Protective Effect of Mangosteen Extract Against Oxidative Stress Induced by β-amyloid in SK-N-SH Cells

Jiraporn Jantaravinid* Dr. Chatchawan Srisawat** Dr. Primchanien Moongkarndi*** Dr. Neelobol Neungton****

ABSTRACT

β-amyloid peptide is the main content of senile amyloid plaque, which is the key role for the pathogenesis of Alzheimer’s disease (AD). Many studies have reported that Aβ induces cytotoxicity and cell death mediated through the reactive oxygen species (Celsi et al.). *Garcinia mangostana* Linn. (mangosteen) has been recognized for strong antioxidant properties. This study aims to investigate the protective effect of mangosteen extract on Aβ(1-42) - induced oxidative cell death in SK-N-SH neuroblastoma cells. The cytotoxicity of Aβ(1-42) was supported from the significant decrease of cell viability by MTT assay. Also, the results from the assay of ROS was definitely increased after an exposure to Aβ(1-42) at 10-20 μM. Mangosteen extract at concentration range of 100-400 μg/ml. showed strong protective effect against Aβ(1-42) confirmed by both assays. Therefore, the data suggest that mangosteen extract might be another good candidate to support the prophylaxis and treatment of Alzheimer’s disease.

Key Words: β-amyloid, Reactive oxygen species, Alzheimer’s disease

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Introduction

Alzheimer’s disease (AD) is the most common cause of dementia in elderly affecting approximately 10-15 % of population at the age over 65 years (Jorm et al., 1987). The incidence increases doubling every 5 years afterwards and reaches more than 50% by the age over 85 (Jorm et al., 1987, Verhey et al., 2007). At present it is a serious problem all over the world since now people live longer. In Thailand, there are approximately at least 200,000 AD patients now. Generally, the disease starts with an insidious onset of neuron loss followed by a gradual and progressive deterioration of brain function then death within about 10 years after diagnosis. Recently there is no definite cause or successful treatment or prevention of AD. In addition to the typical clinical findings, the two hallmarks found in AD patients are the increased accumulation of neurofibrillary tangles and senile plaques in the brain. The main component of senile plaques is β-amyloid peptide (Aβ) which is considered as a major role in the development of AD. This polypeptide consisting of 39-43 amino acid residues is proteolytically cleaved from a transmembrane amyloid precursor protein by β- and γ-secretases. Many studies have demonstrated the in vitro and in vivo toxicities of Aβ as an important role in the pathogenesis of AD (Hensley et al., 1994, Pike et al., 1991). In cell culture study, Aβ can directly induce neuronal cell death and cause neurons vulnerable to excitotoxicity and oxidative insults. Aβ facilitates the generation of free radicals causing the peroxidation of membrane lipids, and increases the production of reactive oxygen species resulting in cell damage and apoptosis (4). At present, considerable efforts have been made to search for the antioxidants that could reduce Aβ induced oxidative stress in AD. Among them, more attention has been drawn to polyphenols that are natural substances obtained from plants, fruits, and vegetables (Zhao, 2005).

Garcinia mangostana Linn. (Mangosteen) is a tropical evergreen tree. It is widely distributed in Northern Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Malaysia, Thailand, and other tropical countries. In Asia, mangosteen is named as the “Queen of Fruits” due to its pleasant flavor. The fruit hull of mangosteen has been used as a traditional medicine for many years in Southeast Asia such as for the treatment of skin infection, wound injuries, dysentery and diarrhea (Gopalakrishnan et al., 1980). Many studies reported that the pericarp of mangosteen containing xanthone, mangostin, tannin, chrysanthemin, garcinone, gartanin, vitamin B1, B2, C and other bioactive substances. The xanthone and its derivatives are classified as polyphenolic compounds which are reported as potent chemicals for anti-inflammatory, antitumour, antioxidant, and antibacterial activities (Williams et al., 1995). Also previous studies reported that mangosteen extract showed the scavenging activity on nitric oxide, hydroxyl and superoxide radicals. The water soluble partition of the methanol extract of mangosteen pericarp demonstrated a significant antioxidant activity in a peroxynitrite-scavenging bioassay (Williams et al., 1995, Weecharangsan et al., 2006). There were reported studies on water extract of some medicinal plants including mangosteen extract, showing neuroprotective
effects in various cell cultures. However, there has been no any report about the inhibitory effect of mangosteen extract on Aβ1-42 induced free radicals generating neurotoxicity. Since Aβ which is a key role in the pathogenesis of AD, then it is interesting to search for the effective suppression of Aβ induced oxidative stress from any natural plant product including mangosteen extract. Considering of its potent antioxidant activity then mangosteen extract should be a good candidate for an antioxidant and neuroprotective agent against oxidative damage especially in AD. Thus the aim of this study is to look for the protective effect of mangosteen extract against the oxidative stress induced by Aβ1-42 in SK-N-SH neuroblastoma cells.

