Neuroprotective Effect of Temulawak (Curcuma xanthorhiza) on Braine Nerve Cell Damage Induced by Lipopolysaccharide (LPS)

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ABSTRACT

Alzheimer’s is a disease characterized by memory loss. This occurred as a result of permanent brain damage. Antiinflammatory and antihypertensive drugs that act by inhibiting an enzyme acetylcholinesterase can inhibit the occurrence of more severe brain damage. Temulawak has been reported to have anti-inflammatory effects. Therefore, temulawak may have ability to work as a protector of the brain nerves. This research was aimed to study the potency of temulawak as neuroprotector through the study of inhibitory activity against acetyl cholinesterase and protective effect against further brain cell damage due to LPS in vitro. The obtained results show that temulawak can inhibit the activity of the enzyme acetyl cholinesterase and prevent further brain damage due to LPS. This result indicates that the temulawak has potential as neuroprotector.

Keywords: Acetylcholinesterase, neuroprotector, anti-inflammatory effect, temulawak

INTRODUCTION

Brain nerve cells play an important role in regulating the activity of the body and intellectual capability. Thus the existent of nerve cell damage in the brain is capable to induce a neurologic disease in which the severity of brain damage is reflected in severity of disease symptoms.

Alzheimer’s disease is one of the brain diseases characterized by loss of intellectual capabilities, including memory and reasoning. Pathological progress of disease involves an imbalance in anabolism and catabolism of protein leading to extracellular accumulation of beta amyloid in the brain. Beta-amyloid protein (Abeta) and intracellular neurofibrillary tangles of hyperphosphorylated tau protein in the brain form extracellular plaques that induce the inflammation. In the absence of an appropriate presynaptic signal, plaque interferen with glutamatergic receptor function to prevent the influx of Ca(2+). This cause a loss of neurons, particularly those of expressing nicotinic acetylcholine receptors (nAChRs). The number of (nAChRs) receptors finally are reduced so that the amount of acetylcholine produced are decreased.

The development of plaques are also associated with microglial cell. It has been hypothesized that microglia cell play a role in plaque evolution. It also has been reported that bacterial lipopolysaccharide (LPS) endotoxins is able to affect microglial cell activity and stimulating the expression of pro-inflammatory factors.

Many plants have been studied for their potency to improve cognitive function and memory including Celastrus paniculatus Willd, Centella asiatica L., Clitoria ternatea L., and Curcuma Longa in India (Jayne 2003). There are also. Ginkgo biloba L., Huperzia serrata Thumb, Lycoris radiate Herb, Magnolia officinalis Rehder & Wilson, Polygala tenuifolia Willd, Salvia miltiorrhiza Bung (Jayne 2003) in china.  In Korean, plants that have been researched intensively are the rhizome Acorus calamus, Gramineus rhizome, Bupleurum falcatum root, Discorea batatas, Epimedium herb koreanum, and Zyzephy jujuba (Oh, 2004)

Curcumin is the secondary metabolite of Curcuma L which are also found in temulawak. Curcumin has antioxidant activity, anti-inflammatory, and inhibitory of-amyloid formation. In studies using animal models for Alzheimer Disease (AD), curcumin could suppress beta amyloid content to a level that was not harmful. Inhibitory activity of curcuma xanthorhiza against amyloid formation couple with the present of vasodilatatory activity against blood flow might be related to its capability to prevent the development of dementia (Jayaprakasha, 2006). Curcumin then is suggested to be able to improve cognitive function.
This research was focused on the study of the effect of temulawak extract on acetylcholinesterase activity and their potential activity to prevent brain damage induced by LPS in vitro. The inhibitory activity against acetylcholinesterase was tested by OH methods and theirs neuroprotector effect was tested using brain nerve cell culture isolated from the juvenile male mice. The curcumin content was also studied using HPLC.

**METHODOLOGY**

Temulawak were sliced, dried and grounded. It was followed by extracting the powdered plants materials by maceration method for two times (1:10) using ethanol 30 % as the solvent. The extract was concentrated in vacuo at 30 o C using a rotary evaporator, and the yield was then calculated. Powder of 50 mg was subjected in to methanol solvent by maceration. The extract was then sonificated and filtered by whatman 0.45 um. The extract was adjusted into volume of 50. mL and injected to HPLC. The curcumin content then was calculated.

Ability to inhibit the enzyme activity is measured by a modified colorimetric method based on the method of Ellman. Acetylcholinesterase and extract formula or physostigmin as positive control added to 2 ml of buffer pH 8 and prae incubated in ice (4 oC) for 30 min (Perry et al. 2000).

