PROTECTIVE EFFECTS OF PHENOLICS FROM JUJUBE (ZIZIPHUS JUJUBA) LEAF AGAINST H$_2$O$_2$-INDUCED OXIDATIVE STRESS IN NEURONAL PC-12 CELLS

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ABSTRACT: We aimed to determine the antioxidant and neuronal cell protective effects of jujube (Ziziphus jujuba) leaf solvent fractions. The butanol fraction from jujube showed potent antioxidant activity in each assay, and its ABTS radical scavenging effect, FRAP, and MDA inhibition increased in a dose-dependent manner. Following H$_2$O$_2$ treatment, intracellular ROS accumulation significantly reduced when butanol fraction from jujube leaf was present in the PC12 cell media. In the MTT cell viability assay, the butanol fraction showed a protective effect against H$_2$O$_2$-induced neurotoxicity and inhibited LDH release into the medium (7.13-43.89%). Total phenols of the three solvent fractions were 72.50 (chloroform), 297.18 (butanol), and 17.93 (water) mg/g GAE. The predominant phenolic compounds in jujube leaves are rutin (57.07 mg/100 g) and quercetin (9.27 mg/100 g). These data suggest that butanol fraction from jujube leaf including phenolics may be useful natural antioxidants to reduce the risk of neurodegenerative diseases including Alzheimer’s disease.

KEY WORDS: Antioxidants, Cell viability, Jujube leaf, Neuronal protective effect

INTRODUCTION

Patients with Alzheimer’s disease (AD) have a high sensitivity to (reactive oxygen species) ROS. Accumulated intracellular H$_2$O$_2$, which is an ROS, causes lipid peroxidation of cell membranes and triggers apoptotic cell death via the activation of caspases (Chun et al., 2005; Chang et al., 2008). AD is one of the most serious threats to human health in aging societies of developed countries. In particular, AD is a major neurodegenerative disease and is characterized by loss of memory and cognition. Around 18 million people in the world suffer from AD, and this number is expected to rise to 34 million by 2025 (Hong et al., 2007). Many studies have demonstrated that the brains of patients with AD are subjected to increased oxidative stress due to free radical damage (Murakami et al., 2000).

Many phenolics protect neuronal cells from oxidative stress induced by ROS or amyloid-β (Aβ) protein, and this may be related to the pathogenesis of AD (Om et al., 2008; Park et al., 2001). Some phytochemicals from natural plant sources, including fruits and vegetables, may reduce the risk of AD because of their antioxidant properties that reduce oxidative insults (Pinzo’n-Arango et al., 2009). Epidemiological observation has revealed that increased antioxidant uptake is inversely related to the risk of incidence of AD (Psotová et al., 2003). Antioxidants are vital substances that have the ability to protect the body from damage caused by free radical-induced oxidative stress (Ozsoy et al., 2008). There is an increasing interest in natural antioxidants (e.g., polyphenols) present in medicinal and dietary plants, which might help prevent oxidative damage (Silva et al.,

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MATERIAL AND METHODS

Chemicals

Folin-Ciocalteu’s phenol reagent, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, 2,4,6-tripyridyl-S-triazine (TPTZ), trichloroacetic acid (TCA), thiobarbituric acid (TBA), vitamin C, persulfate, 2,4,6-tripyridyl-S-triazine (TPTZ), trichloroacetic acid (TCA), and ethylenebrine-6-sulfonic acid (ABTS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY, USA).

Plant material

Jujube leaves were collected from Jinju in Korea in September 2015, and were authenticated by Institute of Agriculture and Life Sciences, Gyeongsang National University where voucher specimens were maintained. These samples were stored at -20 °C until use.

Jujube leaf extracts

Each solvent fraction of freeze-dried jujube leaf was obtained in the following manner. Powdered jujube leaves (500 g) were put in suspension and extracted using 500 ml of 80% ethanol at 80 °C for 3 h. The extracts were filtered through Whattman No. 2 filter paper (Whatman International Limited, Kent, England) and evaporated to dryness. The dried material was dissolved in 200 mL of distilled water. The solution was consecutively portioned in a separation funnel with the equivalent amount of chloroform, butanol, and water. Each fraction was concentrated in a vacuum evaporator (Eyela NE, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 40 °C. The water filtrate was frozen and lyophilized. The fractions were placed in a glass bottle and stored at -20 °C until use. The lyophilized fractions were dissolved in 10% DMSO to obtain a concentration of 1000 μg/mL.

