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ANALGESIC EFFECT OF *KUMIS KUCING (ORTHOPSIPHON STAMINEUS)* IN MICE (*MUS MUSCULUS)* WITH WRITHING METHOD

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ABSTRACT

Background: Pain is an unpleasant sensation that can range from mild to severe, causing discomfort and potentially disturbing daily activities. Therefore, pain management and treatment are necessary. *Kumis kucing* (*Orthosiphon stamineus*) is a traditional medicinal plant known to contain flavonoids with anti-inflammatory and analgesic properties that can help alleviate pain. **Objective:** This study aimed to investigate the analgesic activity and determine the effective dose of *Orthosiphon stamineus* extract. **Methods:** The design of this study was a true experimental post-test-only randomized controlled group. Pain was induced using a 1% acetic acid intraperitoneal injection to evoke the writhing reflex in mice. The writhing reflex was observed every 10 minutes for 1 hour, and the data were analyzed using one-way ANOVA followed by a post-hoc test. **Results:** The results revealed significant differences in analgesic activity between the P1 and P3 groups, as well as between the P2 and P3 groups ($p < 0.05$), indicating that the *kumis kucing* extract at a dose of 0.24 mg/gBW effectively reduced pain, as demonstrated by a decreased total number of writhing reflexes. The pain protection percentage at the 0.24 mg/gBW dose was 48.75%. **Conclusion:** *Kumis kucing* extract has significant analgesic activity, with the most effective dose being 0.24 mg/gBW.

INTRODUCTION

Pain is a sensation that almost every person has experienced. The International Association for the Study of Pain (IASP), an international organization engaged in pain research and education, defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage[1]. Pain is not a disease in itself, but rather a symptom of various illnesses. As such, pain is often likened to an alarm that protects the body from further tissue damage. The unpleasant sensations caused by pain can be stabbing, burning, electric shock-like, and other forms, which interfere with the quality of life of patients or individuals experiencing pain. Hence, prompt pain management is required[2].

The most common approach for pain management is the use of analgesics from the nonsteroidal anti-inflammatory drug (NSAID) class, such as paracetamol and ibuprofen, which are readily available by prescription or over-the-counter in shops and pharmacies. These drugs work by inhibiting the production of prostaglandins, which serve as mediators of pain[3]. Nevertheless, the use of NSAIDs can cause side effects, especially when used long term, such as hepatotoxicity, upper gastrointestinal bleeding, kidney damage, coagulation disorders, and cardiovascular disturbances[4].

Kumis kucing (*Orthosiphon stamineus*) is a tropical plant that grows abundantly in countries across Asia, such as Indonesia, Malaysia, Thailand, Singapore, the Philippines, Vietnam, Cambodia, and Papua New Guinea. In Indonesia, *kumis kucing* is classified as a traditional herbal plant found throughout the archipelago. Traditionally, *kumis kucing* has been used as a diuretic and for blood glucose control in diabetes mellitus. *Orthosiphon stamineus* is a herbal plant that grows wildly on forest edges, in arid areas, or along roadsides[5].

Phytochemical studies to assess the content of *Orthosiphon stamineus* have been conducted since the

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1930s[6]. Meanwhile, the pharmacological activity of *kumis kucing* has been extensively researched, including its role as an anti-inflammatory, diuretic, antioxidant, antihypertensive, anti-angiogenic, antimicrobial, and antidiabetic agent[7]. Several flavonoid compounds such as sinensetin (SIN), eupatorin (EUP), and 3′-hydroxy-5,6,7,4′-tetramethoxyflavone (TMF), as well as rosmarinic acid (RA), a caffeic acid derivative, have been identified as the main anti-inflammatory compounds in *kumis kucing* leaves, indicating their potential analgesic effects through the suppression of inflammatory pain[5, 6, 8, 9]. A study on the bioactive compounds of *kumis kucing* (*Orthosiphon stamineus*) hexane extract suggests that the leaves have potential analgesic properties [10].

Based on the above, this study was conducted using ethanol extract of *kumis kucing* leaves to reduce pain. The analgesic activity was evaluated using the writhing reflex test by observing the number of writhes in mice, marked by stretching, abdominal constrictions, pressing down towards the floor of the cage, backward leg pulling, tensing one side of the body, or rolling and circling in the cage[11-13]. The protection index was then calculated to assess the effectiveness of the test material as an analgesic.

METHODS

Instruments and Materials

The equipment used included an Erlenmeyer flask for maceration, measuring glasses, water bath, analytical balance, animal scale, animal cages, gastric sonde, disposable syringe, and stopwatch. The materials used comprised 1 kg of *kumis kucing* leaves sourced from a farmer in the Gunungpati area, Semarang, 70% ethanol, 0.5% CMC solution, aspirin, 0.1% acetic acid, male mice, as well as standard feed and drinking water for the test animals.

