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Antidimentia activity of instant granule containing a combination of *Brassica oleracea* L. Var. Italica and *Centella asiatica* L. Urban extracts in male mice (*Mus musculus*) model of dementia

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ABSTRACT

Dementia is a progressive cognitive impairment characterized by cognitive decline and memory loss. Acetylcholine levels in dementia patients decrease due to increased levels of acetylcholinesterase which breaks down acetylcholine into choline and acetate. Gotu kola (*Centella asiatica* L. Urban) contains asiaticoside, while broccoli (*Brassica oleracea* L. Var Italica) contains glucosinolate. Both compounds are believed to have acetylcholinesterase inhibitor activity. This study aimed to evaluate the antidementia activity of instant granules of a combination of *B. oleracea* and *C. asiatica* extracts in male white mice model of dementia. Thirty-five male mice were used in this experimental divided into seven group with five mice in each group. All groups were induced with hyoscine butylbromide intraperitoneally except the normal control group (group I). Group II (negative control) was given Na CMC 0.5% orally, and group III (positive control) was given piracetam intraperitoneally. Groups IV to VII were given instant granules with different formulation, namely namely group IV (25 mg :190 mg dose 1x), group V (25 mg : 190 mg dose 2x), group VI (190 mg : 190 mg dose 1x) and group VII (190 mg : 190 mg dose 2x). Y-maze test for mice was carried out for 5 min by allowing mice to explore the three arms of the Y-maze freely. A significant difference in antidementia activity between groups given the instant granules was observed ($p < 0.05$). The instant granules combination with a ratio of *B. oleracea* and *C. asiatica* extracts (190 mg : 190 mg) had a higher antidementia activity than that combination of 25 mg : 190 mg. The instant granules combination with a ratio of *B. oleracea* and *C. asiatica* extracts (190 mg : 190 mg) at dose 2x had a higher antidementia activity than at dose 1x. In conclusion, the instant granules combination of *B. oleracea* and *C. asiatica* have a potential antidementia activity.

ABSTRAK

Demensia adalah suatu kerusakan kognitif yang bersifat progresif ditandai dengan kemunduran kognitif dan kemunduran memori. Kadar asetilkolin pasien demensia berkurang karena peningkatan kadar asetilkolinesterase yang memecah asetilkolin menjadi kolin dan asetat. Pegagan (*Centella asiatica* L. Urban) mengandung asiatikosida, sedangkan brokoli (*Brassica oleracea* L. Var Italica) mengandung glukosinolat. Kedua senyawa tersebut diduga memiliki aktivitas penghambat asetikolinesterase. Penelitian ini bertujuan mengkaji aktivitas antidimensia granul instan kombinasi ekstrak brokoli dan pegagan pada mencit putih jantan model demensia. Sebanyak 35 ekor mencit yang dibagi menjadi 7 kelompok perlakuan dengan 5 ekor mencit masing-masing kelompok digunakan dalam penelitian. Seluruh kelompok diinduksi dengan hiosin butil bromida intraperitoneal kecuali kelompok kontrol normal (kelompok I). Kelompok II (kontrol negatif) diberikan NaCMC 0,5% peroral, kelompok III (kontrol positif) diberikan piracetam intraperitoneal. Kelompok IV hingga VII diberikan granul instan yang mengandung brokoli dan pegagan dengan kombinasi dan dosis yang berbeda yaitu kelompok IV (25mg : 190mg dosis 1x), kelompok V (25 mg : 190 mg dosis 2x), kelompok VI (190mg : 190mg dosis 1x) dan kelompok VII (190 mg : 190mg dosis 2x). Uji Y-maze mencit dilakukan selama 5 menit dengan membiarkan mencit mengeksplor ketiga lengan labirin Y secara bebas. Terdapat perbedaan aktivitas antidimensia yang signifikan antar kelompok yang diberikan granul instan kombinasi ekstrak brokoli dan pegagan ($p<0,05$). Granul instan dengan perbandingan ekstrak brokoli : pegagan (190 mg : 190mg) memiliki aktivitas antidemensia lebih tinggi dibandingkan dengan perbandingan (25

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mg: 190mg). Granul instan dengan perbandingan konsentrasi ekstrak brokoli : pegagan (190 mg : 190mg) 2x pemberian memiliki aktivitas antidemensia lebih tinggi dibandingkan dengan sekali pemberian, bahkan lebih tinggi dari kontrol positif. Dapat disimpulkan, granul instan yang mengandung ekstrak brokoli dan pegagan memiliki potensi sebagai antidemensia.

