EFFECTIVENESS OF THE COMBINATION OF *CURCUMA XANTHORRHIZA* AND *AVERRHOA BILIMBI* JUICE ON SWIMMING ABILITY AND HISTOLOGICAL OF BRAIN RATS

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ABSTRACT

The medicinal plants Curcuma xanthorrhiza and Averrhoa bilimbi are known to contain compounds that have antioxidant activity. Flavonoids are antioxidant compounds that can overcome fatigue. The objective of study was to investigate the effect of the combination of Curcuma xanthorrhiza and Averrhoa bilimbi on swimming ability and histological of brain Rats. The research method used a Completely Randomized Design using twenty-four male Wistar Rats (Rattus norvegicus) with the following treatment arrangements NC (aquabidest 3 mL), PC (herbadrink dosage 2.5 g/kgbw), P1 (plant juice dosage 20 g/kgbw), P2 (plant juice dosage 15 g/kgbw), P3 (plant juice dosage 10 g/kgbw), P4 (plant juice dosage 5 g/kgbw) given orally for 28 days. The parameters observed were swimming ability and histological of brain rats. The results showed that the administrastion combination of C. xanthorrhiza and A. bilimbi can improve swimming ability in rats but based on histological observations there is necrosis in neuroglia cells. The conclusion of the study is that although there is neuroglial cell necrosis, the combination administration is not toxic to the brain because it can affect the increase in stamina and the recommended dose is P4 (5 g/kgbw) because it has the lowest percentage of necrosis but also increases the rat's swimming ability.

Keyword: *A. bilimbi, brain of rats, C. xanthorrhiza, histological, swimming ability.*

1. Introduction

The use of medicinal plants in traditional medicine has been carried out by the Indonesian people since time immemorial. *C. xanthorrhiza* and *A. bilimbi* are plants that are often used in various diseases including: cholesterol, diabetes, and cancer [1], treat cough, hypertension, diabetes, toothache, zits, and sprue [2]. The effectiveness of *C. xanthorrhiza* as a therapeutic agent caused by the activity of natural antioxidants [3]. Indonesia people are commonly consumed as fresh fruit of A. bilimbi in order to add sour flavor. Previous research has shown the benefits of a combination extract of *C. xanthorrhiza* and *A. bilimbi* to reduce blood glucose levels in rats induced by Streptozotocin and improve insulitis levels in the pancreas, better when compared to single administration, this is probably due to the synergistic effect of the compounds contained in the two plants [4].

Free radicals can cause damage to tissues and cells. This causes various diseases such as chronic fatigue, heart disease, cancer, allergies, hyperlipidemia, atherosclerosis, kidney damage [5]. Stamina can decrease due to free radicals and weak immunity. The accumulation of free radicals can affect the decrease in stamina, therefore supplements that contain antioxidants are needed. Antioxidants are obtained from medicinal plants which can be used as natural supplements. The activities of the compounds in *C. xanthorrhiza* and *A. bilimbi* are antioxidants. Curcuminoids are antioxidant compounds found in *C. xanthorrhiza* rhizome and has levels of 8.70-11.50%, has an antioxidant activity of 87.01 ppm so it has the potential to be a good natural antioxidant [6]. *A. bilimbi* has antioxidant compounds, including: saponins, terpenoids, vitamin c, vitamin a, riboflavin and tannins [7]. Flavonoids are compounds contained in plants that have activities as antioxidants, immunomodulators, anti-inflammatory, anti-cancer, anti-diabetic [8]. Flavonoid supplementation in rat endurance exercise can reduce muscle fatigue thereby increasing performance [9].

The endurance test for swimming in rats is one of the observations made to determine the state of stamina in test animals. If fatigue occurs, it will have an effect on stamina, this can be overcome by giving a compound from plants that acts as a stamina booster. These compounds have antioxidant activity. The content of flavonoids in plants is a compound that acts as an antioxidant. Based on several research results, flavonoid compounds in ethanol extract of tartary wheat (Fogopyrum esculentum) at a dose of 120 mg/kgbw can increase stamina in mice. The ethanol extract of the noni fruit at doses of 400 and 800 mg/kgbw containing flavonoid compounds has been shown to increase stamina [10]. In hives leaves (Quisqualis indica L.) it can increase the stamina of the mice tested by the swimming endurance test [11]. Phyto- chemicals such as antioxidants absorbed from tea, fruit, and medicinal plants could not only reduce free radical formation and scavenge free radicals but also possess considerable antifatigue activity [12].

