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Stimulant Activity of Arabica Coffee Leaves (Coffea arabica L.) and Clove Flowers (Syzygium aromaticum (L.) Merr. & L.M. Perry) Ethanol Extract Mixture in Male White Mice

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ABSTRACT

Arabica coffee leaves and clove flowers are some of the plants that are used as traditional medicine. Both of these plants are known to have secondary metabolites that have stimulant activity. This study aims to determine the stimulant activity of a combination of coffee leaf extract (Coffea arabica L.) and clove flower (Syzygium aromaticum (L.) Merr. & L. M.Perry). This study used 35 male white mice divided into seven groups. Normal groups, control group caffeine 13 mg/kg BW; coffee leaf extract 400 mg/kg BW; clove flower extract 400 mg/kg BW and a combination of coffee leaf extract and clove flower 300 mg/kg BW: 100 mg/kg BW; 200 mg/kg BW: 200 mg/kg BW; 100 mg/kg BW: 300 mg/kg BW orally. All treatments to mice were carried out for 15 days. The stimulant activity was tested on days 5, 10, and 15. The parameters of the stimulant activity observed were motor and sensory activity using an Automatic hole board, endurance using Tail Suspension Test, and memory using a T-Maze. The results showed that the best stimulant activity for motor, sensory, and endurance was given by a combination of coffee leaf extract: clove flower 300 mg:100 mg ($P<0.05$). The best stimulant activity for memory was given by a combination of coffee leaf extract: clove flower 100 mg:300 mg ($P<0.05$).

Keywords: *Stimulant effect, Coffea Arabica, Syzygium aromaticum, Central nervous system.*

1. BACKGROUND

The central nervous system is the control centre of the entire nervous system (1). Almost all drugs that act on the central nervous system activity on special receptors that regulate synaptic transmission. Drugs on the central nervous system (CNS) act on specific receptors that modulate synaptic transmission (2). CNS stimulation consists of various behaviors, including mild elevation in alertness, increased nervousness and anxiety and convulsions. In general, any hyperexcitability associated with drug administration alters the delicate balance normally maintained in the CNS

between excitatory and inhibitory influences (3). One of the CNS stimulants that are well-known use abused in society is caffeine (4).

Caffeine is an alkaloid from the methylxanthine group, which stimulates CNS activity (5). The caffeine content in coffee is able to stimulate the central nervous system, so it can focus on maintaining concentration. Caffeine can also increase motor nerve alertness and stimulation of the respiratory system and blood vessel and heart systems (6). Caffeine is not only found in the beans but also in the coffee leaves (7).

Coffee leaf has been traditionally used as ethnomedicine (8). In Indonesia, especially Sumatra, coffee leaves have been consumed as tea since the 1800s, known as *kawa daun*. Kawa daun is a traditional beverage made by roasting coffee leaf in a bamboo tube and covered with a bamboo lid made from the black fibres surrounding the trunk of Arenga pinnata (9). Compared to the other two popular beverages, coffee and tea, the significances of coffee leaves to human health and Coffee leaves are also considered as a better antioxidant source than tea (7).

Another plant that is used traditionally as a stimulant is a clove. Cloves (Syzygium aromaticum (L.) Merr.) contain active compounds in their flower buds, like saponins, hydroxybenzoic acids, flavonoids, hydroxyphenyl propens, hydroxycinnamic acids, eugenol, gallic acid and terpenoids (10). Clove has been used as a spice, traditional medicine, and a mixture in Indonesian cigarettes known as kretek. In China and India clove traditionally used as stimulants. In clove flowers, terpenoid and alkaloid compounds can stimulate the central nervous system (11). The pharmacological activities of the clove plant that have been studied include stimulant activity (11)(12), antidepressant activity (13)

Based on the above background, the researchers wanted to examine the effect of a mixture of ethanol extract of Arabica coffee leaves (Coffea arabica L.) with clove flowers (Syzygium aromaticum (L.) Merr. & LM Perry) on the activity of the central nervous system in male white mice given orally.

2. MATERIALS AND METHODS

2.1. Materials

Materials used in this study were fresh *Coffea arabica* leaves approximately 2 kg obtained from Alahan Panjang, Solok, Dried Syzygium *aromaticum* flower bud approximately 2 kg obtained from Solok, 70% ethanol (PT Brataco), aqua dest (PT Brataco), *Natrium Carboxymethyl Cellulose* (Na CMC) (PT Brataco)

2.2. Plant Determination

Determination and identification were carried out in ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

2.3. Extract preparation

2.3.1. Coffee arabica

The extract was made from the dry powder of *Coffee arabica* leaves by maceration using 70% ethanol as a solvent. Three hundred grams of dried *Coffee arabica* leaves powder were put into the macerator plus 3L of 70% ethanol. The maceration process is carried out for 18 hours. The macerate is separated from the pulp using a flannel cloth. The waste obtained is macerated again twice with the same type and amount of solvent. All macerate was collected and evaporated under a vacuum evaporator until a thick extract was obtained. The yield obtained is weighed. Based on the literature, the yield obtained is not less than 31.6% (14).

