Gambir catechins modulates amyloid-β concentration in cerebrospinal fluid of Alzheimer’s model rat

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Abstract. Neurodegeneration in Alzheimer’s disease has been reported as the consequences of amyloid-β accumulation in the brain resulted from oxidative stress. Gambir, which contains catechins as a potential antioxidant, is abundant in West Sumatra. This study aims to evaluate the effect of gambir catechins administration on amyloid-β concentration in cerebrospinal fluid [CSF] of the rats. An experimental study with a post-test only design was carried out on 12-week old female Sprague Dawley, an Alzheimer’s model rat. As many as 30 rats were divided into the control and treatment groups. Four of the six groups were treated with the catechins extract doses of 20 mg, 40 mg, 60 mg, and 100 mg per 200 g BW. Meanwhile, the other two groups were positive and negative control groups. After four weeks, the rats were sacrificed and the CSF was collected. Amyloid-β concentration measurement was using the ELISA method. The statistical analysis was using One-way ANOVA with posthoc test Tukey HSD. Treatment groups exhibited a linear decrease in amyloid-β concentration in all doses, particularly in CC3. The CC3-treated group showed the lowest amyloid-β concentration [13.325±4.625 ng/ml], which is significantly lower [p<0.05] when compared to the PC group [45.280±7.590 ng/ml]. This result provides experimental evidence that gambir catechin reached its optimum neuroprotective effect on amyloid-β level in cerebrospinal fluid by 60 mg/200 g BW.

Keywords: Alzheimer’s disease; antioxidant; catechin; gambir; nature-based

1. Introduction
Alzheimer’s disease [AD] is the most common type of dementia, a neurodegeneration disorder. It is characterized by the accumulation of amyloid-β [Aβ] in the form of plaques in the extracellular compartment and the tau protein in the intracellular compartment [1]. Free radicals and antioxidant imbalance, menopause, ovariectomy, and hyperglycemia [2–4] will precipitate the change to amyloid precursor protein [APP] metabolism [5]. The Aβ plague occurs before the cognitive impairment follows. The cognitive decline that is progressively followed by motoric deterioration also affects the family, community, and government to increase cost and loss in productivity [6].

Essential efforts have been carried out by researchers to find an effective treatment and with excellent efficacy, not yet successful [7,8]. Drug discovery tends to find other solutions, and one of them is by exploring the possibility of a nature-based compound that possesses antioxidant properties, which is also showing a potent ability to scavenge the free radicals [9–11].
Nature-based antioxidants could be found in tea, grapes, chocolate, and gambir, which contain catechins. Gambir [Uncaria gambir] plant is widely spread in West Sumatra, Indonesia, namely gambir cubadak, gambir udang, gambir riau mancik, and gambir riau gadang [12]. The [+]-catechin in gambir is the highest among the other plants and provides potent antioxidant activity even higher than vitamin E [13].

Previous research [14,15] has demonstrated that administration of [+]-catechins orally results in improvement of cognitive function in the animal with AD suggesting that the oxidative stress is mitigated by the catechins [15–17]. Many types of research have shown that green tea catechins exhibited valuable interaction in reducing amyloid in AD [18,19]. However, research is scarce about gambir catechins and their potential as anti-amyloid therapeutics. There are no relevant studies using gambir catechin [Uncaria gambir Roxb] against neurodegeneration in the last decade, Aβ-42 concentration in particular. However, there are abundant sources of tea and green tea catechins studies on its neuroprotective properties [18–21], and several studies that using [+]-catechins on Alzheimer-induced animal [15–17]. This study aims to examine the effect of gambir catechin against amyloid-β in AD model rat.

2. Materials and methods
This post-test-only design experimental study was conducted following the Guide for the Care and Use of Laboratory Animals for Education and Research purpose [Andalas University, Padang]. The Ethical Committee of the Faculty of Medicine of Andalas University approved the procedures, the ethical clearance number 012/KEP/FK/2016.

The gambir catechin extracts were purchased from the Andalas Phytopharmacy Laboratory [Padang, Indonesia]. It contained 97.8% [+] catechin compound according to the Indonesian Herbal Pharmacopeia [22]. Different doses applied to different groups were according to documented catechins efficacy in improving the memory and cognitive function [15,18]. Each treatment groups, received 20 mg/200 g BW [CC1], 40 mg/200 g BW [CC2], 60 mg/200 g BW [CC3], and 100 mg/200 g BW [CC4] respectively. These doses are safety if referring to Material Safety Data Sheet that the Lethal Dose [L50] of [+] catechin for rat and mouse orally are more than 10,000 mg/kg BW [23].