However, if the use of the dose is not appropriate, it can be toxic and can damage organs including the brain, namely the cerebral cortex by observing neuroglia cells. The cerebral cortex is the first part exposed to compounds that enter the brain so it is very vulnerable [13]. The brain is one of the important organs that has functions to control all activities of living things and is a target of toxicity (OECD 2008) [14]. This organ receives approximately 20% of the blood supply from the total cardiac output, which is about 750 ml of blood per minute [2]. Brain observations were made in the prefrontal part of the cerebral cortex. The cerebrum cortex is an area where feedback cycles and relationships between sensory and motor systems are important connected and integrated [15]. The brain has two types of cells, namely neuron cells, and neuroglia cells. Neuron cells function to deliver impulses. If cells in the brain experience necrosis, neuroglia (microglia) cells will be activated to carry out phagocytosis and the number of neuroglia cells will increase [16]. The cells observed in this section are neuroglia cells, because they play an important role in protecting neurons, and have more numbers than neuron cells in the motor area of the brain [17].

Research on the antioxidant activity of medicinal plant compounds is very important because it can be used as a safe and effective compound in overcoming fatigue and increasing stamina. The purpose of this study is to determine the effectiveness of the combination *C. xanthorrhiza* and *A. bilimbi* on swimming ability and histological of brain rats.

2. Methods

2.1. Experimental Animals

The research was carried out in the Laboratory of animal structure and function Department Biology Faculty of Mathematics and Natural Sciences Universitas Padjadjaran. Twenty-four male Wistar rats (*Rattus norvegicus*) of 6-8 weeks weighing 160-170 gram were housed in standard cages and maintained under standard laboratory conditions. In the first stage, the test animals were acclimatized for 7 days so that the rats could adapt to the laboratory environment. Rats were kept in animal houses with 12 hours of light and 12 hours of darkness at a temperature of around 26^0C and humidity of 60-70%. The mouse cage is a plastic box measuring 60 x 40 x 20 cm, covered with iron bars and covered with husks. The Monitoring by observing at general conditions such as body weight. The feed given was in the form of pellets as much as 20 grams/head/day and drinking water in the form of distilled water ad libitum. The cage is cleaned 2 times a week.

2.2. Plant Juice Preparation and Experimental Design

Plant Juice Preparation

The collecting plants sample of *C. xanthorrhiza* rhizome from Indonesian Medicinal and Aromatic Crops Research Institute Manoko Lembang Bandung Indonesia, whereas *A. blimbi* fruits were collected from Arboretum Padjadjaran University in Jatinangor Sumedang Indonesia. For each experiment, *A. blimbi* fruits and *C. xanthorrhiza* rhizomes were weighed by the comparison (1:1). For example, plant juice dosage 20 g/kg bw (P1), therefore *A. blimbi* fruits as much 10 g with *C. xanthorrhiza* rhizomes as much 10 g were mixed. Furthermore, *A. blimbi* fruits and *C. xanthorrhiza* rhizomes were crushed and squeezed. The juice was filtered, mixed with aquabidest until 4 mL, and stored at 0^0C until the experiment.

Experimental Design

The experiment was conducted with Completely Randomized Design, twenty-four rats were divided into six groups; consists of NC (aquabidest 3 mL), PC (herbadrink dosage 2.5 g/kg bw), P1 (plant juice dosage 20 g/kg bw),

P2 (plant juice dosage 15 g/kg bw), P3 (plant juice dosage 10 g/kg bw), P4 (plant juice dosage 5 g/kg bw) given orally for 28 days. The parameters observed were swimming ability and histological of brain rats.

2.3. Parameter observed:

Exhaustive Swimming Test

Before the swimming test, rats fasted for 24 h but allowed to drink water. Then, body weight was weighed. The tank was maintained at 28^0C during swimming process and the endurance for each rat was measured as swimming time recorded from the beginning of the exhaustion, defined by observing uncoordinated movements and failure to return to the surface. The time of floating, struggling were evaluated until possible drowning. The test performed twice, the day before plant juice administered and the last day of the treatment.

