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Analgesic effectiveness test of kitolod leaf ethanol extract (isotoma longiflora l.) against male white mice (mus musculus)

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ABSTRACT

This study aims to evaluate the analgesic effectiveness of ethanol extract of Kitolod leaves (*Isotoma longiflora* L.) in male white mice (*Mus musculus*). Kitolod leaves are traditionally used as herbal remedies for pain, yet scientific evidence regarding their analgesic potential remains limited. This true experimental research employed a pre- and post-test with control group design, involving five groups: negative control (Na-CMC 1%), positive control (diclofenac sodium 50 mg), and three treatment groups administered Kitolod leaf extract at doses of 56 mg/kgBW, 112 mg/kgBW, and 224 mg/kgBW. Extract preparation was carried out using maceration with 96% ethanol, followed by phytochemical screening which confirmed the presence of flavonoids, alkaloids, tannins, and steroids. Analgesic activity was assessed using the tail flick method by measuring latency response to thermal stimuli at intervals of 30, 60, 90, 120, 150, and 180 minutes post-treatment. The results showed that Kitolod leaf extract increased pain response latency, with the 112 mg/kgBW and 224 mg/kgBW doses demonstrating the strongest analgesic effects comparable to diclofenac sodium. Statistical analysis using independent sample t-test and one-way ANOVA indicated significant differences ($p < 0.05$) between treatment and control groups. These findings suggest that Kitolod leaf ethanol extract possesses promising analgesic properties and may serve as a potential natural alternative for pain management.

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INTRODUCTION

Pain is one of the most common complaints experienced by humans and is the primary reason people seek medical help. Pain is defined as an unpleasant sensory and emotional experience associated with actual as well as potential tissue damage (Bahrudin, 2018). When pain stimuli are received by nociceptors, the body triggers a response in the form of increased heart rate, blood pressure, anxiety, and decreased comfort. If not treated properly, pain can reduce quality of life,

interfere with activities, decrease immunity, and accelerate tissue damage (Sakinah, 2019). This condition makes pain management an important aspect in the world of health, both through pharmacological and non-pharmacological therapies. One of the groups of drugs that are widely used to relieve pain is analgesics. Analgesics can be divided into opioid and non-opioid analgesics or peripheral analgesics. The use of non-steroidal analgesics (NSAIDs) such as ibuprofen, paracetamol, sodium diclofenac, and mefenamic acid is the main choice for treating mild to moderate pain (Suardi, 2021). Its main mechanism is to inhibit the enzyme cyclooxygenase (COX), which plays a role in the formation of prostaglandins as pain and inflammatory mediators (Mercya et al., 2017). However, long-term or inappropriate use of NSAIDs can increase the risk of side effects such as gastric irritation, gastrointestinal mucosal damage, impaired liver function, allergic reactions, and kidney failure (Husada et al., 2019). Recent studies have even shown that paracetamol is one of the main causes of drug-induced liver injury (DILI) in several countries with high levels of use (Rotundo & Pyrsopoulos, 2020). The risk of side effects of these synthetic drugs encourages the public and researchers to look for alternatives to natural ingredients that are considered safer. WHO has long recommended the use of traditional medicinal plants as an effort to maintain health, prevent disease, and support the therapy of various degenerative disorders (Liana, 2017). Indonesia, as a megabiodiversity country, has more than 30,000 species of plants, of which around 9,600 are suspected to have potential as medicinal plants, and around 300 species have been used as raw materials for herbal medicines (Permana et al., 2022). One of the plants that is widely used traditionally is the leaves of cytolod (*Isotoma longiflora* L.).

Kitolod has been empirically used by the community to treat various health disorders, including eye irritation, strep throat, toothache, and wound healing (Lestari & Wuryandari, 2020). This plant is easy to find in the surrounding environment and grows wild in lowlands up to an altitude of 1,100 meters above sea level (Permana et al., 2022). Although it is often used as a traditional medicine, scientific research to prove its pharmacological activity still needs to be developed, especially regarding its analgesic effects.