The same procedure was also performed on other tube containing a solution of physostigmin to provide opportunities for interference with the compounds that are tested and to controlled hydrolysis of ACh which was not due to AChE activity. Reaction began with addition of 5.5 ‘-dithio-bis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine (Ach) 20 mL. The mixture was incubated at a temperature at 37oC for 20 min. fisostigmin solution (α, 1 mM) was added to stop the reaction and then measured with a spectrophotometer at a temperature of 25oC with optical density measured at 412 nm. The inhibitory activity of formula against achetylchilinesterase was counted with referred to the activity of physostigmin.

**Newborn Rat Primary Midbrain Culture.**

The midbrain of newborn rat (Sprangue Dawley) was isolated and suspended homogenously in Phosphate Buffered Saline (PBS), followed by centrifugation. The neuron suspension (2x10⁴ cells/ml coverslips were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% Non Essential Amino Acid (NEAA), 10% Newborn Calf Serum (NBCS), and 50 µg/ml gentamycine and plated on to 0.1% gelatine coated tissue dish in 5% CO² incubator, at 37°C, until 50% confluent 

Medium were removed and the attached cells were rinsed with PBS, and the medium was replaced with DMEM without NCBS containing 5 µg/ml Lipopolysaccharide (LPS) for 2 hours. The LPS medium was removed and the attached cells were rinsed with PBS. The medium was replaced with DMEM without NCBS containing 100 ppm temulawak. The cell monolayers were further cultured for 6 days concurrently.

**Evaluation and Characterization.** The effects of temulawak were evaluated on the neuron and microglial proliferation and the neuron axon and dendrit lenght growth. All data are represented as the mean ± s.e.m. The statistical significance of differences was assessed using ANOVA, followed by T-test on Minitab program Student analysis. Statistical significance was defined as P<0.05 for all analysis.

**RESULTS AND DISCUSSION**

Microglial cells are one component of brain nerve cells that have an important role in monitoring the brain health. Microglia is activated and secrets various inflammatory and neurotoxic factor when there was subjected into an infection and nerve cell damage. The damage nerve cell was then eliminated by microglia and their’s chemical mediator (Kreutzberg GW, 1996, Gao et al., 2002, nelson et al., 2002).

![Figure 1](image1.png) Normal: Mid brain nerve cell without LPS confluent 50% at day 5th. Nerve body with glial cells and long axon

![Figure 2](image2.png) LPS Induced brain nerve cell damage indicated by vacuolization inside of microglial cell. Brain nerve cell at day 1st after LPS addition
LPS induced brain nerve cell damage in the media which was indicated by the present of vacuolization in the bodies cell (Fig 1 and 2) The amount of Microglia cell in the present of LPS with or without of temulawak extracts is depicted in Table 1. The number of survival nerve cell in the media containing LPS is lowered than that of nerve cells in media without LPS. LPS induced cell damage could be inhibited by temulawak extract, which was showed by the number of survival cell in the present of temulawak extract was higher than that of survival cell in LPS induced nerve cell damage media. Furthermore, nerve cells formed an axon and dendrite in control media. In the LPS induced damage of nerve cell media, however, axons and dendrites are not found instead of the microglia (Table 2).

Table 1. Level of proliteration and growth in the medium mDMEM and mDMEM+Temulawak

<table>
<thead>
<tr>
<th>Amount of Cell before Treatment</th>
<th>Treatment</th>
<th>Without LPS</th>
<th>With LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mDMEM</td>
<td>mDMEM</td>
<td>mDMEM+Temulawak</td>
</tr>
<tr>
<td></td>
<td>The Final Amount</td>
<td>The Final Amount</td>
<td>The Final Amount</td>
</tr>
<tr>
<td>2 x 10^4</td>
<td>25 x 10^4</td>
<td>2.0 x 10^4</td>
<td>7.0 x 10^4</td>
</tr>
<tr>
<td>2 x 10^4</td>
<td>20 x 10^4</td>
<td>4.5 x 10^4</td>
<td>14 x 10^4</td>
</tr>
</tbody>
</table>

Different superscrip letters on the same line indicate significant differences (P<0.05).