This study used the AD model female Sprague Dawley rats, age 12 weeks, and weight between 150 g and 160 g. The rats were housed in the animal care facility at Bogor Animal Laboratory. All rats consumed 30-50 g of the pellet [protein 14%] and water ad libitum. The rats were induced with bilateral ovariectomy and highly D-galactose according to Hua et al. method [24].

The six groups consisting of five rats. The two control groups were negative control [NC] [normal rats] and positive control [PC] [AD rats]. The other four groups were CC1, CC2, CC3, and CC4 groups. Each treatment rat received gambir catechin extract dissolved in saline solution [NaCl 0.9%] with the calculation of 100mg/ml/kg BW. The extract solution was administered orally to each rat using a stomach gauge; once daily for four weeks. At the end of the fourth week, the specimen was collected following Liu & Duf [25].

Amyloid-β concentration was measured using ELISA methods as suggested in the procedure of RAT Aβ-42 ELISA Kit [Elabscience, China]. This specific rat Aβ-42 has a sensitivity of 0.094 ng/ml with the range of detection 0.156 – 10 ng/ml.

Data expressed as mean±SD after normality test, with Shapiro-Wilk and Levene’s test for the variance. One-way ANOVA was used to determine the mean difference among the groups. Tukey HSD posthoc was following the one-way ANOVA test. P-value < 0.05 was considered significant.

3. Results and Discussion

3.1. Result
Using an antibody against Aβ-42, this study's primary finding was the Aβ-42 concentration in the PC group was the highest and the CC3 group was the lowest [Fig. 1].
3.1.1. Amyloid-β concentration in cerebrospinal fluid after gambir catechin administration. This study found that Aβ-42 concentration in the CC3 group was 13.325±4.625 ng/ml which is significantly lower than PC with Aβ-42 concentration was 45.28±7.590 ng/ml, p-value 0.0001.

Figure 1. The concentration of Aβ-42 after four weeks gambir catechins administration [ng/ml].

Following the data mean differences found to be significant, a post-hoc test using Tukey HSD resulted in the finding of optimum dose for Aβ-42 concentration in the CC3 group, which is 60 mg/200 g bw 300 mg/kg bw [Table 1].

Table 1. Mean differences of the concentration of Aβ-42 using post-hoc test Tukey HSD.

| Aβ-42 concentration | PC   | CC1   | CC2   | CC3     | CC4     |
|---------------------|------|-------|-------|---------|---------|
| PC                  |      | 0.064*|       | 0.0001* | 0.0001* |
| CC1                 | 0.064|       | 0.048*| 0.006*  | 0.047*  |
| CC2                 | 0.0001*| 0.048*|       | 0.950   | 1       |
| CC3                 | 0.0001*| 0.006*| 0.950 |       | 0.950   |
| CC4                 | 0.0001*| 0.047*| 1     | 0.950   |         |

3.2. Discussion
In the present study, the decrease of Aβ-42 peptide concentration in the CC3 group compared to the PC group is the most significant. This study evaluated gambir catechins’ neuroprotective effect on bilateral ovariectomy and highly D-glucose-induced rats. These treatments lead to ROS production and oxidative stress, which will produce higher Aβ-42 peptide toxic to the neuron, although in normal condition Aβ peptide is crucial for synaptic function [5,26]. Amyloid-β peptide accumulation leads to senile plague in the extracellular compartment. This condition could easily be detected in
cerebrospinal fluids since the brain’s chemical changes are directly depicted [27,28]. The result shown in Fig.1 is supporting the study by Lee et al. [2020]. The study on catechins from green tea that processed at high temperature could inhibit ROS and reduce Aβ formation in human microvascular endothelial cells [19].

Anggraini et al. [2018] revealed that all types of gambir in West Sumatra exhibited similar antioxidant activities. Those four types also showed extraordinarily antioxidant properties that enable them to scavenge ROS vigorously [12], which is more potent than vitamin E [13]. Although no direct mechanism could be found regarding gambir catechin against Aβ-42 neurotoxicity, the proposed mechanism could be drawn from other catechins properties, such as green tea [18–20,29]. According to Anggraini et al. [2011] the catechin from the four types of gambir [gambir cubadak, gambir udang, gambir Riau mancik, and gambir Riau Gadang] is safe, which is different from grape seed extract tendency [12].