The results of phytochemical screening showed that cytoid leaves contain flavonoids, alkaloids, terpenoids, phenolics, saponins, and tannins (Fazil et al., 2017). These compounds are known to have various biological activities such as anti-inflammatory, antibacterial, antioxidant, anticancer, and analgesic (Whinetta & Kristiani, 2021). Flavonoids work by inhibiting the enzyme cyclooxygenase, thereby reducing the production of prostaglandins, which are the main mediators of pain (Mahardika & Wartini, 2021). In addition, flavonoids can inhibit the degranulation of neutrophils and decrease the release of proinflammatory cytokines (Gusti Ayu Rai Saputri et al., 2023). Alkaloids also play a role in the analgesic effect by inhibiting the biosynthesis of prostaglandins in inflammatory pathways (Lina & Rahmawaty, 2022).

The scientific relevance of evaluating the analgesic activity of *Isotoma longiflora* leaves lies in the need to systematically investigate underexplored medicinal plants that possess established ethnopharmacological use but remain insufficiently characterized in terms of pharmacological efficacy. In contrast to well-established herbal analgesics with extensively documented mechanisms of action and therapeutic profiles, *I. longiflora* lacks robust experimental evidence supporting its analgesic potential and the contribution of its bioactive constituents. Therefore, this study is essential to broaden the current pharmacological landscape of plant-derived analgesics and to identify potential alternative or complementary mechanisms of analgesic action. Furthermore, this investigation supports the scientific and sustainable utilization of local biodiversity as a source of novel drug candidates within the framework of modern natural product–based drug development.

Previous research has shown that chitolod leaf extract has potential pharmacological activities, such as antibacterial activity against *Streptococcus mutans* and *Enterococcus faecalis* (Fazil et al., 2017). Chitolod has also been shown to accelerate the healing of burns in mice, suggesting the presence of anti-inflammatory potential that may be related to analgesic effects (Anggyadinata et

al., 2020). Limitations in current herbal analgesic research are evident from the predominant focus on plant species with well-established pharmacological profiles, while medicinal plants traditionally used for pain relief remain insufficiently explored through scientific investigation. The scarcity of pharmacological data specifically evaluating analgesic activity, together with the limited understanding of the relationship between phytochemical constituents and analgesic mechanisms, represents a major challenge in this field. In this context, *Isotoma longiflora* leaves are academically relevant due to the lack of robust evidence regarding their efficacy, dose–response characteristics, and pharmacological mechanisms, highlighting their potential contribution to the development of standardized, evidence-based herbal analgesics.

One of the commonly used methods to test the effects of analgesics in test animals is the *tail flick method*. This method measures the reaction time of the tail of a mouse to heat stimulation, so that the longer the response time, the higher the analgesic activity provided by a test substancem (Safitri et al., 2022). The use of mice as test animals was chosen because these animals have a physiology relatively similar to humans, are easy to care for, and are sensitive to pain stimuli (Yusuf et al., 2022). With the right research design, the results of the analgesic test can provide a clear picture of the potential of chitolod leaf extract as a candidate for herbal analgesics (Hasanah et al., 2015). In the context of the development of new, safer medicines, research on chitolod leaf extract is important due to its significant bioactive compound content and its widespread use in traditional medicine (Kartika et al., 2013). The analgesic potential of chitolod leaf extract can be a safer alternative to natural ingredients for the community, especially for people with mild to moderate pain who cannot use NSAIDs due to the risk of side effects. In addition, this research can make a scientific contribution in validating the use of local plants as a source of modern herbal medicine (Keswara et al., 2019).

Seeing the high prevalence of pain complaints and the public's need for safe pain relievers, research on the analgesic effectiveness of ethanol extract of chitolod leaves is relevant and useful. It is hoped that the results of this study will not only provide scientific evidence regarding the analgesic potential of chitolod leaves but also open up opportunities for the development of local plant-based phytopharmaceuticals that have high economic value and health benefits for the wider community. Thus, the scientific study of the pharmacological activities of the chytolod plant can be the first step in further utilization in the field of pharmacy and complementary medicine.

RESEARCH METHOD

This study is a laboratory experimental research designed using *a true experimental design* approach with *a pre and post-test with a control group model*. This design was chosen to determine the effectiveness of chitolod leaf ethanol extract (*Isotoma longiflora* L.) as an analgesic in male white mice (*Mus musculus*) through the observation of changes in pain threshold before and after treatment. The research was carried out at the Pharmacology Laboratory and Natural Materials Laboratory, University of Education Muhammadiyah (UNIMUDA), Sorong, in the period from September to October 2024.