Materials and methods

β-amyloid preparation

Aβ1-42 was purchased from American Peptide Company, USA. Aβ1-42 stock solution of 1 mM was prepared in 5% NH4OH and stored at -20 °C. The stock solution was diluted to various concentrations immediately before use.

Cell culture

SK-N-SH human neuroblastoma cell line was obtained from American Type Culture Collection (ATCC HTB-11). Cells were cultured in MEM medium containing 10% fetal bovine serum (v/v), 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, streptomycin (100 µg/ml) and penicillin G (100 units/ml) at 37 °C in a humidified 95% air and 5% CO2.

Mangosteen extract

The mangosteens were collected from Chantaburi province, Thailand. The pericarp were removed and dried for 5 days then crushed into powder and kept at room temperature for further extract. All mangosteen powder (1 kg) was macerated with 5 L of methanol at 60°C. The crude extract was filtered and stored at 4°C for 2 days. Then, the solvent was concentrated by vacuum centrifugation on a vacuum rotary evaporator. Concentrated crude methanol extract was partitioned with ethylacetate (EtOAc) and water (H2O) to separate the active constituents based on their polarity. Both EtOAc extract and H2O extract were preliminarily analyzed for some specific chemical activities followed by further purification. The water soluble extract used for our experiments was determined for total phenolic compounds by Folin Ciocalteau Phenol reagent method. The phenolic compounds containing must not less than 179 mg of Gallic acid equivalent per gram. The amount of the α-mangostin found in H2O extract when determined by Thin layer chromatography was generally less than 2%. Mangosteen extract after preparation, was dissolved in DMSO at a concentration of 1,000 µg/ml. then diluted into 100-400 µg/ml before experiment.

Assay for cell viability

Cell viability was assayed by quantitative spectrophotometry with MTT 3-(4, 5-dimethylthiazoly1 -2-yl)-2, 5-diphenyl-tetrazolium bromide). SK-N-SH cells
(2 x 10^4 cells/100 μl) were grown overnight in 96-well plate then preincubated 30 minutes with mangosteen extract treating with Aβ_{(1-42)} for 24 h. MTT solution was added to each well at a final concentration of 500 μg/ml. Formazan crystals formed by the living cells were dissolved in DMSO and measured by microplate reader at 570 nm.

Measurement of intracellular ROS

The level of intracellular ROS was quantified by using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), a non-fluorescence probe to estimate the levels of cytosolic and mitochondria ROS. DCF-DA is deacetylated by viable cells into fluorescent 2',7'-dichlorofluorescein (DCF) by the activation of hydrogen peroxide produced in the cells (Halliwell and Whiteman, 2004). Cells (2 x 10^5 cells/ml) were seeded in 24-well plate and treated with various concentrations for 24 h. Afterwards, cells were harvested and washed once with PBS. DCFH-DA in PBS (10 μM) was added and incubated in dark at 37°C for 30 min. After washing cells with PBS, the fluorescent DCF in each well was quantified by spectrofluorometer at 485 nm excitation wavelength and 530 nm emission wavelengths.

Statistical analysis

All data were presented in means and standard error of means (S.E.M.). Statistical analyses were carried out using SPSS version 16.0 (SPSS Inc. Chicago, Il). The data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett t-test to determine whether multiple group were significance. Differences were considered to be significant at p<0.05. For the ROS was calculated by non-parametric analysis, Mann-Whitney U analysis.

