative stress, such as Alzheimer's disease, stroke and Parkinson's disease, BNO increases cell damage through the formation of highly reactive peroxynitrite (Guix *et al.*, 2005). Dorheim *et al.* (1994) reported that the elevated level of BNO may result from the activation of nitric oxide synthase (NOS) which was also elevated in the brain of patients with AD, indicating that BNO may play a role in neuronal cell injury in this disease. One of the possible mechanisms by which Al could induce BNO elevation in brain tissue may be related to Al-induced nitric oxide synthase (NOS) activity with consequent increase in BNO products in rat brain tissue and microglial cells (Bondy *et al.*, 1998). Guix *et al.* (2005) demonstrated that cerebellar levels of inducible NOS (iNOS), not neuronal NOS (nNOS) protein in rats were significantly elevated following both short-term and extended Al dosing.

Some reports suggest that Al-induced AD could interface with signal transduction pathways associated with NMDA receptors (Platt *et al.*, 1995). In this pathway, activation of NMDA receptors leads to increased intracellular calcium in the postsynaptic neuron, which, in turn, binds to calmodulin and triggers the activation of the NOS enzyme opening a gate for the electron flux into the active center of the NOS in brain tissue (Canales *et al.*, 2001). It has been demonstrated that all three isoforms of NOS (nNOS, eNOS, and iNOS) are aberrantly expressed during Al intoxication. This gives rise to the elevated levels of BNO that are apparently involved in neurodegeneration by different mechanisms, including oxidative stress and activation of intracellular signaling mechanisms (Lüth *et al.*, 2001).

The preliminary studies conducted by this work revealed the non-toxic nature of *J. montana* and *J. candicans* on normal rats. This result is greatly supported by that of Hussein (2008). The current results revealed that brain MDA level was significantly lower in the groups of Alzheimer's disease group treated with *J. montana* and *J. candicans* extracts compared to Alzheimer's disease group. The above result suggests that both *J. montana* and *J. candicans* extracts may exert antioxidant activities and protect the tissues from lipid peroxidation (Hussein, 2008). It is most likely that the potential effect of *J. montana* and *J. candicans* extract in reducing the lipid product represented by MDA level is a consequence of the modulatory influence of these extracts on the biotransformation enzymes of detoxification. High content of flavonoids and phenolic compounds has been demonstrated in *J. montana* extract (Hussein and Abdel-Gawad, 2010) as well as in *J. candicans* one (Hammerschmidt *et al.*, 1993) which may be responsible for free radical scavenging activity. The phenolic compounds such as quercetin and kaempferol which are present in high concentration in each of *J. montana* (Soliman *et al.*, 2009) and *J. candicans* (Hammerschmidt *et al.*, 1993) have been found to exihibit antilipid peroxidative effect (Dasgupta and De, 2007; Liu *et al.*, 2008) due to their ability to inhibit H_2O_2-induced lipid peroxidation (Ammar *et al.*, 2009). Flavonoids are able to inhibit lipid peroxidation on the mitochondrial membrane, thus these compounds possess good antilipidperoxidat activity (Sugihara *et al.*, 1999) which indicates the pharmacological potential of flavonoids against pathological processes related to oxidative stress (Andarwulan *et al.*, 2010).

The ability of each of *J. montana* and *J. candicans* total extracts to reduce brain nitric oxide (BNO) level in Alzheimer's disease-induced rats in the present study could be attributed to the efficacy of flavones content in these extracts to interfere with expression of the inducible nitric oxide synthase (iNO). Therefore, they were considered as a powerful inhibitor for BNO production without BNO scavenging activity (Cerqueira *et al.*, 2008). Hussein and Abdel-Gawad (2010) study greatly supported our results in this concern as they stated that BNO level is significantly depleted after *J. montana* treatment in rats-induced cholestasis. The high content of flavonoids and phenolic compounds in *J. montana* extract (Hussein and Abdel-Gawad, 2010) and *J. Candicans* (Hammerschmidt *et al.*, 1993) may contribut in this effect.