INTRODUCTION

Dementia is a progressive cognitive impairment characterized by cognitive decline accompanied by symptoms of memory decline or recall, difficulty thinking properly, inability to solve problems and often uncontrolled emotions.[1-3] The elderly population worldwide diagnosed with dementia is 6%, this number is expected to triple by 2050.[4] According to WHO data, in 2023 more than 55 million people will be affected by dementia and there will be an increase of 10 million new cases each year.

In dementia patients, amyloid-β is found in aggregate form while in normal humans it is found in soluble form.[5] In addition, there is an increase in acetylcholinesterase levels which break down acetylcholine into choline and acetate.[6] One of the plants that can be used as a traditional medicine in the treatment of dementia is gotu kola (*Centella asiatica* L. Urban). The leaves of the *C. asiatica* contain the main compound triterpenoid.[7] One of the total triterpenoid compounds is asiaticoside which works as an acetylcholinesterase inhibitor.[8] In addition, the flavonoid content of the *C. asiatica* is believed to improve cognitive and emotional disorders, because it can degrade amyloid-β plaques.[9] Another plant that can be used as an antidementia is broccoli (*Brassica oleracea* L. Var Italica) which has glucosinolate content that can act as an acetylcholinesterase inhibitor.[10] Utilization of both plant extracts combination has been developed in the form of instant granule preparations with a ratio of 190 mg : 190 mg.[11] After being tested hedonic, the preparation has a very strong *B. oleracea* aroma and can not be covered with essence. Therefore reformulation was carried out by changing the composition of *B. oleracea* and *C. asiatica* extracts to 25 mg : 190 mg.[12] When the analysis of the flavonoid quercetin levels in the new instant granule formula was carried out, there was a decrease in levels from the original 0.48 mg QE/g ± 0.0111 to 0.42 mg QE/g ± 0.0112. Based on these data, a study was conducted to evaluate antidementia activity of the instant granules in male mice model of dementia using Y-maze method.

MATERIAL AND METHODS

Protocol of the study has been approved by the Research Ethics Committee, Faculty of Mathematics and Natural Sciences, Universitas Pakuan, with letter number 027/KEPHP-UNPAK/06-2024 dated June 14, 2024.

Animals preparation

Animals used in the experimental were 35 male white mice (Mus musculus) balb/c strain aged 4 mo with a body weight of 24-30 g. The mice were acclimatized first for 1 wk in the Laboratory of Pharmacology, Faculty of Mathematics and Natural Sciences, Universitas Pakuan, Bogor so that the animals could adapt to the environment. The cage used was a plastic box with a wire lid, the cage box was washed every two d.[13]

Preparation of test solution

A total of 500 mg of Na-CMC was weighed and dispersed in 20 mL of hot water. The mixture was then diluted with distilled water to a final volume of

100 mL and stirred until a uniform thick suspension was formed.[14]

Preparation of hyoscine butylbromide solution: by dissolving hyoscine butylbromide injection (20 mg/mL) with aqua pro injection to a volume of 200 mL (the concentration obtained was 20 mg/200 mL). The dose of hyoscine butylbromide was 1.5 mg/kgBW intraperitoneally.[13]

Preparation of piracetam solution was conducted by dissolving piracetam injection (1 g/5 mL) with aqua pro injection to a volume of 50 mL (the concentration obtained is 1 g/50 mL). The dose of piracetam given to mice was 400 mg/kgBW intraperitoneally.[15] Two preparations of the instant herbal granule test solution were conducted as follows: one containing a combination of *B. oleracea* (25 mg) and *C. asiatica* (190 mg), and another containing a combination of *B. oleracea* (190 mg) and *C. asiatica* (190 mg). Each instant granule was packed in a sachet container of 5 g each. The dose given to mice was a dose that has been converted from a human dose to an animal dose. The administration of each instant granule had 2 different doses, namely a 1x dose and a 2x dose. Each instant granule formula was prepared by dissolving it in 250 mL of distilled water.