Results and discussion

The cytotoxicity of Aβ_{(1-42)} was determined by MTT reduction. MTT is a tetrazolium salt reduces to formazan by mitochondria dehydrogenase, which is active only in live cell. The viability of SK-N-SH cells, after 24 h of incubation was affected by Aβ_{(1-42)} in dose-dependent. (Table 1. and Fig.1.). The minimum values observed with the concentration of Aβ_{(1-42)} 5 μM were about 68.54±2.33% of cell survival, and attenuated to 55.20±2.06% at concentration 20 μM. Moreover, the cell viability was reduced to constant plateau at 54±2.72% of 48 h. incubation following time dependent ROS was calculated by non-parametric analysis, Mann-Whitney U analysis.

Table 1. Data from MTT assay showing toxicity of Aβ_{(1-42)} at 5-20 μM in SK-N-SH cells

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>24 h.</th>
<th>36 h.</th>
<th>48 h.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>68.54±2.33*</td>
<td>59.71±4.15*</td>
<td>55.68±4.65*</td>
</tr>
<tr>
<td>10</td>
<td>59.03±2.48*</td>
<td>60.40±3.11*</td>
<td>54.93±2.45*</td>
</tr>
<tr>
<td>20</td>
<td>55.20±2.06*</td>
<td>55.75±2.82*</td>
<td>54.00±2.72*</td>
</tr>
</tbody>
</table>

* shows statistically significant with control

Fig.1. The toxicity of Aβ_{(1-42)} in SK-N-SH cell was determined by MTT assay. The cell viability was measured at 24, 36, 48 h with various concentrations of Aβ_{(1-42)} Differences compared to untreated control values were significant at p<0.001 in post hoc test, Dunnett’s analysis.
manner. Under this condition, \( \alpha \beta_{1-42} \) was significantly decreased cell viability compared to control following concentration dependent.

**Table 2.** Data from MTT assay showing protective effect of mangosteen extract against oxidative stress induced cell viability and by \( \alpha \beta_{1-42} \) for 24 h after 4 h.

<table>
<thead>
<tr>
<th>Concentration of mangosteen extract (( \mu \text{g/ml} ))</th>
<th>Cell viability (%)</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60.71±2.69*</td>
<td>10.49±4.83*</td>
</tr>
<tr>
<td>50</td>
<td>71.20±2.97</td>
<td>16.52±4.44*</td>
</tr>
<tr>
<td>100</td>
<td>77.22±2.39*</td>
<td>20.38±4.44*</td>
</tr>
<tr>
<td>200</td>
<td>81.09±1.48*</td>
<td>32.36±4.44*</td>
</tr>
<tr>
<td>400</td>
<td>97.07±2.50*</td>
<td>60.36±4.44*</td>
</tr>
</tbody>
</table>

* shows statistically significant with control

In the protection of cells against \( \alpha \beta_{1-42} \) toxicity, mangosteen extract showed a potent effectiveness nearly complete inhibition of \( \alpha \beta_{1-42} \) induced cell death. There was no toxicity of mangosteen extract at various concentrations on SK-N-SH cells (Fig. 2A.). After 24 h. treatment mangosteen extract, number of viable cells was mostly similar to control. When cells were induced by \( \alpha \beta_{1-42} \) 10 \( \mu \text{M} \) for 24 h cell viability was significantly decreased to 59.03±2.48% relative to control (Table 1. and Fig.1.). Therefore, the concentration of \( \alpha \beta_{1-42} \) at 10 \( \mu \text{M} \) after 24 h. incubation was used for next experiment to induce neuronal cell death. The protective effect of mangosteen extract to protect the cell viability was studied at various concentrations. Pretreatment of the cells with mangosteen extract substantially reduced cell death caused by \( \alpha \beta_{1-42} \) in the concentration dependent manner. Mangosteen at 400 \( \mu \text{g/ml} \) showed the strongest inhibitory effect on the \( \alpha \beta_{1-42} \) induced cell death. (Fig.2B.) Significant protective effects were achieved starting from 50-400 \( \mu \text{g/ml} \) that increased cell viability from 60.71±2.69% to 93.07±6.50%, respectively. Thus, \( \alpha \beta_{1-42} \) caused a decrease in the cell viability in SK-N-SH cells which was almost completely restored by the protection of mangosteen extract.