Test Animals

This study used a post-test only randomized controlled group design, with male mice (Mus musculus) as test subjects. The test animals were males, 6–8 months old, weighing 20–30 g, in normal health, and appearing active.

Extract Preparation

The *kumis kucing* leaf extract was prepared using the maceration method. A total of 1 kg of *kumis kucing* leaves were finely chopped and dried in the shade (avoiding direct sunlight). The dried leaves were weighed at 40 g and soaked in 500 mL of 70% ethanol in an Erlenmeyer flask, shaken until homogenous, sealed, and kept in a dark room for 48 hours. The filtrate was then collected and evaporated over a water bath at 50 °C. The resulting extract was stored in a sterile bottle and kept ready for use.

Treatment of Test Animals

Thirty mice that met the inclusion criteria were acclimatized for 7 days in the laboratory with access to standard feed and drinking water. Prior to treatment, the mice were fasted for 12 hours (with access to water). On the eighth day, the mice were randomly divided into five groups (six mice per group) by simple random sampling. Group 1 (K1) served as the negative control and received 0.5% CMC solution (0.5 mL). Group 2 (K2) served as the positive control and received aspirin orally at a dose of 0.065 mg/g body weight. Groups 3 (P1), 4 (P2), and 5 (P3) were the treatment groups that received *kumis kucing* leaf extract orally at dosages of 0.06 mg/g body weight, 0.12 mg/g body weight, and 0.24 mg/g body weight, respectively. After 15 minutes, all mice from the five groups were administered an intraperitoneal injection of 1% acetic acid at a dose of 0.1 mg/g body weight.

Each mouse was then observed and the number of writhes was counted, defined as the extension of all four legs forward and backward, followed by abdominal contraction towards the floor of the cage, every 10 minutes for 60 minutes. The results were tabulated and statistically analyzed. The writhing response was an indicator of the pain induced by acetic acid.

Statistical Analysis

In this study, data were analyzed using a one-way ANOVA test followed by a Post Hoc test with SPSS 15.0 for Windows Evaluation Version. Initially, the average number of writhes per mouse over 1 hour was calculated using statistical software, followed by the calculation of the percentage of analgesic protection using the Henderson–Forsaith [14] formula:

$$\% \text{ Protection against acetic acid} = [100 - (P/K \times 100)]\%$$

P = Average number of writhes in the treatment group
K = Average number of writhes in the negative control group

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RESULTS AND DISCUSSION

The characteristics of mouse writhing in each treatment group are presented in Table 1. The lowest average number of writhes over 1 hour was observed in the P3 group, while the highest average number was observed in the P2 group.

Table 1. Average number of writhes in mice over 1 hour

G	MSD	*Levene (p^L)*	*Post Hoc Games-Howell (p^h)*			
			K2	**P1**	**P2**	**P3**
K1	24,0 ± 15,6		0,999	0,615	0,503	0,582
K2	25,7 ± 9,0		-	0,379	0,328	0,205
P1	33,8 ± 4,8	0,015		-	0,972	0,016*
P2	36,3 ± 9,2				-	0,013*
P3	12,3 ± 10,4					-

G: Group; MSD: Mean±SD; * p & p^h <0,05 signifikan; *p^L>0,05 homogen

The data on the number of writhes presented in Table 1 were used to calculate the percentage of analgesic protection using the Henderson–Forsaith formula.

Table 2 shows the percentage of protection against acetic acid-induced pain. As indicated in Table 2, the percentage of protection increased in the group treated with the *kumis kucing* leaf extract at a dose of 0.24 mg/g BW (P3). A clear relationship was observed between the average number of writhes and the percentage of pain protection for each group. The higher the average number of writhes, the lower the percentage of analgesic protection, and vice versa.

Table 2. Percentage of protection against acetic acid-induced pain

Group	% pain protection
K1	0%
K2	-7%
P1	-40,8%
P2	-51,25%
P3	48,75%

Referring to Table 2, the percentage of pain protection induced by acetic acid was found to be increased in the treatment group administered *kumis kucing* leaf extract at a dose of 0.24 mg/gBW (P3). It can be seen that there is a relationship between the average number of mouse writhing and the percentage of pain protection for each group. The higher the average number of mouse writhing, the lower the percentage of pain protection in that group. Conversely, the lower the average number of mouse writhing, the higher the percentage of pain protection in that group.

Based on the available data, statistical analysis was conducted to assess the presence or absence of differences between treatment groups. The Shapiro–Wilk test was performed first to assess the normality of the data distribution, and the results showed a normal data distribution where $p > 0.05$. Next, a one-way ANOVA test was used to determine the differences between treatment groups, and homogeneity of data was assessed using Levene's test.

Based on Table 1, the results of Levene's test indicated that the data distribution was not homogenous. Meanwhile, the results of the one-way ANOVA test showed $p < 0.05$, indicating a significant difference between the treatment groups. The data analysis was further conducted using the Games–Howell post hoc test to determine the level of statistical significance for each group.

