RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v54n4.2754
Majalah Kedokteran Bandung. 2022;54(4):191–195

Majalah Kedokteran Bandung (MKB)

Received: March 18, 2022
Accepted: August 11, 2022
Available online: December 30, 2022

Effectiveness of Basil, Lime, Honey, Candlenut, and Tiwai Onion Herbs Combination as Analgesics and Stamina Stimulator

Andriyanto,[1] Sharon Aurellia,[2] Silmy Kamila Widyanti,[2] Hamdika Yendri Putra,[3] Leliana Nugrahaning Widi,[3] Rindy Fazni Nengsih,[3] Aulia Andi Mustika,[1] Wasmen Manalu[4]

[1]Division of Pharmacology and Toxicology, Departement Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[2]Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[3]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
[4]Division of Physiology, Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia

Abstract

Traditional medicine is generally considered to be safer than modern medicine because it has lower incidence of side effects and also relatively easy to obtain. The purpose of this study was to examine the analgesic effect of herbs made of basil, lime, honey, candlenut, and Tiwai onions as well as to evaluate the potential of these herbs as a stamina stimulator. This study was conducted at the Laboratory of Animal Management Unit, Faculty of Veterinary Medicine, IPB University, from January–February 2022. Mice were divided into four groups based on the treatment dose with each group consisted of five mice. An analgesic test was conducted using hot water immersion tail-flick test method and the stamina test was performed with swimming endurance test. Data obtained were analyzed using Microsoft Excel 2019 and Minitab 19. Significant differences of the average of each group were shown statistically using the one-way ANOVA and confirmed with Tukey test. Results showed that mice given the combination herbal medicine infusion of with a dose of 4 g/kg body weight had the highest average tail lift time compared to the controls ($p<0.05$). Mice with a treatment dose of 1 g/kg body weight had the highest analgesic power compared to the other treatment doses. The highest swimming duration was observed in group that received a dose of 1 g/kg body weight. It is concluded that this herbal combination can be used as an analgesic and a stamina stimulator.

Keywords: Analgesic, herbs, mice, traditional medicine, stamina

Introduction

Indonesia is considered one of the countries that have various kinds of diversity, not only from the cultural aspect but also from the flora and fauna. Indonesian locals have known and used plants as medicine alternatives in curing various diseases or as health stimulators since ancient times. The use of traditional medicine has been considered way safer compared to modern medicine because it is relatively easy to obtain and the side effects are much smaller. Other than that, interest in herbs has been rising lately among Indonesians because they are more affordable, both in price and availability in nature.[1] Traditional medicine is commonly used as an alternative therapy or as an additional therapy for modern medicine. One of the traditional medicine that is commonly used is jamu.

Health has always been the most important thing for living beings, especially humans. A decrease in health causes the body to be more susceptible to disease and reduces a person's capability of work, thus disturbing the daily activity of a person. Therefore, people have started shifting and looking for traditional stamina stimulator drinks that are made from herbs, such as jamu. Stamina is the long-lasting ability of the human organism to resist fatigue within a certain time limit. Other than being able to increase stamina, herbal medicine can be used as a pain reliever as well. Pain is defined as a process that happens when tissue damage occurs, and also as a defense response and protection of the body.[2] Drugs that can relieve pain without losing the consciousness of their consumer are defined as analgesics.[3]

This study used jamu made from a mixture of

Corresponding Author:
Andriyanto
Division of Pharmacology and Toxicology, Departement Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia
Email: andriyanto@apps.ipb.ac.id

several ingredients, namely basil leaves (*Ocimum basilicum*), candlenuts (*Aleurites moluccanus*), tiwai onions (*Eleutherine americana*), lime (*Citrus aurantiifolia*) and honey. The mixture can be used to create stimulant effects and analgesic effects due to the chemical compound in them. Basil or Ocimum basilicum contained compounds like tannin, flavonoids, astiri oil, saponin, and pentose that provide a lot of benefits such as scavenging free radicals, improving blood flow, boosting the immune system, relieving nausea and influenza and also curing canker sores.[4] In addition, according to Zagoto et al.[5] basil leaf oil has been known to have an analgesic effect on mice. The second ingredient used is lime (Citrus aurantifolia). Limes contain a lot of beneficial compounds such as citrate acid, atsiri oil, as well as vitamins A, B1, and C. The potential of lime as an analgesic and stamina stimulator is also due to the presence of flavonoid and phenolic acid compounds. This study uses only the juice from lime.