Grouping of the animals

The research design used was a completely randomized design (CRD). The CRD can be used in research with homogeneous environmental conditions, tools, materials and media,[16] with a minimum number per group following the Federer formula: $(t-1)(n-1) \geq 15$

The experimental animals used in this study were 35 male mice divided into 7 groups with 5 mice in each group. The grouping of animals is in TABLE 1.

TABLE 1. Group of the animals

Name of Group		Administration of Test Solution
I (normal control)	:	0.35 ml/20gBW Na CMC 0.5% p.o.
II (negative control)	:	0.03 mg/20gBW hyoscine butylbromide induction i.p.
III (positive control)	:	8 mg/20gBB piracetam i.p and 0.03 mg/gBW hyoscine butylbromide induction i.p.
IV	:	instant granules of *B. olirecae* and *C. asiatica* extracts (25 mg : 190 mg) at dose of 18.2 mg/20gBB p.o (1x dose) and 0.03 mg/20gBB hyoscine butylbromide nduction i.p.
V	:	instant granules of *B. olirecae* and *C. asiatica* extracts (25 mg : 190 mg) at dose of 36.4 mg/20gBB p.o. (2x dose) and 0.03 mg/20gBB hyoscine butylbromide nduction i.p.
VI	:	instant granules of *B. olirecae* and *C. asiatica* extracts (190 mg : 190 mg) at dose of 18.2 mg/20gBB p.o. (1x dose) and 0.03 mg/20gBB hyoscine butylbromide nduction i.p.
VII	:	instant granules of *B. olirecae* and *C. asiatica* extracts (190 mg : 190 mg) at dose of 36.4 mg/20gBB p.o. (2x dose) and 0.03 mg/20gBB hyoscine butylbromide nduction i.p.

Solution administration and Y-maze testing

The mice were given test solutions according to the provisions of each group for 7 d.[17] On the 8th day, induction was carried out using hyoscine butylbromide solution for all groups except the normal control group. The dose of hyoscine-butylbromide inducer given was 1.5 mg/kgBW. Before being given the inducer, 30 min earlier all groups had been given instant granule solution (groups IV to VII) and piracetam solution (positive control). After 1 hr of administering the inducer solution, the effect was observed using the Y-maze tool for 1 hr.[18] (FIGURE 1.)

The Y-maze test lasted for 5 min by allowing the mice to freely explore the three arms of the Y-maze. If the mouse climbed to the maze wall, then the mouse had to be lowered to the last arm left behind. To avoid placement bias, the starting arm of each mouse was placed differently marked with symbols such as A, B, and C. At the end of each session, the three arms of the Y-maze were cleaned using 70% alcohol and allowed to dry. The number of arms entered was recorded with a video camera and the first choice of the Y-maze arm was recorded and counted.

FIGURE 1. Y-maze

The formula for % replacement used is as follows[14]: % Replacement = (Total replacement)/(Total incoming arms - 2) x100%.

The Y-maze used was made from Bogor design black acrylic material with a thickness of 3mm. The size of the Y-maze is adjusted to the protocol from the Stanford Behavioral and Functional Neuroscience Laboratory. The three arms are 40 cm long, 8 cm wide and 15 cm high and have 120° angle equipped with a partition that can be lifted on each wing.

Data analysis

Analysis data using SPSS software includes test of normality, homogeneity, and further test. Further test (Post Hoc) is conducted if the results of the analysis of variance test are declared significant. This test aims to analyze homogeneous or heterogeneous conditions in a sample. The selection of the type of further test is based on the calculation of the percentage of the coefficient of diversity (KK) as follows[19]: 1). Percentage of KK ≥ 10% for homogeneous data and ≥ 20% for heterogeneous data, using the Duncan multiple range test (DMRT). 2). Percentage of KK 5-10% for homogeneous data and 10-20% for heterogeneous data, using the least significance difference test, (LSD test). 3). Percentage of diversity coefficient ≤ 5% for homogeneous data and ≤ 10% for heterogeneous data, using the honestly significance difference test (HSD test; Tukey test) or the honestly significance difference test (BNJ).

