Objectives: This study was performed to investigate the effect of *Jaeumgeonbigagamtang* (JGT) on restraint-induced oxidative stress in the mouse brain.

Methods: After treatment with JGT, CBC, ROS, MDA, TAC, SOD, activity of catalase, and total GSH content were analyzed.

Results: JGT had a strong antioxidant activity by in vitro assay as presented GEAC. JGT treatment significantly ameliorated decrease of blood WBC and increase of platelet count. JGT (50 mg/kg) treatment significantly ameliorated increase of MDA and GSH content level in brain tissue. JGT (100 mg/kg) treatment significantly ameliorated increase of MDA and activity of TAC level in brain tissue. JGT (200 mg/kg) treatment significantly ameliorated increase of ROS, MDA, activity of TAC level and depletion of catalase level in brain tissue.

Conclusion: The present study demonstrated antioxidant activity in brain tissue. This result would be consistent with the long clinical efficacy of JGT, and this finding may provide a strong possibility of JGT as a drug candidate for brain-specific multiple disorders and symptoms.

**Key Words**: *Jaeumgeonbigagamtang* (JGT), oxidative stress, antioxidant activity, malondialdehyde (MDA)

Introduction

With a rapidly aging society, degenerative brain diseases and brain vascular disorders such as dementia, Alzheimer's disease, Parkinson's disease, and stroke become abundant. These brain illnesses induce impairment in psychological and physical activity of their victims, and raise serious social troubles of economic cost and total dependency in their final years. So, there are urgent requirements to identify effective prevention and treatment strategies for these degenerative brain diseases.

On the other hand, oxidative stress has been thought to be linked to neurodegeneration and brain injury. Oxidative stress represents an imbalance status between the production of reactive oxygen species (ROS) and a capacity to readily detoxify the reactive intermediates, and this condition cause toxic effects resulting in damage of all cellular components, including proteins, lipids, and DNA. Therefore, many studies have focused on inhibition of oxidative stress to obtain neuroprotective actions.

Many traditional herbal medicines have been also studied for their neuroprotective effects related to the antioxidant properties of drug candidates. Tanshinone IIA (Tan IIA), an active compound of *Salvia miltiorrhiza Bunge*, showed a protective effect against cortical neuron cells via antioxidant activity.
Artemisia absinthium was demonstrated to have pharmaceutical effects on cerebral oxidative stress and damage, and behavioral disturbances induced by cerebral ischemia and reperfusion injury in rats[11].

Jaeumgeonbitang is a traditional herbal formula which is composed of fifteen herbal plants. This drug has been clinically prescribed to patients suffering from symptoms of dizziness and anxiety[12]. So far, several studies have reported on the pharmaceutical effects of Jaeumgeonbitang using animal models such as sedative action and hematomia, indomethacin-induced gastritis, and learning test[13-15]. Jaeumgeonbitang or modified-Jaeumgeonbitang improved brain circulation[6,17].

Jaeumgeonbitang (JGT) is a new herbal formula composed of nineteen medicinal herbs. JGT has been prescribed for patients with various range of dizziness in Oriental Hospital of Daejeon University since 2000, and its clinical efficacy on 70 patients with dizziness was reported[18]. However, no study had been performed to study its antioxidant effect.

In order to support the underlying mechanisms of clinical effect of JGT on brain disorders, the present study investigated the antioxidant activity of JGT in brain tissue using a restraint-rat model.

1. Composition of Jaeumgeonbigagamta (JGT)

JGT is composed of nineteen medicinal herbs (Table 1.). The manufacturing process for JGT follows the process given in over-the-counter Korean monographs by Hanpoong Pharmacy (Seoul, Korea). Briefly, the nineteen herb mixture was boiled in distilled water for 4 hours at 100°C, then filtered using a 300-mesh filter (50μM) and then condensed. We obtained a 23% (w/w) lyophilized JGT aqueous extractions from the dried mixture, and stored it

Table 1. Composition of Jaeumgeonbigagamta (JGT)