ABTS radical scavenging activity

To test the free radical scavenging ability of the three jujube leaf extracts, a 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assay was used. ABTS was dissolved in water to obtain a stock concentration of 7 mM. ABTS working solution was prepared by allowing the ABTS stock solution to react with 2.45 mM potassium persulfate (final concentration) and further allowing the mixture to stand in the dark at room temperature for 12-16 h before use. For the study of leaf extract sample, the ABTS stock solution was diluted with 5 mM phosphate-buffered saline (pH 7.4) to obtain an absorbance of 0.70 at 734 nm. After the addition of 980 μL of diluted ABTS to 20 μL of the sample, the absorbance reading was taken 5 min after the initial mixing (Jeong et al., 2010). This antioxidant activity is stated as percent ABTS scavenging that is calculated as:

% ABTS scavenging activity = \[ \frac{(control \ absorbance - sample \ absorbance)}{(control \ absorbance)} \times 100 \]

Ferric reducing antioxidant power (FRAP) of jujube leaf extracts

The FRAP assay used in this study is described in Jeong et al. (2010). Briefly, 1.5 mL of working, prewarmed FRAP reagent (10 volumes 300 mM acetate buffer, pH 3.6 + 1 vol of 10 mM dihydroxyacetone (DHA), 3-[4,5-dimethoxyanisole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay kit, and the lactate dehydrogenase (LDH) assay kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY, USA).
2,4,6-tripyridyl-S-triazine in 40 mM HCl + 1 vol of 20 mM FeCl₃ at 37 °C was mixed with 50 μL of the leaf extract fractions and standards. This was vortex mixed and the absorbance read at 593 nm against a reagent blank at a predetermined time after sample-reagent mixing. The test was performed at 37 °C and the 0-4 minutes reaction time window was used.

**Malondialdehyde (MDA) assay using mouse brain homogenates**

This assay was carried out using the method described by Chang et al. (2001). The brains of young adult male Balb/c mice were dissected and homogenized in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 12,000 x g for 15 minutes at 4 °C. A 1 mL aliquot of the supernatant was incubated with the test leaf extract samples in the presence of 10 μM FeSO₄ and 0.1 mM vitamin C at 37 °C for 1 h. The reaction was terminated by the addition of 1 mL TCA (28%, w/v) and 1.5 mL TBA (1%, w/v) in succession, and the solution was then heated at 100 °C. After 15 min, the absorbance of the MDA-TBA complex was measured at 532 nm. (+)-Catechin, a well-known antioxidant, was used as a positive control. The inhibition ratio (%) was calculated as follows:

% inhibition = [(control absorbance – sample absorbance) / control absorbance] x 100

**Neuronal cell culture**

The PC12 cell line was derived from a transplantable rat pheochromocytoma. The cells respond reversibly to nerve growth factor (NGF) by induction of the neuronal phenotype. PC12 cells (KCLB 21721, Korea Cell Line Bank, Seoul, Korea) were propagated in RPMI 1640 medium containing 10% fetal bovine serum, 25 mM HEPES, 25 mM sodium bicarbonate, 50 units/mL penicillin, and 100 μg/mL streptomycin.

**Measurement of intracellular oxidative stress**

Levels of intracellular reactive oxygen species (ROS) were measured using the 2',7'-dichlorofluorescein diacetate (DCF-DA; fluorescent probe) assay (Heo et al., 2001). Briefly, cells (10⁴ cells/well on 96-well plates in 100 μL of RPMI. The cells were pre-incubated with the butanol fraction for 48 h before 200 μM of H₂O₂ were added. The cells were treated with or without H₂O₂ for 2 h. The amount of MTT formazan product was determined by measuring absorbance using a microplate reader (680, Bio-Rad, Tokyo, Japan) at a test wavelength of 570 nm and a reference wavelength of 690 nm.