2.3.2. Syzygium aromaticum

The extract was made from the dry powder of Syzygium aromaticum flower bud by maceration using 70% ethanol as a solvent. Three hundred grams of dried Syzygium aromaticum flower bud powder were put into the macerator plus 3L of 70% ethanol. The maceration process is carried out for 18 hours. The macerate is separated from the pulp using a flannel cloth. The waste obtained is macerated again twice with the same type and amount of solvent. All macerate was collected and evaporated under a vacuum evaporator until a thick extract was obtained. The yield obtained is weighed.

Based on the literature, the yield obtained is not less than 31.6% (14)

2.4. Phytochemical screening test

2.4.1. Alkaloid identification

500 mg of extract was weighed, then 1 ml of 2N hydrochloric acid and 9 mL of water were added. Then heated in a water bath for 2 minutes, cooled and separated the filtrate. Three drops of the filtrate were transferred into the watch glass, adding two Bouchard drops *at* LP. If with *Mayer* LP, a white or yellow condensed precipitate is formed that dissolves in methanol P. And with *Bouchard at* LP, a brown to black precipitate is formed. There is a possibility that an alkaloid is present (14).

2.4.2. Flavonoids identification

0.5 grams of extract were dissolved in 1 ml to 2 ml ethanol (95%), 0.1 g of magnesium powder and ten drops of hydrochloric acid were added; the colour is red-orange to red-purple, indicates flavonoids. If there is an orange-yellow colour, it indicates flavones presence (15).

2.4.3. Saponins identification

0.5 grams of extract were dissolved into the tube reaction, 10 mL of hot water were added, cooled, then the extract was shaken vigorously for 10 seconds. Positive results are indicated by forming a stable foam for not less than 10 minutes at 1-10 cm height. With the addition of 1 drop of 2N hydrochloric acid, the foam does not disappear (15)

2.4.4. Phenolic Identification

0.5 grams of extract were dissolved into the tube reaction, a few drops of neutral ferric chloride (FeCl3) solution were added to the tube. The formation of a red, blue, green, or purple colouration indicates the presence of phenols (15)

2.5. Animals and experimental design

This study has obtained ethical approval from the Research Ethics Committee, Faculty of Medicine Andalas University No. 215/KEP/FK/2019. The experimental animals used were male BALB/c white mice. A total of thirty-five mice aged 2-3 months with a bodyweight of 20-30 grams were used in this study. Before being treated, the mice were acclimatized to standard laboratory conditions with 12:12 light/dark cycles for one week and provided standard chow and water ad libitum. Thirty adult male white mice were divided into seven groups; each group was containing five animals. Normal control group were given Na CMC as vehicle, positive control group mice caffeine dose 13 mg/kg, BW and another group was given mixture of extract coffee:clove dose of 300 mg/kgBW:100 mg/kg BW; coffee:clove 200 mg/kg BW : 200 mg/kg BW; coffee:clove 100 mg/kg BW: 300 mg/kg BW; coffee dose 400 mg/kg BW and clove dose 400 mg/kg BW. All animals were treated orally for 15 days.

2.5.1. Automatic hole board test

The automatic hole board protocol use in this study was the modification of hole board protocol by Laboot, et al (2015). For 30 minutes, then observe the motor and sensory activity by placing the experimental animal on the Automatic Hole Board for 5 minutes. Observations were made in a room that was free from sound interference and had five watts of light (16).

2.5.2. T-Maze test

T-Maze tests were performed according to the protocol in Current Protocols in Neuroscience (1998). Observation time for each mouse was limited to 10 minutes. If, to this limit, the mice have not been able to find food, then the observation is stopped, and it is considered that the time taken by the mice is 11 minutes (17).

2.5.3. Durability Test

The durability test was performed according to the modification of Tail Suspension Test. The animal was isolated and suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Durability time was recorded during a 6-min period. Observed and recorded how many minutes the animals stayed on the resistance test equipment until the mice fell (18).

2.6. Data analysis

The research data were analyzed statistically using the two-way ANOVA. Analysis between time and dose, followed by Duncan's test (Duncan's Multiple F Test).

3. RESULT AND DISCUSSION

3.1. Plant determination

Based on the results of plant determination that has been carried out, it shows that the plant identified are *C. Arabica* from the Rubiacea family dan *S. Aromaticum* from the Myrtacea family.

3.2. Results of phytochemical screening

The results of the phytochemical screening test are shown in Table 1.