Honey is a kind of medicine that can enhance energy and health as well. As one of the choices to protect health and prevent the presence of disease around the body, people usually use honey as a stamina stimulator. Minerals in honey are needed to keep the body healthy, meanwhile, the vitamins contained in honey play a role in the metabolism of protein. Nutrition in honey that works as antioxidants are vitamin C, organic acid, enzymes, phenolic acid, flavonoid, and beta-carotene. They are all considered highly functioning antioxidants. Candlenut contains compounds such as flavonoids, polyphenols, vitamins, folate, proteins, carbohydrates, tannin, alkaloids, saponins, steroids, and terpenoids. People use candlenuts to stimulate hair growth, cure wounds on the skin, ulcers, diarrhea, and asthma, and enhance the effect of analgesics.[6,7]

The last ingredient used is tiwai onion (*Eleutherine americana Merr.*). Beneficial compounds that are contained in tiwai onion are carbohydrate, protein, aldehyde, ketone, carboxylate, tannin, glycoside, and phenol. The bulbs of tiwai onions are used widely as traditional medicine for coronary vasodilatation, prothrombin reduction, anti-fertility, and the healing of the wound.

The study aimed to examine the analgesic effect of jamu made from basil, lime, honey, candlenut, and tiwai onion. On the other hand, the study was also conducted to evaluate the potential of jamu as a stamina stimulator. The study is expected to provide an overview of the potential of jamu made from basil, lime, honey, candlenut, and tiwai onion as an analgesic and stamina stimulator. In addition, the results of this study are expected to be useful as a basic consideration of the use of jamu as an efficacious drug.

Methods

This research was conducted at the Laboratory of Animal Management Unit of the Faculty of Veterinary Medicine IPB University. This research was held from January–February 2022. The research has met the rules of research ethics from the Committee of Animal Ethics, Faculty of Veterinary Medicine, Bogor Agricultural University, followed by the ethical clearance certificate number 058/KEH/SKE/VIII/2019. The tools used in this research were a digital scale, syringes, gastric probe, water bath, alcohol thermometer, and digital thermometer. While the materials used are candlenuts, limes, honey, basils, tiwai onions, 20 mice, and aquades. Mice were divided into 4 groups based on the treatment dose and each group consisted of 5 mice. The mice were divided into 4 groups. There are control, dose 1 g/kg body weight, dose 2 g/kg body weight, and 4 g/kg body weight. After that, the suspension was administrated orally with a gastric tube.

The collection of ingredients that are used in this research is obtained from different places. Candlenuts, limes, and basils are obtained from Dramaga traditional market. Tiwai onions are obtained from Kalimantan meanwhile, pure forest honey is obtained originally from the Baduy tribe, Lebak, Banten.

The process of making starts with drying 500 grams of basil leaves, 500 grams of candlenuts, and 500 grams tiwai onions in the sun. Before the drying process, tiwai onions were peeled and cut into small pieces. After basil leaves, candlenuts, and tiwai onions were dried, each of them was mashed and weighed as much as 50 g. Basil leaves and candlenuts were boiled and added with 100 mL aquades, meanwhile, 500 mL of tiwai onions are boiled under the temperature of 90°C for 15 minutes at a separate place. The result of the boiling was cooled and filtered using tea filter, and then put into a container.

Limes are washed clean with running water and cut into two pieces. Each piece was squashed with an orange squasher so that the lime juice was obtained. The juice then was filtered with a tea filter, so that the pulp and the seed could be separated. The juice that has been filtered was put into a container. Honey was not processed

further because the honey used was pure honey obtained from the forest. All ingredients that had been processed then were mixed, each ingredient as much as 20 mL, so that a total of 100 mL was obtained. The jamu was shaken so that the ingredients become homogenous. Jamu was then stored inside a refrigerator at a temperature of 4–5°C.