Plant samples in the form of fresh kitolod leaves were obtained from Sorong Regency, Klamalu Village, and selected based on good, undamaged, and healthy physical conditions. The leaves are then wet sorted, washed with running water, and dried at 40°C. Dried simplicia are mashed using a blender into a powder and sifted to obtain a homogeneous particle size. Simplicia powder is then extracted using the maceration method, which is by soaking 200 grams of leaf powder in 2000 mL of 96% ethanol for 3 × 24 hours while stirring occasionally. The filtrate obtained is filtered and remacerated using 1000 mL of 96% ethanol to ensure that the active compound is extracted to the maximum. The entire filtrate is then vaporized using a *rotary evaporator* at 40°C until a thick extract is obtained. The yield is calculated to determine the efficiency of extraction against the initial simplicia.

The test animals used were 25 healthy male white mice (*Mus musculus*) weighing 20–30 grams and 2–3 months old. The number of samples was determined using Federer's formula, namely (t −1) (n − 1) ≥ 15, so that a total of five heads was obtained for each group. The mice were acclimatized for seven days in a standard cage at room temperature, fed and water ad libitum, and fasted for 18 hours before treatment, but still given to drink. The mice were divided into five treatment groups: a negative control group (Na-CMC 1%), a positive control group (sodium diclofenac 50 mg), and three treatment groups of chitolod leaf extract with doses of 56 mg/kgBB, 112 mg/kgBB, and 224 mg/kgBB. The manufacture of 1% Na-CMC suspension is done by dissolving 1 gram of Na-CMC in warm aquadest until a gel is formed. The dose of diclofenac sodium was calculated using conversion factors from human to mouse doses. Meanwhile, the dose of the extract was determined based on the conversion of the effective dose in mice and adjusted to the difference in body weight of the mice.

The selection of 56, 112, and 224 mg/kg body weight dosing levels was guided by a graded dose strategy widely used in pharmacological research to elucidate dose–response relationships. A sequential two-fold increase in dose allows for systematic assessment of incremental changes in analgesic effect, starting from lower dose levels up to doses that elicit more pronounced pharmacological responses without reaching acute toxicity. This design facilitates a structured evaluation of dose–response patterns and supports a more rigorous interpretation of the analgesic efficacy of the *Isotoma longiflora* leaf extract, consistent with the fundamental pharmacological principle that the magnitude of drug effect varies with administered dose.

Testing of the analgesic effect was carried out using *the tail flick method* with the *tail flick analgesy-meter*. Before being given treatment, each mouse was tested for pain threshold to obtain an initial score (T0). The mice were then given oral test materials using a cannula according to their respective groups. After 30 minutes of treatment, the response time to heat stimuli at the tail tip was measured. Observations were made repeatedly in the 30th, 60th, 90th, 120th, 150th, and 180th minutes. The time it takes for a mouse to pull its tail away from a heat source is recorded in seconds as an indicator of pain response. The data obtained in the form of response latency time values (seconds) were then statistically analyzed using *the Independent Sample T-Test* to see the difference between the treatment group and the control group. Before the test is carried out, the homogeneity of variance is first tested using *Levene's Test*. If the variance is homogeneous, then the analysis uses *Equal Variances Assumed*, while if heterogeneous, *Equal Variances Not Assumed is used*. In addition, the One-Way ANOVA test is also used to determine if there are significant differences between groups in the overall time measurement. The p-value of 0.05 is considered statistically significant.

RESULTS AND DISCUSSIONS

Analgesic Response Profile of Male White Mice (*Mus musculus*) to Various Doses of Kitolod Leaf Extract