Moreover, we demonstrated that mangosteen extract prevented \( \alpha \beta_{1-42} \)-induced cell death in SK-N-SH cells by the suppression of \( \alpha \beta_{1-42} \) – induced ROS generation. The ROS production after \( \alpha \beta_{1-42} \) exposure was measured by DCF fluorescence. Cells after treated 24 h. with \( \alpha \beta_{1-42} \) 10 \( \mu \text{M} \) and 20 \( \mu \text{M} \) showed significant increase of intracellular ROS levels from
22.00±1.29 units to 105.75±9.71 and 251.75±39.76 units, respectively (Table 3. and Fig.3.). The increased levels of fluorescence from ROS, approximately 5-10 folds, were significantly different when compared to the untreated cells. The enhancement of ROS generation by $A\beta_{(1-42)}$ was reduced in the presence of mangosteen extract at 400 μg/ml. ROS production from 10 μM of $A\beta_{(1-42)}$ induction was significantly decreased from 43.02% to 76.04% by the protection of 400 μg/ml mangosteen extract against $A\beta_{(1-42)}$ 10 μM and 20 μM.

Table 3. Protective effect of mangosteen extract against $A\beta_{(1-42)}$ induced ROS production in SK-N-SH cells

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Fluorescence intensity(units)</th>
<th>Protection (%) (approximately)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>22.00±1.29</td>
<td></td>
</tr>
<tr>
<td>$A\beta_{(1-42)}$ 10 μM</td>
<td>105.75±9.21*</td>
<td></td>
</tr>
<tr>
<td>$A\beta_{(1-42)}$ 20 μM</td>
<td>251.75±39.70*</td>
<td></td>
</tr>
<tr>
<td>MS400 mg/ml+ $A\beta_{(1-42)}$ 10 μM</td>
<td>59.75±5.10*</td>
<td>43.02</td>
</tr>
<tr>
<td>MS400 mg/ml+ $A\beta_{(1-42)}$ 20 μM</td>
<td>60.50±8.39*</td>
<td>76.06</td>
</tr>
</tbody>
</table>

* shows statistically significant with control

Fig. 3: Protective effect of mangosteen extract on ROS production determined induced by $A\beta_{(1-42)}$ 10-20 μM in SK-N-SH cells for 24 h. Levels of ROS were measured using the fluorescence probe 2',7'-dichlorofluorescein (DCF). The results showed mean±S.E.M. (n=4) non-parametric test, Mann-Whitney U analysis, $p<0.05$ versus the group treated with $A\beta_{(1-42)}$ only.

respectively. These results corresponded with a decrease in cell viability to 55.02-68.54% of the control and recovered to 97.07% from the protection of mangosteen extract (Table 1). Therefore, the data supported the protective effect of mangosteen extract against $A\beta_{(1-42)}$ - induced neurotoxicity on the aspect of oxidative stress. Considering the strong correlation between $A\beta_{(1-42)}$-induced oxidative stress and cytotoxicity. The decreases in mitochondria membrane potential from previous study indicated the disruption of mitochondrial integrity (Butterfield et al., 1999). Afterwards, ROS produced in mitochondria may then leak to cytoplasm, leading to oxidative stress and initiate apoptosis via activation of apoptosis signaling (Zhu et al., 2007, Roth, 2001). Many studies have demonstrated that ROS involved in apoptotic mechanisms and triggered in process of $A\beta$-mediated neurotoxicity in some models of AD (Chauhan and Chauhan, 2006, Pike et al., 1991). Several antioxidants had been shown to protect cells from β-amyloid toxicity. The present data supported the protection of mangosteen extract against $A\beta_{(1-42)}$ induced toxicity in SK-N-SH cells from the suppression of ROS production. Our previous study of the antioxidative activity from mangosteen extract at 200 μg/ml against H$_2$O$_2$ toxicity at 150 μM had been compared with the protection from vitamin C in SK-N-SH cells. However the concentration of vitamin C must be as high to mega dose to be comparable with the same protection from the mangosteen extract (data not shown). Besides, the antioxidant activity might be one of the neuroprotective effects of mangosteen extract, especially from beta amyloid induced toxicity. Preliminary result from this study will lead to further identification of other
protective pathways of our mangosteen extract for future application in AD prevention and/or treatment.

Conclusions

*Garcinia mangostana* Linn. has been reported from many studies to have a protection against oxidative stress, infection, inflammation, etc. Our study in SK-N-SH cell line significantly supported the neuroprotective effect of mangosteen extract against \( \text{A}^\beta_{(1-42)} \) induced intracellular oxidative stress. Thus, the protective effect from the fruit hull of mangosteen may provide a pharmacological benefit in prevention or decreased progression of the process of AD.

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References