Cage preparation, acclimatization, and rearing of mice were done by cleaning the cages a week before the mice were introduced. The cages used were plastic boxes, measuring about 55x37x17 cm and covered with wire. Each cage was equipped with a drink container and wood husks as the base. Acclimatization was carried out for 7 days so that mice could adapt to the new cage condition, to reduce stress. During the process of acclimatization, mice were given drink via ad libitum, they were also fed, dewormed, and administered vitamin C and amoxicillin.

Each group was given a different dose. namely 1,2, and 4 g/kgBW, the average body weight of the mice used was 30 grams and the concentration of the preparation was 200 mg/mL so the total volume used for each mouse was 0.15, 0.3, and 0.6 mL. Administration of the preparation is done orally by mouth using a gastric probe. This aims to ensure that the suspension given is not wasted and can be completely absorbed into the body.

An analgesic test was done by administering the mice based on the treatment dose, after the administration, we waited for 5 minutes to let the test material be evenly distributed in the body of the mice. An analgesic test was carried out using the hot water immersion tail-flick test. This method was done by immersing half of the mouse tail inside the water bath until the tails flicked by themselves. The tail-flick test method has been widely used to measure the nociception degree experienced by laboratory animals. The response given is in the form of sudden snapping and pulling of the tail of the laboratory animals.[8]

Tests to determine the effect of analgesic were done by handling the mice on the nape of the neck or the skin behind the neck, then half of their tails were submerged in a water bath with a temperature of 50°C. The time needed from the start of the tail being submerged until the tail flicked was calculated using a stopwatch. The mean time of each group was counted and compared with the control. The test was repeated three times on different days with an interval of one day.

A stamina test was conducted 5 minutes after the analgesic test. A stamina test was done with the swimming endurance method. The swimming endurance method is a pharmacological screening method to determine the effect of drugs that work on the coordination of movement, both on the test for decreased central nervous control or increased central nervous control.[9] Mice were left to swim until they stopped as a sign of tiredness. The time spent by the mice swimming until they stopped was counted with a stopwatch and recorded. The average time of each group was calculated and compared with the control group. The test was repeated three times with an interval of one day. The parameter observed in the analgesic test was the time taken for the mice to flick half of their tail. In the stamina test, the parameter observed was the duration of swimming endurance of the mice.

Data were analyzed using Microsoft Excel 2019 and Minitab 19. A significant difference in the average of each group was shown statistically by analysis of variance (ANOVA) one way and Tukey test.

Results

The result of the research showed that mice that were administered with an infusion of jamu combination experienced a greater increase

Table 1 The Effect of Different Dosages of Jamu Combined Infusion on the Time (Seconds) of Tail Lift in Mice

Repeat	Group			
	Control	1	2	4
1	$(0.76\pm0.21)^{b}$	$(0.94\pm0.09)^{ab}$	$(1.02\pm0.13)^{ab}$	$(1.14\pm0.15)^{a}$
2	$(1.30\pm0.20)^{b}$	$(1.08\pm0.04)^{ab}$	$(1.36\pm0.05)^{a}$	$(1.48\pm0.15)^{a}$
3	$(1.66\pm0.09)^{b}$	$(1.46\pm0.38)^{ab}$	$(1.82\pm0.19)^{ab}$	$(2.2\pm0.45)^{a}$
Group Average	$(1.24\pm0.42)^{b}$	$(1.16\pm0.31)^{ab}$	$(1.4\pm0.36)^{ab}$	$(1.61\pm0.53)^{a}$

Note: Different superscripts a, ab, and b within the same row showed significant differences ($p<0.05$)

Table 2 The Effect of *Jamu* Combination on Swimming Time (seconds) on Mice

Repeat	Group			
	Control	1	2	4
1	(53.40±23.10)[a]	(74.40±9.89)[a]	(51.40±2.07)[a]	(69.40±7.60)[a]
2	(83.00±4.85)[a]	(89.20±7.92)[a]	(64.20±8.67)[b]	(88.40±4.93)[a]
3	(111.60±12.03)[b]	(148.20±30.70)[a]	(100.40±10.78)[b]	(122.60±17.13)[ab]
Group average	(82.67±28.39)[ab]	(103.93±37.48)[a]	(72±22.74)[b]	(93.47±25.03)[ab]