The effect of *J. montana* and *J. candicans* total extracts on the total antioxidant capacity in the brain revealed that these extracts could significantly increase brain total antioxidant capacity in the Alzheimer’s disease-treated rats. It has been found that *Jasonia* species can either increase the biosynthesis of glutathione or reduce the oxidative stress leading to less degeneration of glutathione or has both effects (Hussein, 2008). Also, this species has been demonstrated to improve the activities of the antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) attributed to the reduction of reactive oxygen free radicals. Moreover, Jasonia species could increase the activities of glutathione peroxidase (Gpx) and glutathione-s-transferase (GST) in the various tissues of rats (Hussein, 2008). Furthermore, Hussein and Abdel-Gawad, (2010) reported that Jasonia species extract effectively normalize the impaired antioxidant status in rats. The phenolic constituents sharing flavonoids in enhancing the total antioxidant capacity in Alzheimer's disease- treated rats. These compounds exhibit both antioxidant activity and antiradical capacity (Liu *et al.*, 2008; Esmaeili *et al.*, 2010), which are responsible for the increased total antioxidant capacity (Robaszkiewicz *et al.*, 2007).

Expression of APP, BACE1, BACE2 and COX-2 genes

The present results revealed a significant decrease of gene expression levels of *APP, BACE1, BACE2* and *COX-2* genes in untreated male rats (negative control) compared with those treated with $AlCl_3$ (Figs 1-4). The same trend was showed in rats supplemented with *J. montana* and *J. candicans*. Whereas, the expression of *APP, BACE1, BACE2* and *COX-2* genes showed low levels in *J. montana* and *J. candicans* groups compared with those treated with $AlCl_3$ (Figs 1-4).

In contrary, the expression levels of *APP, BACE1, BACE2* and *COX-2* genes in rats treated with $AlCl_3$ (0.3 g/L for 4 months) were significantly higher than those found in untreated rats or in rats supplemented with *J. montana* or *J. candicans* (150 mg/kg b.w.) alone (Figs 1-4).

The results showed also that *J. montana* and *J. candicans* were able significantly to reduce the expression level of AD related-genes in rat groups treated with $AlCl_3$ plus *J. montana* or treated with $AlCl_3$ plus *J. candicans* (Figs 1-4).

The formation of Alzheimer's Aβ peptide is initiated when the amyloid precursor protein (APP) is cleaved by the *BACE1* and *BACE2* enzymes (Vassar and Citron, 2000). In AD brains, it has been also reported that the expression of *COX-2* mRNA and protein was elevated (Yasojima *et al.*, 1999).

The current results revealed a significant increase of gene expression levels of *APP, BACE1, BACE2* and *COX-2* enzymes in rats treated with $AlCl_3$ induced AD disease compared with those in untreated rats or in rats supplemented with *J. montana* or *J. candicans*. In contrary, the expression levels of *APP, BACE1, BACE2* and *COX-2* genes in rats treated with $AlCl_3$ plus *J. montana* or *J. candicans* were significantly lower than those found in rats treated with $AlCl_3$ alone.

In agreement with our results, Hardy and Selkoe (2002) reported that the generation of amyloid β-peptide (Aβ) is widely held to play an early and critical role in the pathogenesis of Alzheimer's disease**.** Aβ is generated from the large precursor protein amyloid precursor protein (APP) by the sequential action of two proteases, β and γ-secretase. β-secretase has been identified as β-site APP-cleaving enzyme 1 (BACE1) which, together with its homolog BACE2, forms a novel subfamily of transmembrane aspartic proteases within the pepsin family (Vassar and Citron, 2000). The formation of Alzheimer's Aβ peptide is initiated when the amyloid precursor protein (APP) is cleaved by the enzyme β-secretase (BACE1); inhibition of this cleavage has been proposed as a means of treating Alzheimer's disease.

Remarkably, *COX-2* is continuously expressed within a distinct population of neurons in the brain (Breder *et al.*, 1995), which is an attribute common in enzymes involved in physiological functions of the central nervous system such as memory, sensory integration, and autonomic regulation and may suggest this role for *COX-2* (Kaufmann *et al.*, 1997). On the other hand, in various neuropathological conditions accompanied by inflammatory reaction, such as stroke (Iadecola *et al.*, 1999; Tomimoto *et al.*, 2000) and amyotrophic lateral sclerosis (ALS) (Yasojima *et al.*, 2001), *COX-2* upregulation is thought to mediate neuronal damage presumably by producing excessive amounts of harmful prostanoids and free radicals. In AD brains, it has been reported that the expression of *COX-2* mRNA and protein was elevated (Yasojima *et al.*, 1999). However, it is not yet completely delineated how and in what type of cells COX-2 is increased in the AD brain.