These antioxidant activities of gambir catechin tend to increase with the dose of the catechin [12]. As depicted in Fig.1, the neuroprotective effect of gambir catechins in the present study was linear on the dose 20, 40, and 60 mg per 200 g bw. This result supports previous research by Wei [2004] and Sutherland [2005], who found the catechin dose-dependent effect on neurodegeneration-induced animals [30,31]. However, the dose of 100 mg per 200 g bw was otherwise, where the CC4 group exhibited a slight increase in Aβ-42 concentration, despite the value being significantly lower than the PC group. This result aligns with research on mice that exhibit a higher catechin dose was not followed by the significant neuroprotective effect [15]. The hypothesis suggested is the saturation of reaction between the oxidative stress with catechin’s neuroprotective effect on the dose 60 mg/200 g bw. However, to understand the exact mechanism of this neuroprotective effect, further study is needed.

4. Conclusions
Oral gambir catechin reached its optimum neuroprotective effect against Aβ-42 in Alzheimer’s disease by 60 mg/200 g bw.

Acknowledgments
This research was supported by the Research and Technology of Higher Education Ministry [DIPA # 1042.01.02.400928/2016]. We thanked the Biomedical Laboratory of Faculty of Medicine, Andalas University for the technical assistance during the research.

Conflicts of interest
The authors reported no potential conflict of interest in this study.