Table 1. Phytochemistry screening test result of *C. Arabica* and *S. Aromaticum*

Chemical Substances	Result	
	Coffee Leaf Extract	Clove Flower Extract
Alkaloid	(+)	(+)
Flavonoid	(+)	(+)
Saponin	(+)	(+)
Phenolic	(+)	(+)

3.3. Stimulant Acitivity of C. Arabica leaves and S. Aromaticum flower bud

The samples that have been used for this research are coffee leaves and clove flowers that have been identified at Herbarium Andalas (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University Padang, West Sumatra, Indonesia. The results of the identification of the species coffee leaf (Coffea arabica L.) and family Rubiaceae and clove flower bud (Syzygium aromaticum (L.) Merr.) and family Myrtaceae.

The stimulant effect can be seen from motoric activity in the automatic hole board test as seen in figure 1. The positive control group had the highest average motor activity on day 5, day 10, day 15, which was 31.20; 32.60; and 37.20, followed by the coffee: clove dose 300 mg/kg BW:100 mg/kg BW, which shows a stimulating motor activity close to the positive control group on day 5, day 10, and day 15, namely 23.20; 28.00; and 31.00 ($p<0.05$). The normal control group had the lowest motor activity, followed by the coffee: clove dose 100 mg/kg BW:300 mg/kg BW. It is because the negative control group was only given Na CMC suspension. Based on the duration of administration of the test solution, the 15th day give the highest motor activity. There was a significant difference on the 5th day ($p<0.05$) compare to the 10th, and day 15th gave almost the same motor activity. Physiologically, motor activity or body movement contains an emotional component because to be able to move requires initiative as a stimulus (Graham, 1971)

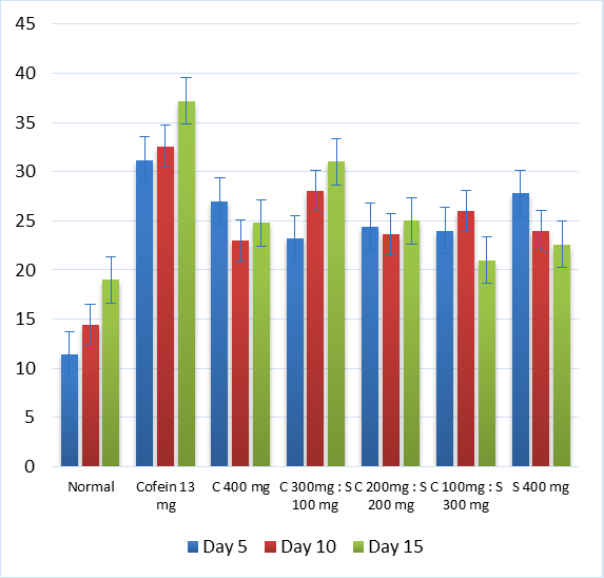

Figure 1. Motoric activity in Automatic hole board

The sensory activity was seen from the number of mice visiting the hole on the Automatic Hole Board in 5 minutes. The positive control group had the highest average sensory activity on day 5, day ten and day 15, namely: 46.40; 46.20; 51.40 times, followed by the coffee: clove dose 300 mg/kg BW:100 mg/kg BW, which shows a stimulating sensory activity close to the positive control group on day 5th, day 10th and day 15th, namely: 40.20; 41.80; 43.20 times ($p<0.05$). The negative control group had the lowest sensory activity compared to other groups with the average value of sensory

activity on day 5, day 10, and day 15, namely: 15.60; 15.60; 16.20. There was no significant difference on the 5th day ($p>0.05$) compare to the 10th, and day 15th gave almost the same sensory activity as we see in figure 2.