Histological of the Brain and Observation

Histological incisions of organs were performed using the paraffin method consisted of fixation, washing, dehydration, purification, infiltration, planting, trimming, slicing, sticking, staining, closing, and labeling stages. On day 29 the brain (cerebrum) was isolated and rinsed using physiological NaCl solution, then fixed in Bouin's fixative solution for 72 hours. Next, the brain is cut transversely on the back 1/3 with a thickness of 3-5 mm so that the cortex is obtained [18]. The brain fragments were washed in 70% ethanol solution for 24 hours. Brain organs were dehydrated in an increasing series of ethanol solutions 70%, 80%, 90%, and absolute with one repetition for 30 minutes. The next stage is the purification of the organs by immersing the pieces of brain organs in a mixture of absolute ethanol and xylol in a ratio of 3:1, 1:1, 1:3 and pure xylol for 5 minutes each. The infiltration stage was carried out in an oven with a temperature of ± 60˚C by immersing the brain pieces in xylol paraffin solution with a ratio of 3:1, 1:1, 1:3 and pure liquid paraffin was carried out for 15 minutes each. The brain fragments were then placed in a cardboard box filled with liquid paraffin until all organs were immersed in paraffin. The finished paraffin block is allowed to stand for ±24 hours until it freezes. In the trimming process, the frozen paraffin block is removed from the box and then the excess paraffin is cut in several parts, then pasted on the wooden block until it freezes (±30 minutes). Frozen paraffin blocks that already contain organ fragments are sliced using a microtome. The organ incision was attached to a slide that had previously been given Meyer albumin and warm water, then dried on a heating plate at 37-40˚C. The tissue sections were stained with hematoxylin and eosin (H&E). Histological observations of the brain were performed under a microscope by measuring the total number of neuroglia, mitotic cells, and necrotic cells with 5 fields of view on each preparation.

2.4. Data Analysis

Results were expressed as mean ± standard deviation (S.D). Statistical significance was analyzed using one-way ANOVA followed by Duncan's multiple range test. P values less than 0.05 were considered significant.

3. Results

3.1. Swimming Ability

Observation of the swimming ability of rats is to determine the effect of supplementary a combination of C. xanthorrhiza and A. bilimbi which act as stimulants. Data on the ability to swimming time (table 1) to determine the motor strength of rats while swimming. Data were collected twice, namely at the beginning before giving the combination juice and at the end after the 28th day of giving the combination juice.

Table 1. Average of Swimming Time

Treatments	Average of Swimming Time ± SD (s)
NC	49.25 ± 8.732 [a]
PC	140.5 ± 9.399[c]
P1	151.25 ± 19.120[c]
P2	132.75 ± 18.246 [c]
P3	89.5 ± 15.927 [b]
P4	80.75 ± 12.659 [b]

Note: Data were analyzed using one-way ANOVA followed by Duncan multiple range test. Difference alphabet in the same column showed P values less than 0.05 and considered significant. NC (aquabidest 3 mL), PC (herbadrink dosage 2.5 g/kg bw), P1 (plant juice dosage 20 g/kg bw), P2 (plant juice dosage 15 g/kg bw), P3 (plant juice dosage 10 g/kg bw), P4 (plant juice dosage 5 g/kg bw).

Based on the data in table 1 there is an increase in swimming time in all treatments when compared to the control. The highest increase in swimming time was in treatment P1 and the lowest was in P4.

3.2. Histological of the Brain Examination

Histological incision of the cerebrum was taken in the frontal lobe, and observed in the cortex. The histological of brain of the neuroglia cells presented on figure 1. Based on observations of histological, neuroglial cell necrosis was calculated by counting from 100 cells in 5 different fields of view with the aid of a microscope so that the results obtained are shown in table 2.