Table 2. The average length of axons and dendrites of neurons in the medium mDMEM and mDMEM+Temulawak

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without LPS</th>
<th>With LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mDMEM</td>
<td>mDMEM</td>
</tr>
<tr>
<td>Axons</td>
<td>120 ± 10</td>
<td>Microglia</td>
</tr>
<tr>
<td>Dendrites</td>
<td>30 ± 10</td>
<td>Microglia</td>
</tr>
</tbody>
</table>

Choi et al. (2011) reported that microglial cells have a dual role in the central nervous system in neurotoxic and neuroprotective effects. Uncontrolled and excessive activation of microglia often contributes to inflammation-mediated neurodegeneration. The rate of lives cell in the present of temulawak extract is higher in the media without temulawak extract. Temulawak extract then was suggested to inhibit the neurotoxic effect of chemical mediator which was secreted by activated microglial cell without affecting the survival microglial cell.

The extracts of temulawak contained curcumin, Desmethoxy curcumin. The amount of these components are 7.46, 6.23, 0.73 mg / kg respectively. The curcumin (Filipovic and Zecevic 2005) was reported to protect pre-oligodendrocytes from activated microglia in vitro and in vivo. He et al (2010). It was also found that apoptosis of pre-oligodendrocytes and expression of either iNOS or NOX in the LPS-activated microglia were inhibited by curcumin (Pendurthi et al., 1997, Weber et al.2006).

Thus the higher level of nerve brain cell of temulawak treated cell in the present of LPS was obtained in line with the anti-inflammatory effect of curcuminoid containing temulawak extract.

Figure 3. Inhibitory activity of temulawak extract on acetylcholinesterase

The cognitive symptoms of Alzheimer’s disease (AD) are caused not only by the loss of neurons in the cholinergic neural systems but also by the irregular functioning of surviving neurons in these systems. Aberrant cholinergic functioning in AD has been linked to deficits in the neurotransmitter acetylcholine (Wenk, 2006). The potential activity of temulawak to control of memory in AD was found in the figure 3. Temulawak affected the acetylcholinesterase, the enzyme that breaks down a parasympathetic neurotransmitter acetylcholine to be choline and acetate. The acetylcholinesterase inhibitory activity of temulawak causes the prolongation of acetylcholine activity.

Table 3. Curcuminoid content of Temulawak extract

<table>
<thead>
<tr>
<th>No</th>
<th>Tests</th>
<th>Parameter</th>
<th>Result</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>value</td>
<td>17.596</td>
<td>%</td>
</tr>
<tr>
<td>2</td>
<td>Yield</td>
<td></td>
<td>14.7554</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Curcumin</td>
<td></td>
<td>7.46</td>
<td>mg/g</td>
</tr>
<tr>
<td></td>
<td>Desmetoksicurcumin</td>
<td></td>
<td>6.23</td>
<td>mg/g</td>
</tr>
<tr>
<td></td>
<td>Bisdesmetoxicurcumin</td>
<td></td>
<td>0.75</td>
<td>mg/g</td>
</tr>
<tr>
<td></td>
<td>Xanthorrizol</td>
<td></td>
<td>67.698</td>
<td>mg/g</td>
</tr>
</tbody>
</table>

Inhibitory activity of temulawak extract can reach 80% at a concentration of 30 ppm. The increment of concentration, however, was not parallel with the increment of the inhibitory activity of temulawak extract against acetylcholinesterase.

HPLC analysis on curcurminoid content in temulawak extract showed that temulawak extract consist of curcumin, Desmetoksicurcumin, bisdesmetoxicurcumin and Xanthorrizol. The highest content of xanthorrizol has been reported for their antibacterial activity against bacterial induced teeth plaque. The curcurminoid and curcumin, desmetoksicurcumin as well as bisdesmetoxicurcumin of Curcuma longa L have been reported to be able to prevent cognitive disorder by interferent with acetylcholinesterase activity.

The lowest inhibitory activity against acetylcholinesterase among curcuminoid was found in curcumin. Unlike other curcuminoid, activity of curcumin against acetylcholinesterase has been reported as not being dose dependent. Since the profile of inhibitory activity of temulawak extract against acetylcholinesterase was not dose-dependent, therefore the inhibitory activity of temulawak extract against acetylcholinesterase was suggested to be dominated by curcumin.

CONCLUSION
Temulawak extract has inhibitory activity against acetylcholinesterase enzyme. Temulawak extract has also protective effect against LPS-induced brain nerve cell damage. Therefore, temulawak extract has potential activity as neuroprotector.

REFERENCES


Choi, DK. Koppula S. and Su, K...2011. Inhibitors of Microglial Neurotoxicity: Focus on Natural Products, Molecules, 16;1021-1043.


