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Descriptive Analysis the Analgesic Activity of Chloroform Fraction from *Acorus Calamus L.* Leaf Extract on Male White Mice (*Mus Musculus*) Induced by Acetic Acid

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ABSTRACT
Pain management is a critical aspect of pharmaceutical research, and Acorus calamus L., commonly known as jeringau, has garnered attention for its potential analgesic properties. This study focuses on evaluating the analgesic activity of the chloroform fraction from ethanol extract of jeringau leaves (Acorus calamus L.) on male white mice induced by acetic acid. Chronic and acute pain significantly impacts individuals' quality of life, emphasizing the need for effective and safe analgesic alternatives. While Acorus calamus leaf extract has shown pharmacological potential, especially the chloroform fraction, a dedicated investigation into its analgesic effects on animal models is lacking. Male white mice, recognized for their reproducible and measurable responses to painful stimuli, serve as relevant subjects in this study. The population of 25 mice, aged 2-3 months and weighing 20-30 grams, is divided into 5 treatment groups, including negative and positive controls. The study employs three dosages of the chloroform fraction, administered orally, with observations made on writhing behavior following intraperitoneal injection of 0.5% acetic acid. Statistical analysis will evaluate significant differences between treatment groups, providing insights into the analgesic potential. Results indicate that Dosage III (44.8 mg/40g BW) exhibits the highest analgesic efficacy at 73.99%, showcasing a dose-dependent response. The mechanism involves flavonoids inhibiting the cyclooxygenase enzyme, aligning with established analgesic actions. Ibuprofen, the positive control, and jeringau leaf extract demonstrate analgesic effects, reinforcing traditional knowledge. This research contributes to scientific understanding, establishing a foundation for further exploration.

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INTRODUCTION

Pain is a sensory and emotional experience that is unpleasant, and its management is becoming increasingly crucial in the field of pharmacy. Acorus calamus L., commonly known as sweet flag or jeringau, has long been utilized in traditional medicine to alleviate

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various health disorders, including conditions related to pain (Fitzcharles, M. A., 2021). Previous research indicates that the leaf extract of Acorus calamus, particularly the chloroform fraction, possesses significant pharmacological potential. However, there has been no in-depth study specifically evaluating the analgesic activity of the chloroform fraction from Acorus calamus leaf extract on animal models (Sharma, V., 2020).

Chronic and acute pain have a significant impact on an individual's quality of life. The use of conventional analgesics often comes with the risk of side effects that can affect other bodily systems. Therefore, recent research is increasingly focused on the identification and evaluation of natural analgesic agents that can provide therapeutic effects without causing adverse impacts (Glare, P., 2020).

The extract of jeringau leaves, containing several bioactive compounds, has garnered attention in this context. Although some studies indicate its pharmacological activities, such as anti-inflammatory and antioxidant properties, there has been no specific research examining the potential analgesic effects of the chloroform fraction from jeringau leaf extract (Prawanayoni, S. S., 2020).

Male white mice (*Mus musculus*) are considered a relevant model in analgesic research as their responses to painful stimuli can be reliably reproduced and objectively measured. The use of mice as an animal model in this study is expected to provide better insights into the analgesic effects of the chloroform fraction from jeringau leaf extract (Setiaji, J., 2022).

This research is not only aimed at identifying the analgesic potential but also at understanding the underlying mechanisms of these effects (Ardianto, N., 2023). By deepening this understanding, it is hoped that doors will be opened for the development of more targeted and efficient pain therapies (Kovalenko, A. M., 2022). Through this study, we strive to make a significant contribution to the scientific understanding of the therapeutic potential of Acorus calamus L. while simultaneously creating opportunities for the discovery and development of analgesic agents that can help meet the needs of patients experiencing pain (Wang, J. F., 2021).

In this context, this research aims to address this knowledge gap by systematically examining the analgesic activity of the chloroform fraction from Acorus calamus leaf extract on male white mice (*Mus musculus*) induced by acetic acid. A profound understanding of the analgesic effects of this chloroform fraction is expected to make a positive contribution to the development of more effective and sustainable pain therapies (Islam, M. M., 2021).

This study not only contributes to the scientific literature regarding the therapeutic potential of Acorus calamus but also provides a strong foundation for further development in the fields of pharmacy and traditional medicine (Akbar, S., 2020). With this research, it is anticipated that opportunities will arise for further exploration of the analgesic properties of the chloroform fraction from Acorus calamus leaf extract as a basis for the development of new drugs that can effectively and safely mitigate pain (Elshikh, M. S., 2022).