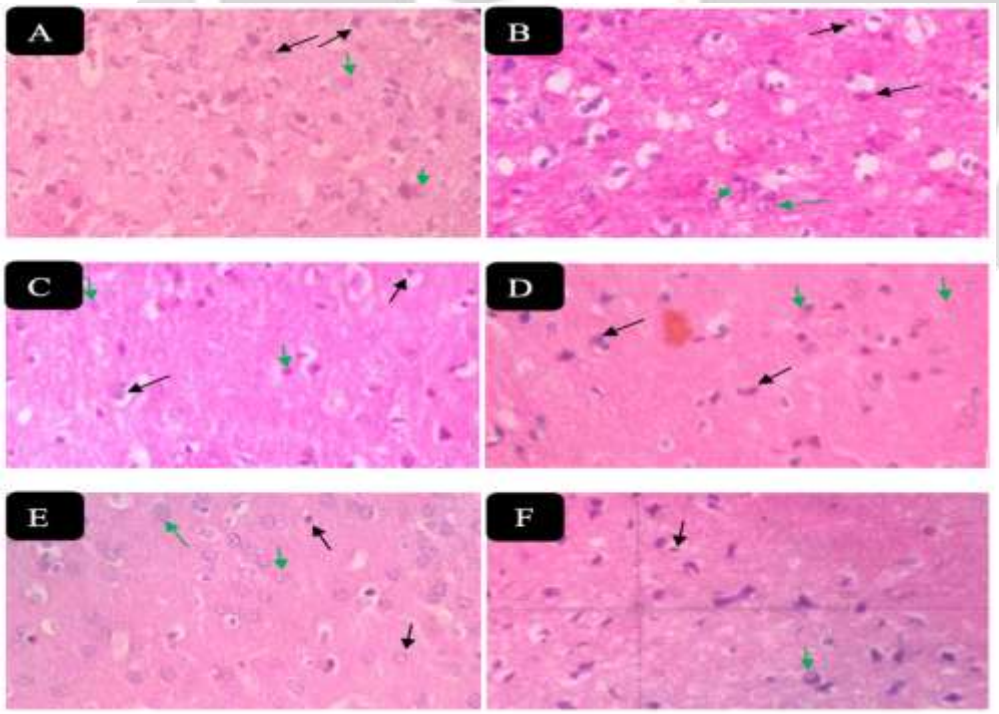

Figure 1. *Histological of Brain rats***.** A=PC; B=P1; C=P2; D=P3; E=P4; F=NC. Black arrow= necrosis neuroglia cell; Green arrow= normal neuroglia cell. 100×.

Table 2. Percentage of Necrosis Cells of Brain

Treatments	Number of Necrotized Cells ± SD (%)
NC	17.24 ± 2.545 [a]
PC	27.69 ± 4.050[b]
P1	33.46 ± 2.902 [c]
P2	23.95 ± 6.586 [b]
P3	17.49 ± 0.875 [a]
P4	12.33 ± 3.619 [a]

Based on the data in table 3, the highest percentage of necrosis was found in treatment P1 and the lowest was in P4, this indicates a decrease in the percentage of necrosis cells in line with the decrease in the dose of plant juice given.

4. Discussion

The swimming time performance was an increase in all treatments giving the combination of *C. xanthorrhiza rhizomes* and *A. bilimbi* fruits juice compared with control. The score of the swimming time were 151.25 ± 19.120, 140.5 ± 9.399, 132.75 ± 18.246, 89.5 ± 15.927, 80.75 ± 12.659 seconds, for P1 (20g/kg bw), P2 (15g/kg bw), P3 (10g/kg bw), P4 (5g/kg bw) the combination treated compared with control (49.25 ± 8.732). *C. xanthorrhiza* rhizomes and *A. blimbi* fruits were hypothesized as plants that can increase the stamina, at low doses P4 (5 g/kg bw) still showed an increase in swimming time compared to control. A similar study found that an aqueous leaf extract of *M. oleifera* increased swimming capacity by delaying the onset of physical exhaustion in rats [19]. The treatment of P1 was most effective, this indicates that combination of *C. xanthorrhiza* rhizomes and *A. blimbi* fruits enhanced the swimming capacity by delaying the onset of physical fatigue in rats, this is because the compounds contained in the two plants work synergistically. Similar results have been obtained by other researchers who tested *C. xanthorrhiza* rhizomes to maintain stamina and the antifatigue potential. In clinical trials, it was shown that the administration of *C. xanthorrhiza* extract can reduce the population of B lymphocyte cells so that *C. xanthorrhiza* indicated in decreased humoral immune function [20]. *C. xanthorrhiza* has the largest content in the form of curcumin of 27.19% and antioxidant activity of 87.01 ppm [3]. Curcumin and flavonoids are compounds that have antioxidant effects. The effect of curcumin as an antioxidant is indicated by the increased activity of NrF2 (Nuclear Factor Erythroid Like-2), detoxifying enzymes (Cytochrome P450 complex), cytoprotective enzymes HO-1 [21]. Meanwhile, flavonoids can activate NrF2, where the activation of NrF2 plays a role in regulating the production of endogenous antioxidant enzymes [22]. Whereas, *A. blimbi* fruits the greatest antioxidant levels were found in flavonoids by 0.568% and saponins 3.582% [7]. Saponin in *A. blimbi* fruits is a type of triterpen saponin, the flavonoid is a type of flavon and flavonol [23]. Flavonoid compounds are the same as noni fruit, flavonoids and saponins play a role in providing tonic effects on the body [10]. Acording to [24]. described that the flavonoid compound could extend the exhaustive swimming time of rats and inhibit the increase of blood lactic acid. Antifatigue agents have been shown to effectively work by delaying lactate accumulation by reducing the glycolytic process or by increasing the rate of removal of blood lactate [25;26]. Plant juice from *C. xanthorrhiza* rhizomes and *A. blimbi* fruits could effectively delay the onset of fatigue through one or both of these mechanisms. Another mechanism for increasing stamina is the presence of antioxidant activity that inhibits fat oxidation. Antioxidants play a role in reducing free radicals and preventing damage from oxidative reactions [27]. The inhibition of fat oxidation by antioxidants causing vasodilation and prevent atherosclerosis with increased blood flow. Therefore, the metabolic rate is increased to produce energy through aerobic respiration.