In the current study the results revealed that the expression levels of *APP, BACE1*, *BACE2* and *COX-2* genes in the brain samples collected from rats supplemented with *J. montana* plus $AlCl_3$ were significantly lower ($p \leq 0.01$) than those observed in rats treated with $AlCl_3$ alone. Furthermore, the expression levels of *APP* and *BACE2* genes in the brain samples collected from rats supplemented with *J. candicans* plus $AlCl_3$ were significantly lower ($p \leq 0.01$) than those observed in rats treated with $AlCl_3$ alone. However, these levels of expression of *BACE1* and *COX-2* genes the brain samples collected from rats supplemented with *J. candicans* plus $AlCl_3$ were not significantly lower ($P > 0.05$) than those observed in rats treated with $AlCl_3$ alone.

The expression levels of all genes tested except *BACE1* gene in the brain samples collected from rats supplemented with rivastigmine were significantly lower ($P < 0.01$) than those observed in rats treated with $AlCl_3$ alone.

Jasonia species occurs in the Mediterranean and surrounding areas (Merxmuller *et al.*, 1977), including the Sinai Peninsula (Tackholm, 1974). The herb has a strong aromatic odor and is used in traditional medicine for diarrhea, stomachache, and chest diseases. A literature survey indicated that some mono- and sesquiterpenes (Ahmed and Jakupovic, 1990; Ahmed, 1991), flavonoids (Ahmed *et al.*, 1989), and essential oils (Hammerschmidt *et al.*, 1993) have been reported from the plant. The different extracts of the plant were also screened for hypoglycemic and antidiabetic activities (AL-Howiriny *et al.*, 2005). Hussein (2008) reported that the extract of this plant exerted rapid protective effects against lipid peroxidation by scavenging of free radicals. Thus, this plant showed a powerful antioxidant activity. Moreover, four sesquiterpenes isolated from *Jasonia glutinosa* D.C. (Asteraceae), namely lucinone, glutinone, 5-epikutdtriol and kutdtriol, have been evaluated for their *in vitro* anti-inflammatory activity in cellular systems generating cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) metabolites (Benito *et al.*, 2002). López *et al.* (2008) have been demonstrated the antioxidant and antifungal activities of this plants. Therefore, the antioxidant activity of these herb used in the present study may contribute to the reduction of the oxidative activity of $AlCl_3$ induced AD. Whereas, *J. montana* and *J. candicans* down-regulated the AD-related genes and reduced the oxidation status (MDA and BNO levels) as well as increased the antioxidant capacity (TAC level).

In conclusion, Administration of *J. candicans* and *J. montana* extracts showed decrease in brain MAD, BNO and expression level of APP, BACE1, BACE2 and COX-2 genes and increase in brain TAC in comparison with control group. The results suggest that the antioxidant activity of *J. montana* and *J. candicans* may be main reasons to reduce the oxidative activity of $AlCl_3$ induced AD.

SUMMARY

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, unusual behaviour, personality changes and a decline in thinking abilities. In this disease, the capacity to memorize is seriously reduced because of compromised neuronal transmission. *Jasonia* species are reported to possess a variety of activities, including antioxidant effects. Thus, the anti-Alzheimer and antioxidant effects of *Jasonia montanta* and *Jasonia candicans* extracts were evaluated via brain malondialdehyde (MDA), brain nitric oxide (BNO) and total antioxidant capacity (TAC) assays as well as quantitative real time-polymerase chain reaction (qRT-PCR) for amyloid precursor protein (APP), β-site APP cleaving enzyme 1(BACE1), β-site APP cleaving enzyme 2 (BACE2) and cyclooxygenase (COX-2). Seventy male rats were allocated in several groups. Untreated control rats and those treated with *J. montanta* and *J. candicans* were supplemented with 0.3% of $AlCl_3$ drinking for forty five days to induce Alzheimer's disease (AD). Afterwards, two groups of them were given orally for further forty five days 150 mg/kg b.wt./day of *J. montanta* or *J. candicans* extract. Another group was given 0.3 mg/kg b.wt. of Rivastigmine for further forty five days. The results revealed that administration of *J. candicans* and *J. montana* extracts showed decrease in brain MAD, BNO and expression level of APP, BACE1, BACE2 and COX-2 mRNAs and increase in brain TAC in comparison with control group. Moreover, treatment of AD induced rats with *J. can-*