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METHODS

This study is an experimental research conducted in a laboratory setting with the aim of delving into a phenomenon or effect that arises as a result of specific treatments. The focus of this research is the evaluation of the analgesic activity of the chloroform fraction from ethanol extract of jeringau leaves (*Acorus calamus L.*) on male white mice.

Picture 1. *Acorus Calamus L.* Leaf

Within the framework of this research, the involved population consists of 25 male white mice (*Mus musculus*) with an age range of 2-3 months and a weight between 20-30 grams. This population is divided into 5 different treatment groups. The negative control is administered with Na CMC suspension, while the positive control receives ibuprofen suspension. Furthermore, the dosage orientation of the extract is conducted by applying 3 variations of doses. A chemical method of pain induction will be implemented to evaluate the extent of analgesic efficacy possessed by the chloroform fraction of the ethanol extract of jeringau leaves. In order to measure the level of analgesia, specific parameters will be carefully observed and measured (Saristiana, Y., 2023). Thus, this research aims not only to assess the analgesic activity of the chloroform fraction from ethanol extract of jeringau leaves on mice but also to gain a deeper understanding of the responses and effects generated by the treatments. Overall, this study is expected to make a valuable contribution to the context of developing more advanced and targeted analgesic therapies.

Picutre 2. White Mice (*Mus Musculus*)

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The testing methodology will encompass careful and structured stages. Firstly, mice within the treatment groups will receive doses according to predetermined parameters. Both negative and positive control groups will also undergo specific treatments. Subsequently, pain induction using a chemical method will be applied to all treatment groups. Thorough observations will be conducted on the mice's responses to pain stimuli and the potential analgesic effects that may arise after the administration of the extract. Measured parameters will include reaction time, frequency of avoidance behaviors, and the discomfort level exhibited by the mice.

Meticulous statistical analysis will be employed to evaluate significant differences between the treatment groups. This will allow for accurate conclusions regarding the effectiveness of the jeringau leaf extract as an analgesic agent. A profound understanding of the mice's responses to pain and the analgesic potential of the extract is expected to provide a robust foundation for the expansion of further research. With this comprehensive approach, it is anticipated that this study will make a valuable contribution to the scientific understanding of the therapeutic potential of jeringau leaf extract and pave the way for the development of more focused and effective pain therapy strategies. This research is expected to establish a more solid scientific basis for the development of new and innovative drugs in the field of analgesics.

In the final stage of the research, a thorough data analysis will be conducted to evaluate the experimental results. Each measured variable will be statistically analyzed using appropriate methods, such as analysis of variance (ANOVA) or independent t-tests, to identify significant differences between the treatment groups. The results obtained from the statistical analysis will be carefully interpreted to draw robust conclusions. The potential impacts or implications of these findings on the development of analgesic therapies will be extensively discussed, detailing the potential strengths and weaknesses of jeringau leaf extract as a candidate analgesic agent. Findings will be compared and contrasted with previous research in the scientific literature to highlight the unique contributions and added value of this study. This discussion will aid in formulating a more comprehensive understanding of the therapeutic potential of jeringau leaf extract in managing pain.

Additionally, recommendations for further research and development will be proposed. Exploring more in-depth mechanisms of action, examining more detailed dosage influences, or even considering the use of alternative animal models may be subsequent steps to deepen the understanding of the analgesic potential of jeringau leaf extract. By concluding this research in a systematic and detailed manner, it is hoped that its scientific contributions will provide valuable guidance to the scientific community and the pharmaceutical industry in their efforts to develop more effective and safer analgesic alternatives.

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RESULTS AND DISCUSSION

The The plant utilized in this research is the Acorus calamus L. leaf, commonly known as jeringau, which has been identified through determinations conducted at the Material Medika Laboratory in Batu city. The determination results confirm that the sample used is Acorus calamus L. from the genus Acorus and the Araceae family. This plant determination aims to ensure the accuracy of the plant used in the research material, preventing errors in the collection process, and avoiding any contamination with other plants that could potentially influence the research outcomes.

The mice used in the study were subjected to an 18-hour fasting period prior to treatment, with access to water to prevent any interference from food. The mice were divided into 5 groups, each consisting of 5 individuals (Prasetyawan, F., 2023). All treatments were administered orally. The grouping of the test animals was conducted randomly to ensure that each member of each treatment group had an equal chance of being selected as a sample. The positive control group was given ibuprofen, the negative control group received 0.2% Na-CMC. The positive control serves as a benchmark for analgesic efficacy compared to the sample under investigation. Dosage groups were given chloroform fraction of jeringau leaf ethanol extract, with Dosage I receiving 11.2 mg/40g body weight of mice, Dosage II receiving 22.4 mg/40g body weight of mice, and Dosage III receiving 44.8 mg/40g body weight of mice. The observations of the mice's writhing behavior were conducted for 1 hour after the intraperitoneal injection of 0.5% acetic acid.