The Games–Howell post hoc test, as presented in Table 1, showed a significant difference between the P1 and P3 groups, indicating a significant difference in the analgesic ability of mice administered *kumis kucing* leaf extract at a dose of 0.06 mg/gBW (P1) compared with those administered *kumis kucing* leaf extract at a dose of 0.24 mg/gBW (P3). A significant difference was also found between the P2 and P3 groups, indicating a significant difference in the analgesic ability of mice administered *kumis kucing* leaf extract at a dose of 0.12 mg/gBW (P2) compared with those administered *kumis kucing* leaf extract at a dose of 0.24 mg/gBW (P3).

Meanwhile, no significant differences were observed between the K1 group and the K2, P1, P2, and P3 groups, nor between the K2 group and the P1, P2, and P3 groups. The P1 group and the P2 group also showed no significant difference. This lack of significance means that there was no meaningful difference in the analgesic ability between those groups.

The difference in the analgesic activity between the P1 and P3 groups, and between the P2 and P3 groups, was significant, which can be interpreted as the *kumis kucing* leaf extract administered at a dose of

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0.24 mg/gBW (P3) reducing the degree of pain in mice as indicated by the decreased number of writhing movements in response to acetic acid-induced pain.

The calculated percentage of pain protection from the *kumis kucing* leaf extract administered at a dose of 0.24 mg/gBW was relatively high, approaching 50%, at 48.75%. This is due to the activity of flavonoids present in the *kumis kucing* leaf extract. Flavonoids are known to work as anti-inflammatories by inhibiting the cyclooxygenase-2 (COX-2) enzyme and the synthesis of nitric oxide [15].

This research is in line with the findings of Akowuah and Zhari, who used 50% methanol extract of *kumis kucing* leaves in rats to assess its anti-inflammatory and analgesic activity [16]. The results showed that oral administration of the extract at a dose of 1000 mg/kgBW demonstrated anti-inflammatory effects, evidenced by a reduction in paw edema caused by carrageenan in rats. The analgesic activity was confirmed in the acetic acid-induced writhing test and formalin-induced licking test in mice and rats. Meanwhile, no effects were observed in the tail-flick and hot plate tests, even when the extract was administered at a dose of 1000 mg/kgBW [16].

Yam et al. conducted a study that investigated the anti-inflammatory activity of the chloroform fraction of *kumis kucing* leaves induced by carrageenan. The results indicated that the oral administration of the flavonoid-rich chloroform fraction at doses of 500 mg/kgBW and 1000 mg/kgBW significantly reduced paw edema in rats. Phytochemical screening confirmed the presence of SIN and EUP, which are flavonoids [8]. Another related study by Prayoga aimed to assess the anti-inflammatory effects of the methanol extract of *kumis kucing* leaves in male Wistar strain rats induced with 0.1% carrageenan. The results showed that the *kumis kucing* leaf extract possessed lower anti-inflammatory activity compared with sodium diclofenac [17].

Previous studies on the analgesic activity of *kumis kucing* leaf extract have not been extensively reported. In Indonesia, no such studies have been conducted. Nevertheless, the analgesic activity is related to its anti-inflammatory activity, as the release of prostaglandins as pain mediators is also triggered by the inflammatory process. The mechanism of action of the *kumis kucing* leaf extract as an analgesic is presumed to be in line with its mechanism as an anti-inflammatory, by inhibiting the formation of prostaglandins as pain mediators through the cyclooxygenase-2 (COX-2) pathway.

The differences in analgesic activity between the P1 and P2 groups were found to be not significant. In terms of the calculated percentage of pain protection, the P1 group showed a protection of -40.8%, and the P2 group -51.25%. This can be interpreted as the *kumis kucing* leaf extract administered at doses of 0.06 mg/gBW and 0.12 mg/gBW having no analgesic activity, possibly due to the low dosage used. This is a limitation of this study.

Another limitation is that the data collected were subjective, relying on the researchers' interpretation of the writhing behavior itself. Moreover, the relatively large sample size posed a challenge for observations, leading to the use of a camera to record mouse writhing. However, due to camera limitations, the recordings were only captured from one angle.

CONCLUSION

Kumis kucing leaf extract (*Orthosiphon stamineus*) has an analgesic effect in mice, and there is a difference in the effectiveness of its analgesic ability at doses of 0.06 mg/gBW, 0.12 mg/gBW, and 0.24 mg/gBW, where the most effective dose is 0.24 mg/gBW with a pain protection rate of 48.75%, while the 0.06 mg/gBW and 0.12 mg/gBW doses have no analgesic activity. Thus, there is no dose–effect relationship observed for the analgesic effect of *kumis kucing* leaf extract across the three dose levels in this study.

ETHICAL APPROVAL

The study was conducted with ethical clearance from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Diponegoro University, No. 110/EC/H/FK-UNDIP/IX/2021.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Joana Anjelia Nai Da Cruz: investigation, resources, data curation, writing—original draft

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