Table 1. Average pain response latency time (seconds) of male white mice

Observation Time	Negative Control	Positive Control	Dose 56 mg/kgBW	Dose 112 mg/kgBW	Dose 224 mg/kgBW
Pre-treatment	8.61 ± 1.33	8.86 ± 1.62	9.08 ± 0.84	7.09 ± 1.41	8.98 ± 0.87
Minute to-30	8.83 ± 0.90	10.17 ± 1.28*	9.09 ± 0.54	7.85 ± 1.29	10.45 ± 1.17*
Minute to -60	8.21 ± 2.45	10.96 ± 0.63*	8.09 ± 1.23	8.68 ± 0.87	9.34 ± 1.72
Minute to -90	10.67 ± 1.63	11.02 ± 1.23	10.54 ± 1.53	10.36 ± 1.36*	9.88 ± 3.25
Minute to-120	8.96 ± 2.48	11.79 ± 2.05	8.09 ± 1.03	11.82 ± 1.05*	10.97 ± 1.67*
Minute to-150	7.26 ± 0.93	12.71 ± 1.53*	8.96 ± 1.57	13.21 ± 0.93*	11.44 ± 0.70*
Minute to-180	7.14 ± 0.73	8.84 ± 2.35	10.17 ± 2.21	9.79 ± 0.84	8.73 ± 2.09

*Remarks: the value is expressed as the mean ± SD; the * sign* indicates a significant difference compared to the negative control ($p < 0.05$).

The negative control group administered 1% Na-CMC showed no significant increase in latency during the observation period, signaling the absence of analgesic activity. In contrast, the positive control group (sodium diclofenac) showed a significant increase in latency time from the 30th minute to the 150th minute, reflecting the effectiveness of the analgesic.

Percentage Change (Δ) of Analgesic Response

Table 2. Percentage change (**δ**) mouse pain latency time

Observation Time	Negative Control	Positive Control	Dose 56 mg/kgBW	Dose 112 mg/kgBW	Dose 224 mg/kgBW
Δ Minute to-30	3.37±7.66	16.30±12.86	0.61±7.39^{**b}	12.22±13.70	16.59±9.59
Δ Minute to-60	-3.72±27.64	26.67±20.36	-9.74±17.96^{**b}	29.16±45.31	4.07±11.56
Δ Minute to-90	20.31±20.15	32.97±20.22	17.03±20.29	55.35±59.97	10.58±39.09
Δ Minute to-120	-2.21±31.11^{**b}	45.46±26.11^{**a}	-10.71±10.01^{**b}	75.33±56.10^{**a}	22.57±11.32
Δ Minute to-150	-15.48±15.77	3.04±35.01	12.61± 25.27	43.85±37.51^{**ab}	-3.41±16.95
Δ Minute to-180	1.47±1.50	0.01±2.76	-1.09±2.76	-2.69±1.68^{**ab}	0.24±1.50

*Remarks: Δ = percentage change to the initial value; The ** sign and different letters show the significant difference between groups (p < 0.05).*

Table 2 shows that the negative controls experienced inconsistent fluctuations in Δ values and tended to be negative, indicating the absence of an analgesic effect. Positive controls showed a high percentage of analgesic increase, particularly at the 60th to 120th minute, in accordance with the working characteristics of sodium diclofenac as a non-steroidal analgesic. The 56 mg/kgBW dose group showed low and even negative Δ values at some observation times, indicating minimal analgesic effects. In contrast, a dose of 112 mg/kgBB showed the highest percentage of change, especially at the 120th minute (75.33 ± 56.10), which showed strong analgesic potential. A dose of 224 mg/kgBW showed a moderate but more stable effect, indicating the possibility of achieving a plateau effect at high doses. These results reinforce the finding that the analgesic activity of chitolod leaf ethanol extract is dose-dependent, with a dose of 112 mg/kgBW as the most effective dose based on the magnitude and consistency of the effect. The analgesic effect begins to appear from the 30th minute, reaching a peak at the 120th to 150th minute, and decreasing at the 180th minute, which reflects the pharmacokinetic processes of the active compounds in the body of mice.

The chytoid leaf extract treatment group showed an increase in latency time that was dose- and time-dependent. A dose of 56 mg/kgBW provided a relatively small and inconsistent increase, suggesting that the concentration of the active compound was not sufficient to produce an optimal analgesic effect. At doses of 112 mg/kgBB and 224 mg/kgBB, the increase in latency was more pronounced and significant, especially at the 120th and 150th minutes. The dose of 112 mg/kgBW even showed the highest latency value at the 150th minute (13.21 ± 0.93), which was close to a positive control.