RESULTS

Two formulations were evaluated in this study i.e. 1) instant granules containing combination *B. oleracea* and *C. asiatica* extracts at a composition of 190 mg:190 mg, and 2) instant granules containing *B. oleracea* and *C. asiatica* extracts at a composition of 25 mg:190 mg.[11,12] The characteristics of the instant granules are presented in TABLE 2.

tment were tested using the Y-maze tool on the 8th day. The memory ability of mice to test the antidementia effect was assessed based on the percentage of alternations made by the mice during the test. The total arms visited by the mice were calculated when all four limbs of the mice were inside the arms or passed through the center circle area.[23] The average results of the percentage of alternations in the Y-maze test are presented in TABLE 3.

TABLE 2. Characteristics of instant granules of *B. oleracea* dan *C. asiatica* extracts combination A total of 7 groups with each trea

Parameters	*B. oleracea* 190 mg : *C. asiatica* 190 mg	*B. oleracea* 190 mg : *C. asiatica* 190 mg	Requirements
Organoleptic : colour, taste, odor	Light green, sweet, specific aroma of coffee	Light green, sweet, specific aroma of coffee	No
Water content (%)	3.73	4.76	2-5%
Flavonoid content (mg QE/g)	0.48	0.38	No

TABLE 3. Observation results of substitution in Y-maze test

Treatment group		% Substitution
I	Normal Control	44.78 ± 1.25[b]
II	Negative Control	28.43 ± 1.64[a]
III	Positive Control	64.88 ± 1.68[f]
IV	*B. oleracea* 25 mg : *C. asiatica* 190 mg (1x dose)	53.84 ± 1.37[c]
V	*B. oleracea* 25 mg : *C. asiatica* 190 mg (2x dose)	59.93 ± 1.90[d]
VI	*B. oleracea* 190 mg : *C. asiatica* 190 mg (1x dose)	62.24 ± 1.95[e]
VII	*B. oleracea* 190 mg : *C. asiatica* 190 mg (2x dose)	72.01 ± 1.94[g]

Note: Each number followed by a different superscript letter in a different column indicates a significantly different effect.

DISCUSSION

The selection of the age of the experimental mice aged 4 mo because they have experienced an increase in oxidative and are not significantly different from mice aged 12 mo. The increased oxidative can trigger oxidative stress which is an important pathogenesis in cognitive decline and the development of dementia.[20] All test animals (35 animals) were acclimatized for 1 wk and weighed every day during the acclimatization period. The body weight of all test animals was calculated to find the coefficient of variant (CV) value before and after acclimatization to ensure that the body weight of the test animals was homogeneous. The CV value obtained before the acclimatization period was 7.57% and after the acclimatization period was 7.52%. Both results stated that the test animals were homogeneous because the CV value was ≤20%.[21] In the first three days of the acclimatization period, the weight of several test animals tended to decrease or remain the same. This can happen because the test animals experience stress when placed in a new environment. However, on the following day, the weight of the test animals increased again, indicating that the test animals had become accustomed to the environment, indicating that acclimatization had been successful.[22]

Antidementia activity

The data obtained showed that the highest percentage of turnover was obtained by the instant granule group

of *B. oleracea* dan *C. asiatica* extracts combination (190 mg : 190 mg) at dose 2x (TABLE 3). The decrease in the percentage of turnover in the Y-maze test observed in the negative control group indicated that the mice experienced dementia after the hyoscine butylbromide induction. Hyoscine butylbromide (butyl-scopolamine) is an anticholinergic substance used as a standard drug to stimulate cognitive deficits in healthy humans and animals.[24,25] Hyoscine butylbromide can affect short-term memory,[26] because it can increase the activity of the acetylcholinesterase (AChE) enzyme[27] and act as a muscarinic receptor antagonist that affects the nerve transmission process related to memory.[28] The decreasing effect caused by hyoscine butylbromide induction can be overcome by administering piracetam (positive control group) which is indicated by an increase in the percentage of turnover in the Y-Maze test. Piracetam works by inhibiting acetylcholinesterase activity, thereby increasing acetylcholine levels in the brain.[29] Administration of the instant granule test solution of *B. oleracea* dan *C. asiatica* extracts combination can overcome the antidementia effect in mice due to hyoscine butylbromide induction indicated by an increase in the percentage of changes in the Y-maze test (groups IV to VII in TABLE #).