dicans or *J. montana* extracts or rivastigmine produced significant decrease in brain MDA, BNO and expression level of APP, BACE1, BACE2 and COX-2 mRNAs accompanied with significant increase in brain TAC when compared with the untreated AD induced group. However, AD induced rats showed increase in brain MDA, BNO and expression level of APP, BACE1, BACE2 and COX-2 mRNAs associated with decrease in brain TAC in comparison with the control group. The results suggest that the antioxidant activity of *J. montana* and *J. candicans* may be main reasons to reduce the oxidative activity of $AlCl_3$ induced AD.

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Table (1): Effect of *J. candicans* and *J. montana* extracts on brain oxidant/antioxidant status in male rats received $AlCl_3$ in drinking water. Data are represented as Mean ± SE of 10 rats/group.

Groups	MDA	BNO	TAC
	(nmol/mg protein)		
Control	5.6 ± 0.3	24.5 ± 0.97	14.2 ± 1.3
J. candicans	4.1 ± 0.5[a]	20.7 ± 0.8[a]	15.2 ± 0.8
J. montana	4.5 ± 0.3[a]	24.0 ± 0.6	14.3 ± 0.2
$AlCl_3$	8.6 ± 0.5[a]	35.9 ± 1.0[a]	7.5 ± 0.5[a]
$AlCl_3$ +*J. candicans*	6.2 ± 0.3[b]	24.9 ± 1.0[b]	13.7 ± 0.4[b]
$AlCl_3$+ *J. montana*	6.9 ± 0.3[b]	25.4 ± 0.6[b]	13.0 ± 0.9[b]
$AlCl_3$+ Rivastigmine	6.2 ± 0.3[b]	25.0 ± 0.7[b]	13.5 ± 0.6[b]

[a]: Significant at P< 0.05 in comparison with the negative control group; [b]: Significant at P< 0.05 in comparison with the untreated AD induced group.

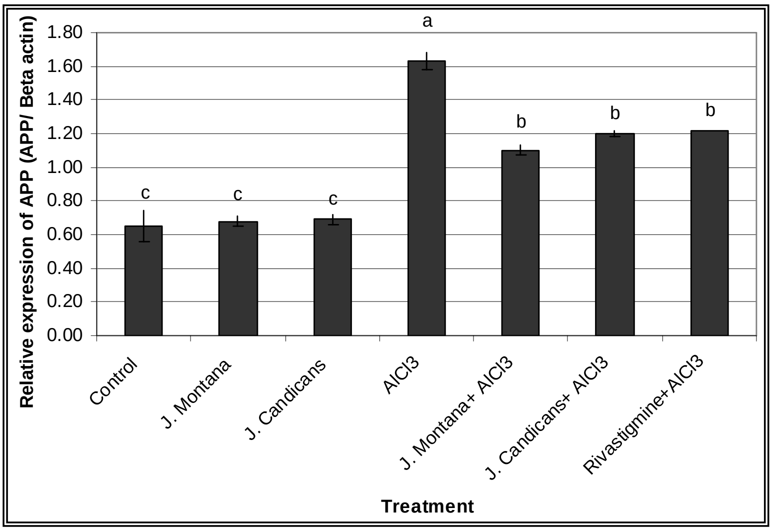

Fig. (1): Semi-quantitative Real Time-PCR analysis of amyloid precursor protein (APP)-mRNAs in brain tissues collected from male rats (n=10) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, within tissue, differ significantly (P ≤ 0.05).