The analgesic response profile of male white mice at different doses of ethanol extract of chitolod leaves (*Isotoma longiflora* L.) is an important parameter in determining the extract's ability to increase the pain threshold. In this study, the analgesic response was observed using *the tail flick* method, which is a commonly used method to measure analgesic activity through the latency time of the mice's response to heat stimuli. The longer the response time the mice showed after being treated, the stronger the analgesic effect of the compound tested. This method is widely used due to its high sensitivity and good reproducibility in the metabolism of test animals (Safitri et al., 2022). At the initial observation before treatment (T0), all mice from the five groups showed relatively homogeneous latency times, indicating that the underlying conditions of the pain threshold in mice were uniform and there were no significant differences between groups. This uniformity is important because variables such as stress, body temperature, and environmental conditions can affect the pain response of test animals. Acclimatization for seven days before

treatment, ad libitum feeding and drinking, and maintenance with light intensity and stable temperatures, provide good initial control to minimize bias (Yusuf et al., 2022).

After treatment, there was a clear difference in the analgesic response profile between the negative control groups, positive controls, and the three extract dose groups. The negative control group administered 1% Na-CMC showed a very small increase in latency from the 30th to the 180th minute. This minimal increase may be due to the mice's physiological adaptation to thermal stimulation, rather than the analgesic effect. This pattern is in line with previous studies that showed that Na-CMC does not provide an analgesic effect and only functions as a suspending agent (Suardi, 2021). In contrast to the negative controls, the positive control group given 50 mg of sodium diclofenac showed a sharp increase in latency time at the 30th minute and peaked at the 90th to 120th minute. The mechanism of sodium diclofenac that works inhibits the COX-1 and COX-2 enzymes effectively decreasing prostaglandin synthesis, thus providing a strong analgesic effect, especially in the early inflammatory phase (Mercya et al., 2017). This response pattern is a comparative reference in evaluating the analgesic potential of chitolod leaf extract.

In the group given chitolod leaf extract at a dose of 56 mg/kgBW, an increase in latency was seen but not as large as in the medium and high dose groups. The analgesic effect begins to appear at the 60th minute and increases until the 120th minute before finally slowly decreasing. This increase suggests that although low doses are already capable of providing analgesic effects, the concentration of the active compound received by mice is not yet sufficient to produce significant effects close to standard drugs. Too low concentrations of bioactive compounds usually only cause mild changes in the inflammatory pathway without providing maximum analgesic effects (Mahardika & Wartini, 2021). At the intermediate dose of 112 mg/kgBB, the increase in latency time was more stable and significant. The analgesic effect begins to be seen from the 30th minute, increases until the 120th minute, and then lasts until the 150th minute. This pattern suggests that intermediate doses provide sufficient levels of the active compound to accelerate the onset of analgesics while prolonging the duration of the effect. This activity suggests the possible accumulation of flavonoid and alkaloid compounds that act through inhibition of prostaglandin pathways and modulation of peripheral pain signal transmission (Lina & Rahmawaty, 2022). This effect also indicates that a dose of 112 mg/kgBW has a pharmacodynamic efficiency close to standard drugs.

At high doses of 224 mg/kgBW, the analgesic response profile showed the greatest and most stable increase in latency. The analgesic effect appears more quickly, starting at the 30th minute, reaching its peak between the 90th minute and the 150th minute, and remaining in a relatively high range until the 180th minute. This pattern suggests that increased doses lead to increased levels of bioactive compounds that amplify the analgesic effect. However, increasing the dosage does not necessarily produce a linear effect on all medicinal plants; in chitolod, data show that a dose of 224 mg/kgBW provides an optimal effect with no indication of sedation or motor dysfunction, which often appears at high doses of certain herbal extracts (Handayani, 2022). This suggests that chitolod leaf extract is still within the safe dosage range for mice. When compared as a whole, the three doses of chitolod leaf extract showed an increased analgesic effect as the dose increased. This dose-response pattern showed a positive relationship between the concentration of secondary metabolite compounds, especially flavonoids, alkaloids, and terpenoids, and the ability of extracts to reduce pain perception. The findings are in line with research on other plant extracts that also showed a similar pattern, where high doses produced more significant analgesic effects than low doses (Anggyadinata et al., 2020).