The term necrosis is used to describe any morphological finding of cell death in the histological section, regardless of the cell death pathway however it can be used as a modifier to determine the dominant cell death pathway, if appropriate. The basic process of necrosis involves loss of cell membrane integrity, which allows for an influx of extracellular ions and fluid, with resultant swelling of the cell and its organelles [28]. Based on table 3, there is a decrease in the percentage of necrosis cells along with a decrease in the dose given, this shows the effect of giving a combination of plant juices *C. xanthorrhiza* rhizomes and *A. blimbi* fruits in the P4 (5 g/kgbw) treatment giving the lowest percentage of necrosis even compared to the control. The highest percentage of cell necrosis was found in treatment P1 (20 g/kgbw), possibly high doses could cause cell necrosis due to the effect of the toxicity of compounds contained in the plant, this is also related to the decrease in the relative weight of the brain in the P1 treatment. Cells observed were neuroglia cells, which consisted of astrocytes, oligodendrocytes, and microglia [29]. Astrocytes play a role in maintaining blood flow in the brain, repairing nerves, and regulating ion levels. Microglia have a phagocytic function from pathogenic infections. Oligodendrocytes play a role in coating the axons by producing myelin [30]. Based on the picture above (Fig. 1) found many neuroglia cells that experience necrosis. Necrosis of neuroglia cells can cause several disorders of these cell functions. Large damage to neuroglia can trigger damage to neurons that deliver motor impulses. Neurons undergoing necrosis showed pyknosis and karyorrhexis. Necrosis occurs as a result of acute and irreversible cellular injury; this is due to failure of cellular metabolism along with rapid depletion of ATP [28]. Some common features of necrotic cells morphologically such as increased translucent cytoplasm, swelling of organelles, dilatation of nuclear membrane and condensation of chromatin into small, irregular, well-defined patches, and increased cell volume [31]. This increase in cell volume will ultimately result in disruption of the plasma membrane with uncontrolled leakage of cellular components (with inflammatory mediators) into the cytosol and interstitial space and subsequent recruitment of inflammatory cells [32].

Based on the results of the study, the highest percentage of cell necrosis was found in P1 treatment but did not cause significant damage, this was indicated by the increase in swimming ability in rats which was very effective. The lowest percentage of necrosis cells was found in the P4 treatment which also affected the increase in swimming ability compared to the control. Therefore, decreasing plant juice dosage could prevent cell necrosis and could make plant juice combination function more effective on swimming ability. The compounds contained in the combination of plant juices can protect the damage to neuroglia cells so that it does not decrease the swimming ability of rats. It is suspected that the content of flavonoid compounds plays a role in increasing stamina. Tonicum compounds (flavonoids, alkaloids, saponins, curcumin, etc.) can increase stamina by blocking adenosine A1 receptors. The blocking mechanism is mediated by intracerebroventricular tonic compounds and adenosine receptors to decrease the effect of fatigue by binding to adenosine receptors. This mechanism occurs in the central nervous system to increase stamina [33].

5. Conclusions

The conclusion of this study is that although neuroglial cell necrosis occurs, the combination is not toxic to the brain because it can affect the increase in stamina, this can be seen from the swimming ability of rats after 28 days of treatment. The combination of C. xanthorrhiza rhizome and A. blimbi fruit has antifatigue properties and increases exercise endurance involving the antioxidant activity of plant juices.

Conflict of Interest

No conflicts are declared.

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