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Part used</th>
<th>Amount (g)</th>
<th>Composition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrodia elata</td>
<td>Rhizoma</td>
<td>6</td>
<td>13.8</td>
</tr>
<tr>
<td>Adenophora triphylla</td>
<td>Radix</td>
<td>6</td>
<td>13.8</td>
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<tr>
<td>Atractylis japonica</td>
<td>Rhizoma</td>
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<tr>
<td>Citrus unshiu</td>
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</tr>
<tr>
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<td>Rhizoma</td>
<td>2</td>
<td>4.6</td>
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<tr>
<td>Poria cocos</td>
<td>sclerotum</td>
<td>2</td>
<td>4.6</td>
</tr>
<tr>
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<td>Radix</td>
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<td>4.6</td>
</tr>
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<td>2</td>
<td>4.6</td>
</tr>
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<td>Radix</td>
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<td>4.6</td>
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<td>4.6</td>
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<td>4.6</td>
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<tr>
<td>Ledebouriella seseloides</td>
<td>Radix</td>
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<td>Fructus</td>
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<td>Rhizoma</td>
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<td>4.6</td>
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<tr>
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<tr>
<td>Poria cocos</td>
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<td>1.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Glycyrrhiza uralensis Fisch</td>
<td>Radix</td>
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<td>3.4</td>
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<tr>
<td><strong>Total amount</strong></td>
<td></td>
<td><strong>43.6</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Effect of Jaeumgeonbigamamtang (JGT) on Restraint-induced Oxidative Stress in Mouse Brain

2. High performance-thin layer chromatography (HP-TLC)-based fingerprinting

In order to produce the fingerprint of JGT, the HP-TLC procedure was adapted using the CAMAG application system (Muttenz, Switzerland). Aqueous extracts of JGT, and ginsenoside Rg1 and Rg3 as control, were dissolved in HPLC-grade methanol and applied to pre-washed 60 F254 HP-TLC plates (silica gel thickness 2 mm, from Merck, Darmstadt, Germany) with an automated applicator (Linomat IV; CAMAG). JGT were separated (migration distance 65 mm) using HPLC-grade chloroform:ethyl acetate:methanol:water=17:46:25:12 and then were visualized under white light after derivatization with 10% sulfuric acid solution. Photos were taken using Reprostar 3 with a digital camera and densitograms were generated (Fig. 1.).

3. Determination of total antioxidant capacity (TAC) of JGT in vitro

TAC of JGT in vitro was determined according to the modified Kambayashi method. 90 μl of 10 mM phosphate-buffered saline (pH 7.2), 50 μl of myoglobin solution (45 μM), 20 μl of 3 mM ABTS solution, and serially diluted JGT (5 μg to 1,000 μg/ml) sample were added to 96-well microplates and mixed well at 25°C. Then 20 μl of H2O2 was added to each well, and incubated for 5 minutes. The absorbance was read using a plate reader at 600 nm (Molecular Device Corp, USA). Gallic acid was used as a control and antioxidant activity was expressed as gallic acid equivalent antioxidant capacity (GEAC).

4. Animals and experimental design

Specific pathogens free six-week old BALB/c male mice were purchased from a commercial animal breeder (Coatech, Gyeongido, Korea). 35 animals were acclimated for 1 week and housed in an environment-controlled room at 22±2°C, on a 12 hours light/dark cycle, and provided commercial pellets (Samyang Feed Co., Korea) and tap water ad libitum. The experiments were designed and performed in strict accordance with the regulations for laboratory animal care (NIH publication No. 85-23, revised 1985) and were approved by the Institutional Animal Care and Use Committee of Daejeon University (animal ethical clearance number: DJUARB 2010-065).

35 animals were divided into 5 groups; normal, induced, JGT 50, JGT 100, and JGT 200 mg/kg group, with 7 mice in each group, respectively. Except the normal group, four groups were orally administrated with 50, 100, 200 mg/kg of JGT or water daily for 5...
days, and then each animal was completely tied down on the wire rack for 6 hours in order for induction of severe stress. All animals were sacrificed by whole blood collection from abdominal aorta under ether anesthesia. The cerebrum was removed and frozen in a deep-freezer.

5. Observation of complete blood count (CBC)

Blood was collected from the abdominal aorta of each mouse and complete blood count was analyzed by blood cell counter (HEMAVET; CDC Technologies, Irvine, CA, USA).