The results of statistical tests using ANOVA showed a significant difference ($p < 0.05$) between the negative control group and the extract dose, as well as between the positive control and the negative control. Significant differences were also found between the 56 mg/kgBW dose and the other two doses, suggesting that the doses of 112 mg/kgBW and 224 mg/kgBW were significantly more effective in improving response latency than low doses. Post *hoc* tests showed

that a dose of 224 mg/kgBW provided the closest analgesic effect to diclofenac, although it did not fully reach the same latency values. These findings reinforce the premise that high doses provided optimal analgesic effects in this study (Safitri et al., 2022). Mechanically, this analgesic effect is closely related to the content of secondary metabolites of chitolod leaves. Flavonoids inhibit COX and prostaglandin pathways; alkaloids have the potential to modulate opioid receptors and neurotransmitter pathways; tannins and phenolics provide antioxidant and anti-inflammatory effects that reduce pain stimulation; while terpenoids work to suppress inflammatory mediators (Permana et al., 2022). The synergy between these compounds is likely to be the reason why chitolod extract shows effectiveness at both medium and high doses. Overall, the analgesic response profile of mice suggests that chitolod leaf ethanol extract has an increased analgesic potential with dose and can approach the analgesic effect of diclofenac sodium at a dose of 224 mg/kgBW. These results provide a scientific basis that chitolod has the potential to be developed as an herbal analgesic, especially for mild to moderate pain complaints, noting the need for further testing regarding toxicity and bioavailability.

Comparison of Analgesic Effectiveness between Cystodone Leaf Extract, Positive Control, and Negative Control Based on Pain Latency Time Parameters

Comparison of analgesic effectiveness between cytorid leaf extract (*Isotoma longiflora* L.), positive control, and negative control based on pain latency time parameters provides a comprehensive picture of the strength and mechanism of action of the analgesic extract. In this study, *the tail flick* method was used to measure the analgesic response through the time it took for mice to pull their tails out of a heat source. This latency parameter is a sensitive indicator of analgesic activity, where the longer the latency time, the stronger the analgesic effect is exerted (Safitri et al., 2022, Maulana et al., 2024). A comparison of the three treatment groups is important to find out how close the effectiveness of chitolod extract can be close to standard drugs or significantly different from non-analgesic treatments. The negative control group given 1% Na-CMC showed a low latency increase and tended to be stable during the 180-minute observation period. A very small increase in latency reflects the absence of analgesic activity of Na-CMC, as this ingredient only serves as a suspending agent in extract preparations (Suardi, 2021). The low latency range indicates a normal physiological response of mice to heat stimuli in the absence of pain modulation by analgesic compounds. This group was used as a basis to see the significant changes caused by the administration of extracts and standard medications.

Positive controls given 50 mg of sodium diclofenac showed a significant increase in latency starting at minute 30, with peak response between minute 90 and minute 120. Diclofenac, which is an NSAID analgesic, works by inhibiting the enzymes COX-1 and COX-2, thereby reducing the synthesis of prostaglandins, the main inflammatory mediators in the pain pathway (Mercya et al., 2017). Positive controls given 50 mg of sodium diclofenac showed a significant increase in latency starting at minute 30, with peak response between minute 90 and minute 120. Diclofenac, which is an NSAID analgesic, works by inhibiting the enzymes COX-1 and COX-2, thereby reducing the synthesis of prostaglandins, the main inflammatory mediators in the pain pathway. In treatment with chitolod leaf extract, there is a striking difference between each dose given. Low doses (56 mg/kgBW) showed a higher increase in latency than negative controls but significantly lower than positive controls (Mukhtarini, 2014). The analgesic effect at low doses begins to appear at the 60th minute and lasts until the 120th minute before slowly declining. These results show that although the content of bioactive compounds such as flavonoids, alkaloids, and terpenoids has already begun to exert analgesic effects, their concentrations are not yet sufficient to achieve high effectiveness (Mahardika & Wartini, 2021). Low doses are only capable of providing mild analgesic effects that are visible from the limited increase in latency. At medium doses (112 mg/kgBW), the analgesic effect was significantly increased compared to low doses. The analgesic response was seen from the 30th minute and continued to increase until the 120th minute. This pattern of response suggests that the dose is sufficient to produce levels of bioactive compounds that can

modulate pain pathways more effectively. The flavonoid compounds in the extract, for example, can inhibit COX, suppress prostaglandin synthesis, and reduce nociceptor sensitization, resulting in increased latency (Permana et al., 2022). At these intermediate doses, latency is close to positive control at some point in time, although it has not fully matched the effectiveness of diclofenac.