**Lactate dehydrogenase assay**

Neuronal PC12 cells, treated as described in the above paragraph, were precipitated by centrifugation at 250 x g for 4 min at room temperature, 100 μL of the supernatant were transferred into new wells, and lactate dehydrogenase (LDH) concentration was measured using the in vitro toxicology assay kit (TOX-7, Sigma Co., St. Louis, MO, USA). Damage to the plasma membrane was evaluated by measuring the amount of the intracellular enzyme LDH released into the medium.

**Determination of total phenolics**

Total phenolic content was determined using spectrophotometric analysis (Jeong et al., 2010). Briefly, a 1 mL portion of the appropriately diluted extract was added to a 25 mL volumetric flask containing 9 mL of deionized distilled water (ddH₂O). A reagent blank using ddH₂O was prepared. One mL of Folin-Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of a 7% Na₂CO₃ solution was added and the solution was mixed. The mixed solution was then immediately diluted to a volume of 25 mL with ddH₂O and mixed thoroughly. After 90 min at 23 °C, the absorbance was read at 750 nm. The standard curve for total phenolics was made using gallic acid standard solution (0-100 mg/L) following the same procedure as above. Total phenolics in each solvent fraction were expressed as milligrams of gallic acid equivalents (mg GAE/g) of the sample.

**Determination of phenolics**

Phenolic compounds in the butanol fraction were measured at 280 nm using 11 phenolics standard solution using a diode array UV-visible detector (Agilent 1100 series, Agilent Co., Santa Clara, CA, USA). Separation was achieved with a LiChrospher 100 RP-18 column (250 mm x 4.6 mm id, 5 μm, Merck Co., Darmstadt, Germany). The elution solvents were (A) 0.01 M-potassium phosphate buffer adjusted to pH 3.0 by phosphoric acid, and, (B) methanol. The solvent gradient elution program used was as follows: initial 90% (A), hold for 9.5 min; linear gradient to 68% (A) in 3.5 min; linear gradient to 67% (A) 17 min; linear gradient to 20% (A) 1 min; linear gradient to 90% (A) 1 min, and hold for 10 min. The flow rate was 1.5 mL/min. Phenolics were identified by comparison of their retention time (RT) values and UV spectra with those of known standards and quantified by peak areas from the chromatograms. All analyses were run in triplicate and mean values were calculated. The content of phenolic compounds was expressed in mg/100 g extract.
Statistical analysis

All data are expressed as mean ± SD (n = 3). Data from each experimental were analyzed using a one-way analysis of variance (ANOVA) and Duncan’s multiple-range test (p<0.05). (SAS program, SAS Institute, Cary, NC, USA)

RESULTS AND DISCUSSION

Antioxidant activity of jujube leaf extracts as assessed by the ABTS and FRAP assays

All fractions from jujube leaf exhibited varying amounts of ABTS radical-scavenging activity in a concentration-dependent manner although the activity levels of all of the tested samples were lower than that of vitamin C. The butanol fraction from jujube leaf showed the highest radical-scavenging activity in the ABTS assay (Fig. 1A). In contrast, the chloroform and water fractions showed low radical-scavenging activity; these were approximately 3-4 times lower than the activity of the butanol fraction. Fig. 1A shows a steady increase in the percentage inhibition of the ABTS radicals by the butanol fraction of jujube leaf and maximum inhibition was achieved when the extract concentration was above 1000 μg/mL. The water fraction showed a percentage inhibition of less than 50% at the highest concentration studied (1000 μg/mL). In addition, the chloroform fraction from jujube leaf did not show a leveling off at the highest concentration; however, its radical scavenging activity was much lower than that of the butanol fraction. Free radical (ABTS) scavenging activity of jujube leaf might be due to the presence of high molecular phenolics such as flavonoids (Lin et al., 2008).