6. Oxidative stress-related parameters in brain tissue

1) Determination of total reactive oxygen species (ROS)

Total ROS level in brain tissue were determined according to the method of Hayashi20). Briefly, 100 mg of brain tissue was homogenized with RIPA buffer and centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant fraction was removed into a clean tube and diluted with 10 mM phosphate-buffered saline (pH 7.2). Hydrogen peroxide was used for generating the calibration curve as standard. DEPPD solution and ferrous sulfate solution (100 μg/ml of DEPPD and 4.37 μM of ferrous sulfate was dissolved in the 0.1 M sodium acetate buffer separately) were prepared beforehand. Five microliters of standard solution or 1:10 diluted liver homogenate supernatant were added to 120 μl of 0.1 M sodium acetate buffer (pH 4.8) in each well of 96-well plates. After 5 minutes incubation at 37°C, 100 μl of DEPPD and ferrous mixture solution (scale factor of 1/25 was used) were added to each well. The level of ROS was determined at 505 nm using a spectrophotometer with catalytic capability for transition metals, and calculated as equivalent to levels of hydrogen peroxide (1 unit = 1 μg H2O2/ μl).

2) Determination of malondialdehyde (MDA)

Lipid peroxidation levels in the brain tissue were determined using the method of thiobarbituric acid reactive substances (TBARS)21). The concentration of TBARS was expressed as μM MDA/g tissue using 1,1,3,3-tetraethoxypropane (TEP) as a standard. Briefly, 0.2 g brain tissue was homogenized in 2 ml ice-cold 1.15% KCl, and 0.13 ml homogenate was mixed with 0.08 ml 1% phosphoric acid and 0.26 ml 0.67% thiobarbituric acid (TBA). After heating the mixture for 45 minutes in a dry oven (100°C), 1.03 ml n-butanol was added followed by a vigorous vortexing and centrifugation at 3,000 rpm for 15 minutes. The absorbance of upper organic layer was measured at 535 and 525 nm with a spectrophotometer and compared with TEP standard curve.

3) Determination of total antioxidant capacity (TAC)

TAC levels were determined according to the method of Kambayashi19. Briefly, 100 mg of brain tissue was homogenized with RIPA buffer and centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant fraction was removed into a clean tube and diluted with 10 mM phosphate-buffered saline (pH 7.2). Ninety microliters of PBS was added to 96-well micro plates and 50 μl of myoglobin solution (45 μM), 20 μl of 3 mM ABTS solution, 20 μl of diluted homogenized samples, and gallic acid were added to 96-well microplates and mixed well at 25°C for 3 minutes. Then 20 μl of H2O2 was added to each well, and incubated for 5 minutes. The absorbance was read using a plate reader at 600 nm (Molecular Device Corp., USA). The level of TAC was expressed as GEAC.

4) Determination of activity of superoxide dismutase (SOD)

Briefly, 100 mg brain tissue was homogenized with RIPA buffer and centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant fraction was transferred into a clean tube, and used to determine the SOD
activity. SOD activity in the brain tissue was determined using an SOD assay kit (Dojindo Laboratories, Kumamoto, Japan). Bovine erythrocyte SOD (Sigma) was diluted serially from 100 to 0.001U/mL and used as a standard.

5) Determination of activity of catalase
Catalase activity in the brain tissue was determined using the method of Beers and Siezert. Briefly, 100mg brain tissue was homogenized with RIPA buffer and centrifuged at 10,000 × g for 15 minutes at 4℃. The supernatant fraction was transferred into a clean tube, and used to determine the catalase activity. The supernatant or standard solution (100μl) were mixed with 2.9μl of substrate solution (0.0036% hydrogen peroxide), followed by measurement of the absorbance at 240nm after 5 minutes.

6) Determination of total glutathione (GSH) content
Total GSH content was determined according to the method of Ellman. Briefly, 100mg brain tissue was homogenized with RIPA buffer and centrifuged at 10,000 × g for 15 minutes at 4℃. The supernatant fraction was transferred into a clean tube. Duplicate 50μl aliquots of the supernatant (or GSH standard) were combined with 80μl of a previously prepared DTNB/NADPH mixture (10μl 4mM DTNB and 70μl 0.3mM NADPH) in a 96-well plate. Finally, 20μl (0.06U) of GSH reductase solution was added to each well and the absorbance was measured at 405nm after 5 minutes.

7. Statistic analysis
Results were expressed as the mean ± standard deviation (S.D.). Statistical analysis of the data was carried out by Student’s t-test. A difference from the respective control data at the levels of p<0.05 were regarded as statistically significant.

Results

1. Total antioxidant capacity (TAC) of JGT
In order to examine the antioxidant capacity of JGT, TAC was determined using in vitro assay. When the value was expressed as GEAC, the capacity of JGT showed a very high GEAC value from the lowest volume 5μg (110.6μM GEAC) to the highest volume 1,000μg (325.4μM GEAC) of A. capillaris respectively (Fig. 2.).