Statistical data analysis using IBM SPSS software included normality test (Shapiro Wilk method) to see data distribution, homogeneity test (Levene test) to see data homogeneity for each group. The results of the normality test show that the data is normally distributed ($p>0.05$) and the results of the homogeneity test show that the data is homogeneous ($p>0.05$). These statistical results meet the requirements for statistical analysis with Anova. The results of the ANOVA test showed that there are significant differences between groups ($p<0.05$), therefore further testing using Post Hoc test was conducted to see groups that have significant differences. The results of Duncan's test showed that each group has a significantly higher percentage of alternation compared to the negative group ($p<0.05$). This means that the instant granules of *B. oleracea* dan *C. asiatica* extracts combination have antidementia activity in mice model of dementia.

The instant granule groups with different formulations showed significant differences in their antidementia activity, as indicated by variations in the increase of spontaneous alternation percentage after following the granule administration. The instant granule group of *B. oleracea* dan *C. asiatica* extracts combination (190 mg : 190 mg) had higher antidementia activity compared to the combination (25 mg : 190 mg) as showed in TABLE 3. A greater amount of *B. oleracea* extract would provide a better antidementia activity. This effect is presumed to occur due to glucosinolates of *B. oleracea,* which act as acetylcholinesterase inhibitors, and sulforaphane, a potent antioxidant that exerts protective effects on brain cells subjected to oxidative stress, such as in conditions of cerebral ischemia and neuroinflammation. The use of sulforaphane has an effect on improving cognitive function in patients with frontal brain damage.[30] In dementia patients, there is an accumulation of beta amyloid plaque due to oxidative stress and can be reduced by administering sulforaphane.[31,32] The instant granule group of *B. oleracea* dan *C. asiatica* extracts combination at dose 2x had a significant difference in antidementia effect compared to the dose 1x administration. It is indicated that the *B. oleracea* dan *C. asiatica* extracts combination is dose dependent meaning that the higher the dose administration, the higher the antidementia activity produced. Furthermore, the instant granule group *B. oleracea* dan *C. asiatica* extracts combination at dose 2x produced 1.11 time higher of antidementia activity than that the positive control. It is indicated that these instant granules

have the potential to be used in the treatment of dementia.

Mechanism of antidementia of *B. oleracea* and *C. asiatica* combination

The phytochemical content of broccoli extract includes vitamin C, sulforaphane, zinc, carotenoids, vitamin E, iron, pro vitamin A, and calcium.[33] Sulforaphane content can be used as an antioxidant, anti-inflammatory and antitumor.[34] Antioxidants are known to protect brain tissue from oxidative damage and inflammation, in addition to helping the body ward off free radical compounds and repair damaged cells so as to improve focus and memory. Vitamin C as an antioxidant has the ability to change free radicals into ascorbyl radicals, then change them into ascorbate and dehydroascorbate. This ascorbic acid has the ability to capture oxygen free radicals.[35] Glucosinolate compounds that are abundant in broccoli have activity as acetylcholinesterase inhibitors which are triggers for dementia.[36]

Centella asiatica contains alkaloids, flavonoids, tannins, saponins and triterpenoids (asiaticoside, asiatic acid, madecoside, madecasic acid).[37] Asiaticoside has activity as an acetylcholinesterase inhibitor.[8] In addition, triterpenoids in *C. asiatica* can revitalize blood vessels so that blood circulation to the brain is smooth, providing a calming effect, and improving mental and cognitive function.[38] In addition, *C. asiatica* has a protective effect on neurons (neuroprotective), is rich in antioxidants, accelerates the wound healing process, is anti-inflammatory, and improves cognitive function and memory. The neuroprotective effect in *C. asiatica* can reduce the aggregation of β-amyloid so that amyloid-β plaques do not form in the hippocampus that occurs in Alzheimer's patients.[39] Based on the mechanism of action of the two extracts, the combination of *B. oleracea* and *C. asiatica* extracts produces a synergistic effect as an antidementia, due to the acetylcholinesterase inhibitor effect of *B. oleracea* and the ability to break down amyloid-β plaques formed by *C. asiatica,* thus helping the antidementia activity.

CONCLUSION

The instant granule containing of *B. oleracea* dan *C. asiatica* extracts combination has antidementia activity in male white mice model of dementia. The extracts combination of 190 mg : 190 mg had higher antidementia activity compared to the combination of 25 mg : 190 mg. In addition, the *B. oleracea* dan *C. asiatica* extracts combination shows dose dependent activity.

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