In the FRAP assay, jujube leaf extract fractions were used in a redox-linked reaction in which the antioxidants present in the sample act as the oxidants. Reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue color that can be measured at a wavelength of 593 nm. The intensity of the color is related to the amount of antioxidant reductants present in the extracts. The trend for ferric ion-reducing activities of different fractions from jujube leaf in the present study is shown in Fig. 1B. For chloroform, butanol, and water fractions, the absorbance clearly increased due to the formation of the Fe²⁺-TPTZ complex with increasing concentration. The highest reducing activity was observed in the butanol fraction, compared to the other two fractions (Fig. 1B). Similar to the results obtained from the ABTS assay, the butanol fraction showed relatively strong ferric ion-reducing activity. Chloroform and water fractions showed lower ferric ion-reducing activities. A correlation between the mean values of the total phenolic content and FRAP deserves detailed attention, as phenolics

FIGURE 1. ABTS radical scavenging activities (A) and ferric reducing antioxidant power (B) of jujube leaf extract. Ascorbic acid; Chloroform fraction; Butanol fraction. Water fraction. Data are presented as the mean ± SD of 3 independent experiments in triplicate; different letters represent significant differences between means. *p < 0.05

FIGURE 2. Inhibitory effect of the butanol fraction of jujube leaves on both ferric ion and vitamin C-induced lipid peroxidation on mouse brain homogenates. Butanol fraction; Catechin. Data are presented as the mean ± SD of 3 independent experiments in triplicate; different letters represent significant differences between means. *p < 0.05
in jujube leaf were the most likely candidate compounds capable of reducing ferric ions. According to recent reports, there was a strong correlation between total phenolics and antioxidant activity in many plant species (Dasgupta et al., 2004; Dorman et al., 2004). In addition, many phenolics have shown high levels of antioxidant activity.

**Inhibitory effect of jujube leaf extract on lipid peroxidation**

There has been increasing interest in lipid peroxidation because the formation of cytotoxic products such as MDA and 4-hydroxynonenal can influence cellular apoptosis and several human diseases (Sevanian et al., 2000). Therefore, in this assay, antioxidant activities of three fractions from jujube leaf on both ferric ion- and vitamin C-induced lipid peroxidation on mouse brain homogenates were also confirmed. The results, shown in Fig. 2, reveal that the three fractions of jujube leaf had inhibitory effects against lipid peroxidation of the mouse brain homogenates. The inhibitory effects of all fractions against lipid peroxidation decreased in the following order: butanol fraction > water fraction > chloroform fraction. The butanol fraction showed excellent suppression activity against lipid peroxidation in mouse brain homogenates. The butanol fraction had a stronger inhibitory effect than (+)-catechin at all concentrations tested and more than 50% of inhibitory activity against lipid peroxidation was observed at the 25 µg/mL concentration. It is also noteworthy that (+)-catechin, which has an EC$_{50}$ value of 24.08 µg/mL, showed less inhibitory activity than the butanol fraction (EC$_{50}$ value of 19.94 µg/mL). Therefore, the active compounds in the butanol fraction might be potential natural antioxidant supplements for food and pharmaceutical products. They might also be used to stabilize foods against oxidative deterioration.

**Measurement of intracellular oxidative stress**

To examine the intracellular accumulation of ROS in PC12 cells, used as neuronal cell models, 2',7'-dichlorofluorescein diacetate (DCF-DA) was used. The DCF-DA probe, which is freely permeable across cell membranes, is hydrolyzed by cytosolic esterases to non-fluorescent dichlorofluorescein (DCFH). Then, DCFH interacting with ROS is oxidized to a highly fluorescent substance, 2',7'-dichlorofluorescein (DCF). Exposure of PC12 cells to H$_2$O$_2$ for 2 h resulted in a 139.21% increase of ROS levels compared to controls (Fig. 3). Pretreatment of PC12 cells with the butanol fraction significantly prevented them from intracellular ROS accumulation in comparison to control PC12 cells that were treated with only H$_2$O$_2$.

Vitamin C is one of the naturally occurring major nutrients having antioxidant activity (Kim et al., 2002). Vitamin C was used as a positive control. Pretreatment with 200 µM vitamin C of PC12 cells resulted in significantly lower oxidative stress compared to PC12 cells with H$_2$O$_2$ treatment alone (Fig. 3). Oxidative stress in AD may result from aging, energy deficiency, inflammation or excessive production of amyloid β protein (Aβ). Aβ levels can trigger cell death through a mechanism involving hydrogen peroxide (Behl et al., 1994).