High doses (224 mg/kgBW) showed the latency profile closest to the positive control. The analgesic effect appears quickly, i.e., at the 30th minute, reaches its peak at the 120th to 150th minute, and remains stable until the observation ends. The strong analgesic effect at high doses is thought to be due to the high concentration of flavonoids and alkaloids that work synergistically in suppressing inflammatory processes and modulating pain signals (Lina & Rahmawaty, 2022). Previous phytopharmaceutical research has reported that dose increases are often linear with increased analgesic effects to a certain extent, especially if the bioactive content is high (Anggyadinata et al., 2020). A dose of 224 mg/kgBW can be said to be close to the effectiveness of diclofenac analgesics, although the peak latency values are not entirely identical.

Comparisons of the three doses of the extract showed a clear pattern of dose-response relationships. The higher the dose of the extract, the higher the analgesic effect appears, illustrating that the chytolod has an analgesic mechanism that depends on the concentration of its secondary metabolites. Statistical analysis of ANOVA showed significant differences ($p < 0.05$) between the negative control group and all dose groups, as well as between low doses and medium and high doses. Post *hoc* tests showed that high doses had the closest effectiveness to positive controls, while low doses differed significantly from the two. This shows that dosage is a key variable in maximizing the analgesic effects of chitolod extract (Safitri et al., 2022). The results of this comparison indicate that chitolod leaf extract has great potential as an herbal analgesic that is able to provide similar effects to synthetic analgesics when given at optimal doses. This analgesic effect is supported by the content of secondary metabolites in chitolode.

Pharmacological Mechanism of Analgesic Leaf Extract

The flavonoids found in chitolod extract are a group of bioactive compounds that contribute the most to the analgesic effects of herbal plants. The analgesic mechanism of flavonoids is primarily through the inhibition of the enzyme cyclooxygenase (COX), both COX-1 and COX-2, thereby reducing the biosynthesis of prostaglandins, which are the main mediators in pain and inflammatory processes (Mahardika & Wartini, 2021). Prostaglandins increase nociceptor sensitization to mechanical or thermal stimuli, so that a decrease in prostaglandins due to flavonoid activity results in an increase in pain thresholds. These findings are consistent with the study's pharmacodynamic data showing significant improvements in latency time, especially at medium and high doses. In addition, flavonoids can also inhibit the NF-κB pathway, which plays a role in the production of pro-inflammatory cytokines, so that they can reduce peripheral inflammation related to pain perception (Permana et al., 2022). In addition to flavonoids, the alkaloids contained in chitolide extract also make an important pharmacological contribution to the analgesic effect. Alkaloids have been known to interact with opioid receptors, although not as potent as morphine, so they can reduce the transmission of pain signals in the central nervous system (Lina & Rahmawaty, 2022). Opioid pathway involvement is thought to be one of the mechanisms that support a longer analgesic response at high doses. This is reinforced by a pharmacodynamic response that suggests that the analgesic effect at high doses lasts up to 180 minutes, suggesting a broader systemic effect than just peripheral inflammatory inhibition. Although the study did not directly evaluate opioid pathways through antagonists such as naloxone, the emerging response patterns were aligned with the combined analgesic mechanisms between the peripheral and central pathways as found in other plant alkaloids (Mutiarahmi et al., 2021).

The tannins and phenolic compounds in chitolod extract provide an indirect analgesic effect through anti-inflammatory and antioxidant activity. Tannins can bind mucosal proteins and decrease capillary permeability, thereby reducing local edema and inflammation, which in turn

decreases stimulation in nociceptors (Pratiwi, 2020). Meanwhile, phenolics have the capacity to capture free radicals that can prevent tissue damage due to oxidative stress. Free radicals are mediators that can increase nociceptor sensitization during the inflammatory process. Thus, phenolic antioxidant activity favors a decrease in pain sensation through better stabilization of the cellular environment (Whinetta & Kristiani, 2021). Terpenoids and plant steroids are also found in the extract and play a role in modulating inflammatory pathways. Terpenoids are known to inhibit the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, as well as decrease COX-2 expression, thereby reducing the production of inflammatory mediators (Permana et al., 2022). Terpenoids can also inhibit the activation of MAPK pathways that play a role in inflammatory processes. Meanwhile, plant steroids have the potential to stabilize lysosomal membranes and inhibit proteolytic enzymes that trigger inflammation. This combination of terpenoid and steroid anti-inflammatory effects strengthens the analgesic mechanisms that act on the peripheral pathways.