2. Change of complete blood count (CBC)
On the final day, hematological parameters were measured (Table 2.). 6 hours of restraint stress signi-
significantly decreased the number of total white blood cells (WBC) compared with the naive group. In the compositional ratio of 5 white blood populations, neutrophils significantly increased while lymphocyte, eosinophil, and basophil decreased compared to the naive group. The number of platelets significantly increased in the control group compared to the naive group. Pre-treatment with JGT significantly ameliorated these changes; eosinophil, basophil, and platelet counts in the 100 and 200 mg/kg groups.

3. Change of total reactive oxygen species (ROS)

On the last day of the experiment, total ROS levels in rat brain were compared among groups. 6 hours of restraint stress significantly increased total ROS level compared to the naive group (p<0.05). Pretreatment with JGT (200 mg/kg) significantly ameliorated the increase of ROS compared to the control (p<0.05, Fig. 3.).

4. Change of malondialdehyde (MDA)

On the last day of the experiment, MDA levels in

![Fig. 3. Brain total ROS level.](Image)

Mice were pre-treated with water, JGT (50, 100, 200 mg/kg) before restraint stress. On the last day of the experiment, total ROS level in brain tissue was determined. Data are expressed as mean ± S.D. #: p < 0.05, significant differences compared with the naive group. *: p < 0.05, significant differences compared with the induced group.
Effect of *Jaeumgeonbigamgamtang* (JGT) on Restraint-induced Oxidative Stress in Mouse Brain

rat brain were compared among groups. 6 hours of restraint stress drastically increased total MDA level compared to the naive group (p<0.01). Pre-treatment with JGT (50, 100, 200mg/kg) significantly ameliorated this increase of MDA compared to the control (p<0.01 or 0.05, Fig. 4).

5. Change of total antioxidant capacity (TAC)

On the last day of the experiment, total TAC levels in rat brain were compared among groups as GEAC. 6 hours of restraint stress significantly didn't change total TAC level compared to the naive group. Pre-treatment with JGT (100, 200mg/kg) significantly increased the TAC level compared to the control (p<0.05, Fig. 5).

6. Change of activity of superoxide dismutase (SOD)

On the last day of the experiment, SOD levels in rat brain were compared among groups. 6 hours of restraint stress drastically increased total MDA level compared to the naive group (p<0.01). Pre-treatment with JGT (50, 100, 200mg/kg) significantly ameliorated this increase of MDA compared to the control (p<0.01 or 0.05, Fig. 4).

**Fig. 4.** Brain malondialdehyde level.

Mice were pre-treated with water, JGT (50, 100, 200mg/kg) before restraint stress. On the last day of the experiment, total malondialdehyde level in brain tissue was determined. Data are expressed as mean ± S.D. ##: p < 0.01, significant differences compared with the naive group. *: p < 0.01, **: p < 0.05, significant differences compared with the induced group.

**Fig. 5.** Brain total antioxidant capacity.

Mice were pre-treated with water, JGT (50, 100, 200mg/kg) before restraint stress. On the last day of the experiment, total TAC level as GEAC in brain tissue was determined. Data are expressed as mean ± S.D. #: p < 0.05, significant differences compared with the induced group.
restraint stress didn’t induce any change of SOD level compared to the naive group. Pre-treatment with JGT also didn’t affect the level of SOD activity in brain compared to the control (Fig. 6.).

7. Change of activity of catalase

On the last day of the experiment, catalase levels in rat brain were compared among groups. 6 hours of restraint stress significantly depleted total catalase level compared to the naive group (p<0.01). Pre-treatment with JGT (200㎍/㎏) significantly ameliorated the decrease of catalase level compared to the control (p<0.05, Fig. 7.).

8. Change of total glutathione (GSH) content

On the last day of the experiment, total GSH content levels in rat brain were compared among groups. 6 hours of restraint stress didn’t affect total GSH content levels compared to the naive group. Pre-treatment with JGT (50㎎/㎏) significantly increased the GSH content levels compared to the control (p<0.05. Fig. 8.).
Effect of Jaeumgeonbigamamtang (JGT) on Restraint-induced Oxidative Stress in Mouse Brain

Discussion

Oxidative stress represents an imbalance between the production of ROS such as superoxide, hydrogen peroxide, or hydroxy radical and the ability to readily detoxify the reactive intermediates. Production of ROS is unavoidable to all aerobic organisms, thus there are various protective systems including free radical scavengers, SOD, catalase, and the glutathione oxidation/reduction system. Now, it is well recognized that oxidative stress is strongly linked to initiation and progress of diverse diseases, likely fatigue, inflammation, and cancer.