These results suggest that the butanol fraction of jujube leaf has antioxidant activity and may be able to play an important role in reducing oxidative stress, an important risk factor for neurodegenerative diseases such as AD.

**Influence of the jujube leaf butanol fraction on the viability of neuronal cells treated with H$_2$O$_2$**

Alteration in the mitochondrial permeability transition pore protein occurs in cells undergoing apoptosis (Salet et al., 1997) and this is related to the release of cytochrome c (Ott et al., 2001). Mitochondria might be one of the main targets of oxidative stress causing neuronal cell death. Fig. 4 shows the improved cell viability of PC12 cells under oxidative stress, measured using an MTT assay, being mainly attributed to bioactive phenolics derived from the jujube leaf butanol fraction. MTT is converted to purple formazan by living cells, in part, because of mitochondrial processes. A significant color difference was observed between control neuronal cells with no H$_2$O$_2$ treatment and groups treated with the butanol fraction at 100-200 µg/mL followed by H$_2$O$_2$ exposure (Fig. 4). The treatment with H$_2$O$_2$ for 2 h decreased the viability of PC12 cells up to 65.16% compared to the control (100%). At a concentration of 100-200 µg/mL, the butanol fraction provided effective protection that resulted in an acceptable PC12 cell viability against oxidative stress. At 200 µg/mL concentration of the jujube leaf butanol fraction, the viability of PC12 cells significantly increased up to 99.00% that of the control cells. Therefore, these results also suggest that neuronal cell protection conferred by the butanol fraction of jujube leaf extract is partially due to mitochondrial protective mechanisms.

**FIGURE 3. Effect of the butanol fraction of jujube leaves on ROS production in the presence and absence of H$_2$O$_2$ in PC12 cells.** Data are presented as the mean ± SD of 3 independent experiments in triplicate; different letters represent significant differences between means. *p < 0.05
Neuronal cell protective effect of jujube leaf

Protective effect of the butanol fraction of jujube leaves against H$_2$O$_2$-induced membrane damage

The neuronal membrane, containing polyunsaturated fatty acids, is vulnerable to oxidative stress induced by ROS such as H$_2$O$_2$. Lipid peroxidation can alter the fluidity of the plasma membrane. The LDH assay provided an estimate of the percentage of surviving PC12 cells whose cell membranes were intact. The butanol fraction protected the integrity of the cellular membrane at all the concentrations tested (Fig. 5). After oxidative treatment with H$_2$O$_2$ for 2 h, the amount of LDH release from PC12 cells increased to 63.88% compared to that from the untreated controls. However, the various levels of butanol fraction treatment showed protective effects against oxidative stress in neuronal cells and followed a dose-dependent pattern (Fig. 5). Treatment of PC12 cells with 100-200 μg/mL concentration of the butanol fraction resulted in

![FIGURE 4](https://via.placeholder.com/150)

**FIGURE 4.** Protective effect of the butanol fraction of jujube leaves against H$_2$O$_2$-induced cell death in PC12 cells. PC12 cells were pretreated for 48 h with various concentrations of the butanol fraction. The cells were then treated with 200 μM H$_2$O$_2$ for 2 h. Cell viability was measured using the MTT assay kit. Vitamin C (200 μM) was used as a positive control. Data are presented as the mean ± SD of 3 independent experiments in triplicate; different letters represent significant differences between means. *p < 0.05

![FIGURE 5](https://via.placeholder.com/150)

**FIGURE 5.** Inhibitory effect of butanol fraction on LDH release in H$_2$O$_2$-treated PC12 cells. Cells, first treated with the butanol fraction, were then treated with 200 μM H$_2$O$_2$ for 2 h. The LDH activity in culture supernatants was measured with a colorimetric LDH assay kit. Vitamin C (200 μM) was used as a positive control. Data are presented as the mean ± SD of 3 independent experiments in triplicate; different letters represent significant differences between means. *p < 0.05