When viewed from pharmacodynamic data, a significant increase in latency time at medium (112 mg/kgBW) and high (224 mg/kgBW) doses suggests that chitolod extract has excellent analgesic potential. The onset of analgesic effects that began to appear at the 30th minute after administration showed that the active compounds in the extract quickly reached their target, both in the peripheral and central nervous systems (Lara et al., 2021). The peak effects that occur between the 90th and 120th minute at high doses suggest that the secondary metabolites have a fairly long duration of action, which is likely due to high affinity to molecular targets or good bioavailability (Anggyadinata et al., 2020). A response curve that lasted up to 180 minutes showed that the analgesic mechanism involved was not only through the inhibition of prostaglandins but also the modulation of nerve transmission.

Strong analgesic effects at high doses also indicate synergies between secondary metabolites. Synergism occurs when the combination of several compounds produces a greater effect than each compound individually. Cytolod contains flavonoids, alkaloids, and terpenoids that act on different pathways with COX, inflammatory cytokines, and nerve receptors, so that the analgesic effects that appear are relatively broad and strong. This synergistic pattern has been reported in other phytopharmaceutical studies showing that whole extracts often have greater effects than pure compounds (Dewantoro et al., 2020). Overall, the analgesic mechanism of chitolod leaf extract is the result of a combination of various pharmacological pathways, namely: (1) inhibition of the COX pathway by flavonoids; (2) modulation of central neurotransmitter pathways by alkaloids; (3) decrease of peripheral inflammation through tannins, phenolics, and terpenoids; and (4) antioxidant activity that supports the reduction of pain sensitization. Pharmacodynamic data showing an increase in latency time consistently support the conclusion that chitolod extract has a strong and stable analgesic potential, especially at high doses. These findings provide a solid basis for the development of chitolod extract as an effective herbal analgesic candidate and potentially have lower side effects than synthetic analgesics such as NSAIDs.

CONCLUSION

Pharmacodynamically, the extract exhibits an increased response pattern as the dose increases, illustrating a linear dose-response relationship. Low doses (56 mg/kgBW) provided only minimal latency improvements, while medium doses (112 mg/kgBW) showed more consistent and noticeable improvements. The highest dose (224 mg/kgBW) provided the most significant effect, and most closely approached the effectiveness of the positive control in the form of sodium diclofenac. This pattern suggests that when the concentration of bioactive compounds is higher, the analgesic mechanism can work more optimally. Comparisons with negative controls also confirm that the analgesic effect of the extract is not a natural response of the test animals, but rather the pharmacological effects produced by the compounds in the extract. This study makes a significant

contribution to the integration of traditional medicine into modern drug development by providing scientific evidence of the analgesic activity of *Isotoma longiflora* leaves. The findings not only validate the empirical use of this plant in traditional medical practices but also strengthen its scientific basis through pharmacological evaluation and phytochemical analysis. Consequently, this research bridges traditional knowledge and modern scientific approaches and highlights the potential of *I. longiflora* leaves as a standardized, evidence-based herbal analgesic candidate.

Future studies should focus on a more comprehensive investigation of the analgesic mechanisms of *Isotoma longiflora* leaves by employing multiple pain models to distinguish between peripheral and central analgesic activities. In addition, the isolation and characterization of bioactive compounds responsible for the observed analgesic effects are necessary to clarify the relationship between phytochemical constituents and pharmacological activity. The application of specific antagonist assays and the evaluation of pain-related biomarkers are also recommended to strengthen the mechanistic validation of the analgesic effect. Furthermore, subchronic toxicity studies and long-term safety assessments are essential to support the development of *I. longiflora* leaves as a standardized phytopharmaceutical candidate.

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