Oxidative stress also is an underlying basic etiology associated with many physiopathological process of brain aging, neuron injury, and cognitive function. Therefore, many medicinal plants or traditional herbal prescriptions applied for brain disorders have been found effective to prevent cerebral oxidative stress.

For example, Shengmai-San, a traditional formula composed of Panax ginseng, Ophiopogon japonicus and Schisandra chinensis, showed prevention of cerebral oxidative injuries in various animal models.

JGT is a herbal prescription originating from a famous traditional herbal formula, Jaeumgeonbitang. Jaeumgeonbitang is a herbal medicine typically prescribed to patients suffering from symptom of dizziness and anxiety. Jaeumgeonbitang or modified-Jaeumgeonbitang has shown positive effects in brain circulation and learning activities. There are reports that indicate an indirect association between brain oxidative stress and dizziness symptoms. JGT, a modified Jaeumgeonbitang, has been prescribed for patients with various ranges of dizziness in Oriental Hospital of Daejeon University since 2000. In order to explore the underlying mechanisms corresponding to clinical efficacy of JGT, we investigated its antioxidant effect using animal model.

To produce an appropriate animal model having brain oxidative stress state, mice were restrained for 6 hours by strongly tying them down to a wire rack. Psychological stress is well known as a critical factor of oxidative stress production. This restraint-induced stress caused oxidative stress in brain tissue of mice as especially evidenced by increased MDA levels. MDA is an aquantitative biomarker of lipid peroxidation resulted from degradation of polyunsaturated lipids by oxidative stressor including ROS. In this model, brain MDA level drastically elevated by about 40-fold, as in other experiments using a restraint model. As
per the hypothesis, JGT treatment significantly attenuated the increase of MDA levels by half that of the control.

Oxidative stressors should normally be eliminated by various antioxidant systems including catalase, SOD, and the glutathione system\(^41,42\). However, excessive production of ROS overwhelming the capacity of the antioxidant system over prolonged periods results in tissue or cellular damage. In this experiment, total ROS levels significantly increased while catalase levels dramatically depleted. However, JGT treatment significantly attenuated the increase of ROS level and depletion of brain catalase activity, especially at the highest dose (200 mg/kg) of treatment. Catalase functions to catalyze the decomposition of H\(_2\)O\(_2\) to water and oxygen\(^43\). On the other hand, the levels of SOD and GSH in brain tissue were not affected by restraint stress in this model. The 50 mg/kg JGT treatment enhanced the GSH level. This may be in accordance with the result for TAC activity of the drug itself, JGT using \textit{in vitro} assay.

These results indicate that JGT has a notable antioxidant activity, especially in brain oxidative stress status. Oxidative stress and cerebral microcirculation are closely associated with each other, and influence brain injury or pathogenesis of brain disease, including stroke or Alzheimer's disease\(^44,46\). There are several reports that presents the improvement of brain circulation by \textit{Jaeumgeonbitang}\(^16,17,47\). These data may support the antioxidant property of JGT.

Taken together, the present study proves the antioxidant activity in brain tissue. This result would be consistent with the long clinical efficacy of JGT, and this finding may provide a strong possibility of JGT as a drug candidate for multiple brain-specific disorders and symptoms.

**Conclusion**

In order to support the underlying mechanisms of clinical effect of JGT on brain disorders, the present study investigated the antioxidant activity of JGT in brain tissue using restraint-rat model.

1. JGT had a strong antioxidant activity by \textit{in vitro} assay as presented GEAC.
2. JGT treatment significantly ameliorated decrease of blood WBC and increase of platelet count.
3. JGT treatment (200 mg/kg) significantly ameliorated increase of ROS level in brain tissue.
4. JGT treatment (50, 100, and 200 mg/kg) significantly ameliorated increase of MDA level in brain tissue.
5. JGT treatment (100 and 200 mg/kg) significantly increased the activity of TAC level in brain tissue.
6. JGT treatment didn't change SOD level in brain tissue.
7. JGT treatment (200 mg/kg) significantly ameliorated depletion of catalase level in brain tissue.
8. JGT treatment (50 mg/kg) significantly increased the GSH content level in brain tissue.

From these results, the antioxidant property of JGT was evidenced. This study provides a scientific basis for the clinical application and drug development for patients with brain disorders in the future.

**Acknowledgement**

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**References**

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