The negative control group administered with 0.2% Na-CMC exhibited the highest writhing response compared to other control groups. This is attributed to the fact that the negative control lacks active substances capable of reducing pain. Na-CMC is also employed as a suspending agent due to its ability to produce a stable suspension with high clarity without affecting the active ingredients (Susanty., 2014).

Ibuprofen at a dose of 200 mg was chosen for the positive control group as it is widely recognized by the public as a pain-relieving medication with high absorption (Prasetyawan, F., 2022). Ibuprofen is known for its analgesic properties, acting by inhibiting the cyclooxygenase enzyme, disrupting the conversion of arachidonic acid. Ibuprofen inhibits both COX-1 and COX-2, limiting the production of prostaglandins associated with tissue damage, providing analgesic and anti-inflammatory effects (Muhammad, 2012).

The rationale for using three different doses in this study is to examine whether there is a dose-response relationship in the experimental results. If a test substance demonstrates a dose-response relationship, it implies that the larger the dose administered, the greater the effect produced. Hence, it can be concluded that the expected effect originates from the test substance (Winarti and Wantiyah, 2011).

Based on the average number of writhing movements observed in mice over 1 hour, the negative control group yielded the highest average writhing count at 157.6. The positive control group exhibited an average writhing count of 43.6, while Dosage I (11.2 mg/40g BW) resulted in 101 writhing movements, Dosage II (22.4 mg/40g BW) had 54.6, and Dosage III (44.8 mg/40g BW) showed 41 writhing movements.

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The highest analgesic efficacy was observed at Dosage III (44.8 mg/40g BW), with a percentage of 73.99%. Dosage II (22.4 mg/40g BW) exhibited an analgesic effect of 65.92%, while Dosage I (11.2 mg/40g BW) showed a percentage of 35.92%. The ability of jeringau leaves to alleviate pain is attributed to the presence of flavonoids, which operate by inhibiting the cyclooxygenase enzyme. This inhibition hinders the production of prostaglandins, thereby reducing pain (Mohan et al., 2012). This indicates that both ibuprofen and the chloroform fraction of jeringau leaf extract at Dosages I, II, and III have analgesic effects. The greater the concentration of the extract, the greater its ability to alleviate pain.

According to a study by Holifah (2019), ethanol extract of jeringau leaves exhibited analgesic activity with a percentage of 78% at Dosage III, classifying it as having strong analgesic activity. This was compared to the positive control, sodium diclofenac 50mg, which showed an analgesic percentage of 51%. The analgesic ability of the ethanol extract of jeringau leaves is attributed to the presence of flavonoid compounds. The mechanism of action of flavonoids involves inhibiting the cyclooxygenase enzyme, reducing the production of prostaglandins (pain mediators) from arachidonic acid and subsequently diminishing pain sensation (Holifah, 2019).

CONCLUSION

In conclusion, the experimental investigation on the analgesic activity of the chloroform fraction from ethanol extract of jeringau leaves (Acorus calamus L.) in male white mice induced by acetic acid has provided valuable insights. The study involved three different dosages, revealing a dose-dependent response with the highest analgesic efficacy observed at Dosage III (44.8 mg/40g BW). This finding suggests a correlation between the concentration of the jeringau leaf extract and its ability to alleviate pain. The observed analgesic effects of the jeringau leaf extract can be attributed to the presence of flavonoids, which act by inhibiting the cyclooxygenase enzyme and, consequently, reducing the production of prostaglandins. This mechanism aligns with the well-established analgesic action of ibuprofen, the positive control used in the study. The experiment underscores the potential of jeringau leaves as an analgesic agent, supporting traditional knowledge regarding its medicinal properties. Comparatively, the results align with prior research, such as Holifah's study, reinforcing the notion that the ethanol extract of jeringau leaves exhibits strong analgesic activity. The comprehensive evaluation of different dosages and the correlation of the findings with known mechanisms of action contribute to the robustness of the study. In the broader context, these findings contribute to the scientific understanding of jeringau leaves as a potential source of analgesic compounds. The research provides a foundation for further exploration, including investigating specific flavonoids responsible for the analgesic effects and exploring additional animal models. The study's systematic and detailed approach enhances its credibility, offering valuable guidance for future research in the development of effective and safe analgesic alternatives.

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