References
[1] Schultz C, Tredici K Del and Braak H 2004 Neuropathology of Alzheimer’s Disease Alzheimer’s Disease A Physician’s Guide to Practical Management ed R Richter and B Z Richter [Springer] pp 21–31
[2] Chen Y, Deng Y, Zhang B and Gong C X 2014 Deregulation of brain insulin signaling in Alzheimer’s disease Neurosci. Bull. 30 282–94
[3] Yamada K, Tanaka T, Zou L B, Senzaki K, Yano K, Osada T, Ana O, Ren X, Kameyama T and Nabeshima T 1999 Long-term deprivation of oestrogens by ovariectomy potentiates β-amyloid-induced working memory deficits in rats Br. J. Pharmacol. 128 419–27
[4] L. R, G.P. M, A.K. S, M.M. P and R. D M 2018 Alzheimer’s disease and menopause: Is there an association? Int. J. Gynecol. Obstet. 143 728
[5] Selkoe D J and Hardy J 2016 The amyloid hypothesis of Alzheimer’s disease at 25 years EMBO Mol. Med. 8
[6] World Health Organization 2017 Global action plan on the public health response to dementia 2017 - 2025
[7] Alzheimer’s Association 2019 FDA-approved treatments for Alzheimer’s 1–5
[8] Willis O 2020 As another Alzheimer’s treatment fails, experts are divided on where to next *ABC Heal. Wellbeing* -HEALTH
[9] Baptista F I, Henriques A G, Silva A M S, Wiltfang J and da Cruz e Silva O A B 2014 Flavonoids as Therapeutic Compounds Targeting Key Proteins Involved in Alzheimer’s Disease *ACS Chem. Neurosci.* 5 83–92
[10] Bilia A R, Isacchi B, Righeschi C, Guccione C and Bergonzi M C 2014 Flavonoids Loaded in Nanocarriers: An Opportunity to Increase Oral Bioavailability and Bioefficacy *Food Nutr. Sci.* 5 1212–327
[11] Faria A, Mateus N and Ao Calhau C 2012 Flavonoid transport across blood-brain barrier: Implication for their direct neuroprotective actions *Nutr. Aging* 1 89–97
[12] Anggraini T, Tai A, Yoshino T and Itani T 2011 Antioxidative activity and catechin content of four kinds of Uncaria gambir extracts from West Sumatra, Indonesia *African J. Biochem. Res.* 5 33–8
[13] Musdja M Y, Rahman H A and Hasan D 2018 Antioxidant activity of catechins isolate of Uncaria gambier Roxb in male rats *LIFE Int. J. Heal. Life-Sciences* 4 34–46
[14] Fasrini U U, Susanti R and Lipoeto N I 2017 Gambier [Uncaria gambir/Hunter Roxb] Administration Improves Locomotor Activities and Neurocognitive Impairment in Alzheimer’s Model Female Rat *Neurona* 35 17–23
[15] Maryadhi N, Swastini D and Leliqia N 2012 Pengaruh Dosis Minuman Gambir Terhadap Peningkatan Daya Ingat Mencit Galur Balb/c *Portal Garuda* 55–8
[16] Ahmed E, Khan M M, Javed H, Vaibhav K, Khan A, Tabassum R, Ashafaq M, Islam F, Safhi M M and Islam F 2013 Amelioration of cognitive impairment and neurodegeneration by catechin hydrate in rat model of streptozotocin-induced experimental dementia of Alzheimer’s type. *Neurochem. Int.* 62 492–501
[17] Zamani M, Rohampour K, Zeraati M, Hosseinmardi N and Kazemian M M 2015 Pre-training Catechin gavage prevents memory impairment induced by intracerebroventricular streptozotocin in rats *Neurosciences* 20 225–9
[18] Katergaris N, Dufficy L, Roach P D and Naumovski N 2015 Green Tea Catechins as Neuroprotective Agents: Systematic Review of the Literature in Animal Pre-Clinical Trials *Adv Food Technol Nutr Sci Open J* 1 48–57
[19] Lee S-B, Choi E-H, Jeong K-H, Kim K-S, Shim S-M and Kim G-H 2020 Effect of catechins and high-temperature-processed green tea extract on scavenging reactive oxygen species and preventing Aβ 1–42 fibrils’ formation in brain microvascular endothelium *Nutr. Neurosci.* 23 363–73
[20] Gundimeda U, McNeill T H, Fan T K, Deng R, Rayudu D, Chen Z, Cadenas E and Gopalakrishna R 2014 Green tea catechins potentiate the neuritogenic action of brain-derived neurotrophic factor: role of 67-kDa laminin receptor and hydrogen peroxide. *Biochem. Biophys. Res. Commun.* 445 218–24
[21] Kim J, Lee H J and Lee K W 2010 Naturally occurring phytochemicals for the prevention of Alzheimer’s disease *J. Neurochem.* 112 1415–30
[22] Ministry of Health R of I 2008 *Indonesia Herbal Pharmacopeia* [Jakarta]
[23] Santa Cruz Biotechnology 1910 [+]-Catechin [hydrate] 1–11
[24] Hua X, Lei M, Zhang Y, Ding J, Han Q, Hu G and Xiao M 2007 Long-term d-galactose injection combined with ovariectomy serves as a new rodent model for Alzheimer’s disease *Life Sci.* 80 1897–905
[25] Liu L and Duff K 2008 A technique for serial collection of cerebrospinal fluid from the cisterna magna in mouse *J. Vis. Exp.*
[26] Prasansuklab A and Tencommnau T 2013 Amyloidosis in Alzheimer’s Disease: The Toxicity of Amyloid Beta [A β ], Mechanisms of Its Accumulation and Implications of Medicinal Plants for Therapy *Evidence-Based Complement. Altern. Med.* 2013 1–10
[27] Humpel C 2011 Identifying and validating biomarkers for Alzheimer’s disease *Trends Biotechnol.* **29** 26–32
[28] Chintamaneni M and Bhaskar M 2012 Biomarkers in Alzheimer’s Disease: A Review *ISRN Pharmacol.* **2012** 1–6
[29] Wu L, Zhang Q-L, Zhang X-Y, Lv C, Li J, Yuan Y and Yin F-X 2012 Pharmacokinetics and blood-brain barrier penetration of [+]-catechin and [-]-epicatechin in rats by microdialysis sampling coupled to high-performance liquid chromatography with chemiluminescence detection. *J. Agric. Food Chem.* **60** 9377–83
[30] Wei I H, Wu Y C, Wen C Y and Shieh J Y 2004 Green tea polyphenol [-]-epigallocatechin gallate attenuates the neuronal NADPH-d/nNOS expression in the nodose ganglion of acute hypoxic rats *Brain Res.* **999**
[31] Sutherland B A, Shaw O M, Clarkson A N, Jackson D M, Sammut I A and Appleton I 2005 Neuroprotective effects of [-]-epigallocatechin gallate after hypoxia-ischemia-induced brain damage: novel mechanisms of action *FASEB J.* **19** 1–22