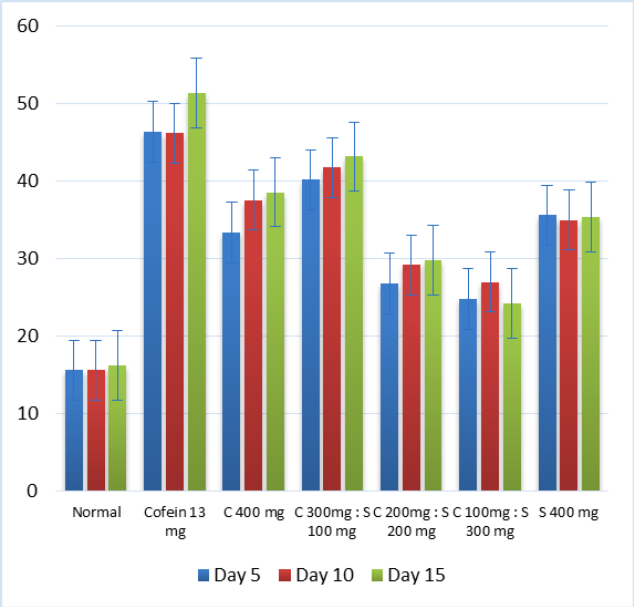

Figure 2. Sensoric activity in Automatic hole board

Endurance activity was seen based on the length of time the mice survived Tail Suspension Test. The positive control group had the highest average on day 5, day 10 and day 15, namely: 35.46; 36.95; and 38.74, followed by the coffee: clove dose 300 mg/kg BW:100 mg/kg BW, which shows a significant improvement in endurance activity close to the positive control group on day 5th, day 10th and day 15th namely: 31.34; 31.80; and 32.04 seconds ($p<0.05$). The negative control group had the lowest endurance compared to the other groups with the average value of endurance on day 5, day 10, and day 15, namely: 10.71; 11.31; and 12.85 seconds. There was no significant difference on the 5th day ($p>0.05$) compare to the 10th, and day 15th gave almost the same endurance activity as see in figure 3.

The memory activity was measured based on the time the mice took to find their food on the T-Maze. The normal control group had the highest memory compared to other groups with the average value of memory on day 5, day 10, and day 15, namely: 42.18; 43.09; and 49.46 seconds, followed by the coffee: clove dose 100 mg/kg BW:300 mg/kg BW which shows a significant improvement in memory namely: 25.17; 26.13; and 25.93 seconds ($p<0.05$).

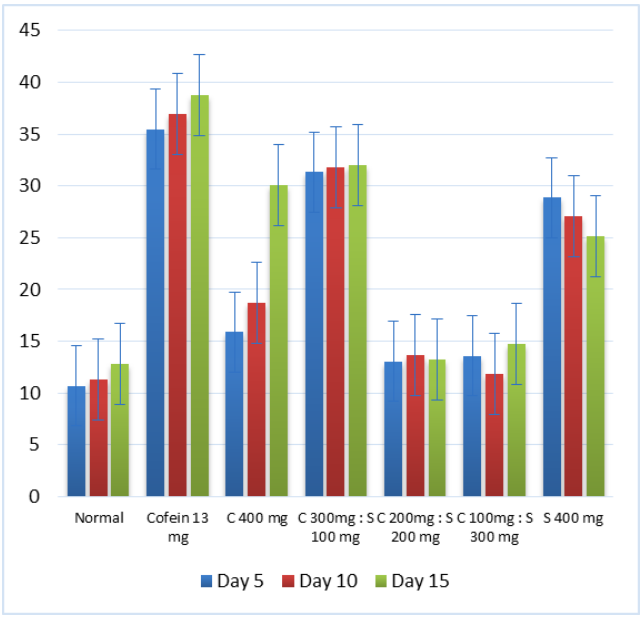

Figure 3. Endurance activity

The positive control group had the lowest memory activity compared to the other groups with the average value of endurance on day 5, day 10, and day 15, namely: 10.70; 11.12; and 11.72 seconds. There was no significant difference on the 5th day ($p>0.05$) compare to the 10th, and day 15th gave almost the same memory activity as see in figure 4.

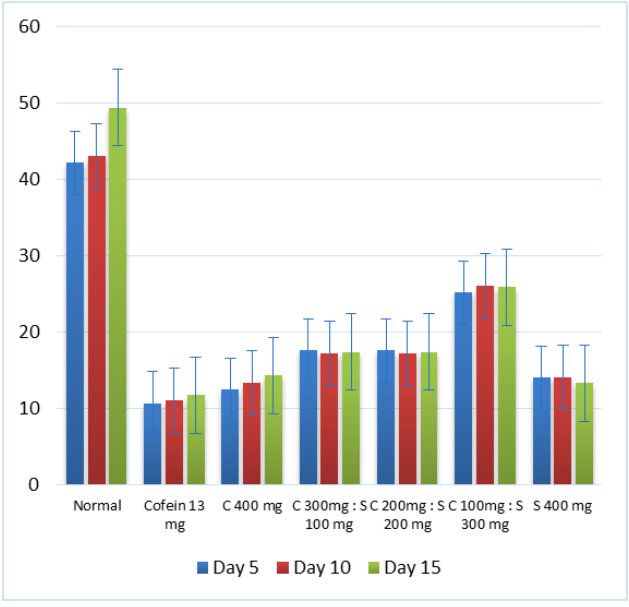

Figure 4. Memory activity in T-Maze test

Based on the results of the study, it was found that at the coffee: clove dose 300 mg/kg BW:100 mg/kg BW almost had the same effect or close to the effect of caffeine, which means that the coffee: clove dose 300 mg/kg BW:100 mg/kg BW was the best dose that gives the stimulant activity.

4. CONCLUSION

The results showed that the best stimulant activity for motor, sensory, and endurance was given by a combination of coffee leaf extract: clove flower 300 mg:100 mg ($P<0.05$).The best stimulant activity for memory was given by a combination of coffee leaf extract: clove flower 100 mg:300 mg ($P<0.05$).

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