Note: Different superscripts a, ab, and b within the same row showed significant differences ($p<0.05$)

in analgesic effect compared to the control ($p<0.05$). The analgesic effect on the treatment dose was greater than the control. The average value of the highest analgesic effect was at a treatment dose of 4 g/kgBW. Treatment doses of 1 g/kgBW and 2 g/kgBW did not show any significant difference in analgesic effect. Based on the result of the study, the average analgesic effect increased altogether with an infusion of jamu combination given to the mice. The larger the dose of infusion of jamu combination, the greater the analgesic effect produced. Data on the analgesic effect on mice that were given the infusion of jamu combination were presented in Table 1.

The result of the study showed that mice that were given jamu combine infusion at the third repeat were able to swim for a longer duration compared to the control. Data analysis from the average of the repeated group showed that the duration of swimming was different between treatment doses ($p<0.05$). Mice with a treatment dose of 1 g/kgBW had the highest stimulation effect compared to other treatment doses. Data on the duration of swimming of the mice that were given jamu combined infusion are presented in Table 2.

Discussion

Based on the result of the study that was gained and analyzed statistically with the ANOVA test, followed by a 95% of confidence level about the effect of combined jamu infusion on male mice, the group of mice that were given combined jamu infusion had a longer tail lift time compared to control group or mice that were not given any combined jamu infusion ($p<0.05$). A stamina effect was shown from the swimming duration of mice that were given combined jamu infusion. The third repeated test showed the longest swimming duration compared to other repeated tests. This can be because the mice had adapted to the combination of jamu ingredients given. The longest swimming duration was found at a dose of 1 g/kgBW.

A stamina test was done by administering the mice with various doses, 1, 2, and 4 g/kgBW. Experiments on giving various doses to mice showed a great effect on increasing stamina. The stamina effect was suspected to come from basil leaves, tiwai onions, candlenut seeds, limes, and honey which were the ingredients of jamu combination infusion. Flavonoid compounds in red onion bulbs are useful in improving the immune defense system. Flavonoid compounds found in plants are proven to be able to stimulate the immune system with increasing macrophage activity and T lymphocyte.[10] The flavonoid compound in tiwai onion is suspected to be able to improve the immune system. Glycosylflavones are a group of flavonoid glycosides that have a special characteristic, namely glycoside compounds that are bound to the basic framework of flavones.

The result of the three combinations showed that the dose of 1 g/kgBW gave a greater tonic effect compared to the dose of 2 and 4 g/kgBW. A combination dose that matches the need will show a greater increase in stamina. Increased stamina is due to the compounds contained in honey, namely carbohydrates, proteins, amino acids, minerals, and vitamins. Other compounds that can increase stamina are fructose, glucose, and maltose which are contained in candlenuts.[12] Lime juice can increase stamina, suspected from its content, of flavonoids and alkaloids, based on another study conducted by Yulianita & Effendi.[11]

Based on the result obtained and analyzed statistically with the ANOVA test, followed by a 95% confidence level about the effect of combined jamu infusion on male mice, mice that were given combined jamu infusion has a longer average tail lift time compared to the control group of mice that were not given any combined jamu infusion ($p<0.05$). The average tail lift time that was given combined jamu

infusion was longer compared to the control. The average tail lift time of the group of mice given the combined jamu infusion increased along with the addition of the combined jamu infusion dose. This shows that combined jamu infusion can provide an analgesic effect on mice. The analgesic effect is suspected to come from a flavonoid compound that is contained in basil leaves, tawai onion, candlenut seeds, lime, and honey which are the ingredients of the combined jamu infusion. According to Meisyayati et al.,[12] the synergistic analgesic effect shown by the combination of a single dose of each ingredient is suspected to be because the flavonoid content alone of the combination is already sufficient to inhibit the action of cyclooxygenase enzyme and prostaglandin. The formulation and composition of the combined ingredients must be appropriate to obtain the desired effect and not cause contradictions.[13] The combined ingredients of combined jamu infusion showed an analgesic effect when tested alone. Research conducted by Al-Ghurabi[14] regarding the analgesic and sedative effect of *Ocimum basilicum* alcohol extract on male mice showed that *Ocimum basilicum* alcohol extract has analgesic and sedative effects. Another research regarding the potential effect of fruit peels from five plants of the Citrus genus done by Malleshappa et al.[15] stated that all Citrus fruits tested have the potential as antiinflammatory and antinociceptive. Another study stated that candlenut methanol extract can increase analgesic activity because it contains alkaloid compounds.