![HPLC chromatogram](https://via.placeholder.com/150)

**FIGURE 6.** HPLC chromatogram of standards (A) and butanol fraction of jujube leaves (B). Retention time 19.916 min: rutin, 20.578 min: quercetin.
Neuronal cell protective effect of jujube leaf

significant protection compared to the group treated with H$_2$O$_2$ only (Fig. 5). This finding suggests that the butanol fraction of jujube leaf might attenuate the extent of oxidative stress damage from H$_2$O$_2$ insult in neuronal PC12 cells and also protect the integrity of the cell's biological membrane. Our results suggest that the phenolics of the butanol fraction might be inhibiting neuronal apoptosis, which is the ultimate consequence of all these cellular dysfunctions. Therefore, phenolics of the butanol fraction may provide an added health benefit by reducing the risk of neurodegenerative diseases such as AD.

**TABLE 1. Total phenolics of three fractions and phenolics content of butanol fraction from jujube leaf.** The data are presented as mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention time (min)</th>
<th>Content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procatechuic acid</td>
<td>5.512</td>
<td>-</td>
</tr>
<tr>
<td>Catechin</td>
<td>7.796</td>
<td>-</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>9.863</td>
<td>-</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>10.021</td>
<td>-</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>11.467</td>
<td>-</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>11.853</td>
<td>-</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>14.032</td>
<td>-</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>15.039</td>
<td>-</td>
</tr>
<tr>
<td>Rutin</td>
<td>18.916</td>
<td>57.07</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>20.578</td>
<td>9.27</td>
</tr>
<tr>
<td>Quercetin</td>
<td>22.933</td>
<td>-</td>
</tr>
</tbody>
</table>

**Total phenolics of three fractions and individual phenolic composition of butanol fraction from jujube leaf**

Phenolic compounds, such as flavonoids, phenolic acid, and tannins are considered major contributors to the antioxidant activity of natural plants. These antioxidants also
possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic activities that may be associated with their antioxidant activity (Chung et al., 1998). The total phenolics of three fractions from the 80% ethanol extract of jujube leaf are presented in Table 1. The butanol fraction had the highest phenolic content (297.18 mg GAE/g). It is approximately 4-fold more than the phenolic content of the chloroform fraction (72.50 mg GAE/g) and 17-fold more than the phenolic content of the water fraction (17.93 mg GAE/g).

Jujube leaf extract fractions were subjected to further analysis by HPLC. The butanol fraction from jujube leaf contained two phenolic compounds. By comparing the retention time and UV spectra of these compounds to those of standards, rutin was identified as the main phenolic compound (Fig. 6). Furthermore, the HPLC results indicated that rutin (57.07 mg/g) was the predominant phenolic compound in the butanol fraction of jujube leaf extract (Fig. 6 and Table 1). Based on the results of the phenolic composition of the butanol fraction, we can conclude that these compounds (particularly rutin and quercitrin) contribute to the antioxidant and cell protective effects of jujube leaf in neuronal cells. The results obtained from this study are noteworthy, not only with respect to the antioxidant and neuronal cell protective effects of jujube leaves but also with respect to its rutin content. The activity of jujube is attributed to these phenolic compounds and specifically rutin. The main phenolic compound found in onion and buckwheat is rutin. Rutin is a compound of interest because it has it a wide range of biological effects that include including antioxidant, anticarcinogenic, antimicrobial activities, and anti-neurodegenerative effects (Dreosti et al., 1997; Jankun et al., 1997; Almajano et al., 2008; Heo et al., 2004).

In this context, the increased cell viability associated with jujube leaf extract may be mainly attributed to rutin, and to a lesser extent, to the other antioxidant phenolics. Finally, our results verified that compounds in the jujube leaf have very strong antioxidant activities, and suggest that the jujube leaf can be utilized as an effective natural antioxidant source and chemopreventive agent against neurodegenerative disease such as Alzheimer’s disease. Further studies are needed to determine the relationship between the specific antioxidant and neuroprotection using in vivo tests.

CONFLICT OF INTEREST DISCLOSURE
We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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