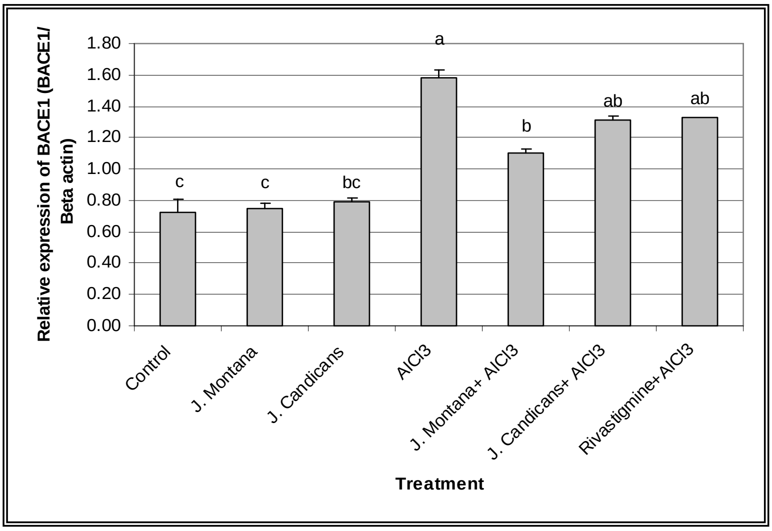

Fig. (2): Semi-quantitative Real Time-PCR analysis of β-site APP cleaving enzyme 1 (BACE1)-mRNAs in brain tissues collected from male rats (n=10) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, within tissue, differ significantly ($P \leq 0.05$).

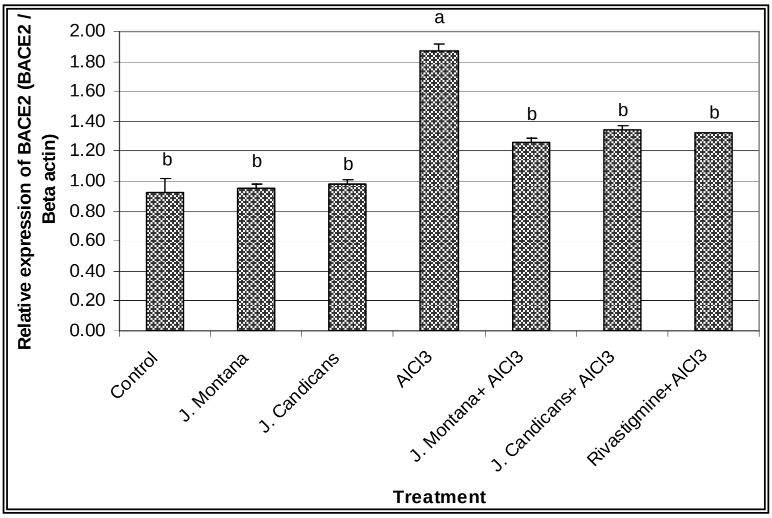

Fig. (3): Semi-quantitative Real Time-PCR analysis of β-site APP cleaving enzyme 2 (BACE2)-mRNAs in brain tissues collected from male rats (n=10) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, within tissue, differ significantly ($P \leq 0.05$).

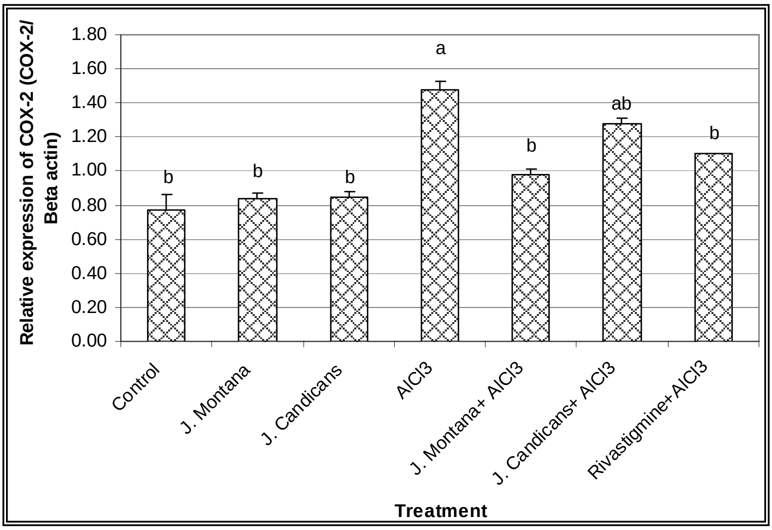

Fig. (4): Semi-quantitative Real Time-PCR analysis of Cyclooxygenase (COX-2)-mRNAs in brain tissues collected from male rats (n=10) supplemented with *J. montana* and *J. candicans* extracts with or without $AlCl_3$. Means with different letters, within tissue, differ significantly ($P \leq 0.05$).