References

1. Nurrani L. Pemanfaatan tradisional tumbuhan alam berkhasiat obat oleh masyarakat di sekitar cagar alam Tangale. Info Balai Penelitian Kehutanan Manado. 2013;3(1):1–22.
2. Wowor PM, Posangi J, Irianty CR. Uji efek analgesik infusa etanol kelopak bunga matahari (Helianthus annuus Linn.) pada mencit swiss (*Mus musculus*). Jurnal E-Biomedik. 2014;2:2.
3. Mita SR, Husni P. Pemberian pemahaman mengenai penggunaan obat analgesik secara rasional pada masyarakat di Arjarsari Kabupaten Bandung. Dharmakarya. 2017; 6(3):193–5.
4. Andareto O. Apotik herbal di sekitar anda (solusi pengobatan 1001 penyakit secara alami dan sehat tanpa efek samping). Jakarta: Pustaka Ilmu Semesta; 2015.
5. Enejoh OS, Ogunyemi IO, Bala MS, Oruene IS, Suleiman MS, Ambali SF. Ethnomedical importance of citrus aurantifolia (Christm) swingle. Pharma Innovation J. 2015;4(8):1–6.
6. Adawiyah R. Uji identifikasi farmakognostik tumbuhan kemiri sunan (Aleurites trisperma) di kebun percobaan Universitas Muhammadiyah Palangkaraya. Anterior Journal. 2017;17(1):60–8.
7. Bilang M, Mamang, Salengke, Putra RP, Reta. Elimination of toxalbumin in candlenut seed (Aleurites molucccana (L) Willd) using wet heating at high temperature and identification of compounds in the candlenut glycoprotein. Int J Agriculture System. 2018;6(2):89–100.
8. Zhou Q, Bao Y, Zhang X, Zeng L, Wang L, Wang J, et al. Optimal interval for hot water immersion tail-flick test in rats. Acta Neuropsychiatrica. 2014;26(4):218–22.
9. Lukman FH, Vivi. Uji anti lelah (anti fatigue) kombinasi nira aren dan air tebu dengan metode ketahanan berenang (natatory exhaustion) pada mencit jantan. Pharmacy. 2013;10(2):124–37.
10. Zalisar L. Flavonoid of phyllanthus niruri as immunomodulator: a prospect to animal disease control. ARPN J Sci Technol. 2013;3(5):29–30.
11. Yulianita, Effendi EM. Uji Efektivitas Jangka panjang kombinasi ekstrak buah cabe jawa dan biji mahoni sebagai penambah stamina pada tikus putih jantan. Acta Veterinaria Indonesiana. 2015;3(2):64–9.
12. Meisyayati S, Immanuel J, Darwis D. Efek analgetik kombinasi ekstrak daun pepaya (carica papaya l) dan ekstrak daun asam jawa (tamarindus indica l) pada mencit putih jantan. Jurnal Ilmiah Bakti Farmas. 2017;2(1):63–70.
13. Herbie, T. Kitab tanaman berkhasiat obat: 226 tumbuhan obat untuk penyembuhan penyakit dan kebugaran tubuh. Cetakan Pertama. Yogyakarta: Octopus Publishing House; 2015.
14. Al-Ghurabi SES. Study the analgesic and sedative effect of Ocimum basilicum alcoholic extract in male rats. Diyala Agricultural Sci J. 2014;6(1):9–22.
15. Malleshappa P, Kumaran RC, Venkatarangaiah K, Parveen S. Peels of citrus fruits: a potential source of anti- inflammatory and anti-nociceptive agents. Pharmacognosy J. 2018;10(6):172–8.