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Analgesic Effectiveness Study Of N-4t-Butylbenzoyl Acetate Compound In Male Balb-C Strain Mice

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ABSTRACT

This study aims to understand the activity and effective dose of N-4t-butylbenzoyl Acetate compound as a pain reliever. The analgesic activity test conducted was divided into 5 treatment groups. The analgesic effect test was performed using male mice from the Balb-C strain. In each test, each group consisted of 5 experimental animals. The first group (negative control) was given 1% Na-CMC suspension; the second group (positive control) received 1.04 mg / 20 grams of mouse body weight ibuprofen; while the third, fourth, and fifth groups were successively given 0.52; 1,04; 1.56 mg as much as 0.2 ml per 20 grams of mouse body weight of N-4t-butylbenzoyl Acetate compound, respectively. After the treatment was given to each test animal, an analgesic test was conducted with a 30-minute interval. After that, it was injected with 1% acetic acid and observed for 180 minutes; every 10 minutes, the movement was observed for all treatments. The analysis was carried out by comparing the number of movements (writing reflex). Data analysis was performed statistically using ANOVA (analysis of variance). The number of mouse activities produced by male mice of the Balb-C strain showed that the N-4t-butylbenzoyl acetate compound at a dose of 1.56 mg had the highest activity, % protection, and % analgesic effectiveness compared to the other two doses. Based on statistical analysis, there was no significant difference in all treatments of the N-4t-butylbenzoyl acetate compound when compared to ibuprofen (positive control).

Keywords: analgesic, effectiveness, ibuprofen, N-4t-butylbenzoyl Acetate, writing reflex

Introduction

Pain is an unpleasant sensory and emotional experience associated with tissue damage or potential tissue damage (Kurniawan, 2018; Taufiq Kadafi et al., 2023). The sensation of pain or soreness can indicate inflammation (rheumatism/gout), bacterial infection, or muscle spasms. Pain can be viewed as a harmful substance in the body because it occurs due to tissue or nerve damage that releases mediators such as histamine, prostaglandins, bradykinins, serotonin, and substances that cause discomfort in the body. The mediators referred to here are known as pain mediators (Suwondo et al., 2017; Arisetijono et al., 2015; Lina & Handayani, 2024).

According to the International Association for the Study of Pain (IASP), pain is described as an unpleasant feeling that involves both the senses and emotions, and it occurs when there is actual or potential harm to body tissues. Based on this definition, pain includes both objective elements, which are related to the physical sensations and bodily functions, and subjective elements, which involve emotions and mental feelings. The first step in treating pain in most cases is the use of analgesic medications. Analgesics are medicines that help reduce pain without making the senses, such as touch or temperature, worse. Nonsteroidal anti-inflammatory drugs (NSAIDs) are one example of a class of drugs used to manage pain (Puteri et al., 2024). However, in the therapy given, there are often various side effects that can have a major impact on the patient's health. One of the effects that often occurs is that it can irritate the stomach. NSAID drugs work by reducing inflammation through inhibition of the cyclooxygenase enzyme (COX), especially COX-1, which is important for maintaining the gastric mucosa. NSAIDs function by inhibiting the synthesis of prostaglandins, which are protective substances for the mucosa in the upper digestive tract. Reduction of prostaglandins can result in decreased mucosal resistance, potentially irritating the stomach (Idacahyati et al., 2019; Jahnavi et al., 2019)

Given the side effects that may be caused by NSAID drugs, the search for new drugs with minimal side effects is very necessary in the pharmaceutical world. The compound N-4t-butylbenzoyl acetate is of interest as a potential candidate in the development of anti-inflammatory drugs. N-4t-butylbenzoyl acetate is a compound that has been modified from 4-ter-butylbenzoyl chloride. The compound N-4t-butylbenzoyl acetate is produced through an acetylation reaction between 4-ter-butylbenzoyl chloride, containing a carbonyl group (C=O), and anhydrous acetic acid, which has an acetyl group (—C(=O)—CH_3) (Mahastari, 2024)

Clinical and preclinical trials are generally conducted to test analgesic drugs. Preclinical trials are conducted using qualified experimental animals. The purpose of using test animals is to obtain defined laboratory animals so that genotypic characteristics, maternal effects, and environmental influences on phenotypes remain consistent. Test animals need to be adjusted so that animal conditions are maintained (Jayanti et al., 2021; Huss & Pacharinsak, 2022). Therefore, it is necessary to test the effectiveness of the N-4t-Butylbenzoyl Acetate compound as an analgesic and anti-inflammatory in experimental animals, namely male Balb-C mice.

The method applied in this study is the chemical induction method. This method is applied to each group, mice are induced with 1% glacial acetic acid i.p, as much as 0.2ml/20gramBW of mice. Furthermore, the mice are placed on the platform, and the number of movements that occur every 10 minutes for 3 hours is counted. Writhing is recorded when the mice begin to feel pain, indicated by rubbing their stomachs on the platform. The results are collected as the movement power of the experimental animals per hour. The strength of the analgesic activity is determined based on the ability to inhibit the movement of the experimental animals (Amir et al., 2023).

Methodology

Instruments and Materials

The tools used in this study were animal scales (OHAUS Triple Beam Balance), 100 ml beaker (pyrex), 25 ml measuring cup (pyrex), 50 ml measuring flask (pyrex), hot plate (HS 4 IKA C), stirring rod (local), mouse cage (local), mouse and rat oral probe (local), syringe (Terumo Syringe 3 cc), and stopwatch (ultrak 1000). The materials used in this study included the compound N-4t-butylbenzoyl acetate, 400 mg ibuprofen (Triman), CMC Na (Sigma Aldrich), NaCl (Sodium Chloride 0.9%) (Analytical Reagent), 1% acetic acid (Analytical Reagent), and distilled water. Male Balb-C mice were used in this study and obtained from breeders in the Malang area.

Analgesic Test

Before the test was carried out, the researcher had obtained an ethical clearance permit from Ethics Committee Faculty of Dentistry, University of Jember. After that, 25 mice aged 2-3 months were adapted in the Laboratory individually and fasted for 8-12 hours. Furthermore, they were divided into five groups, each consisting of five male mice selected randomly, namely the five groups:

a. Negative control group K (-): mice received CMC-Na 1% / 20 grams of mouse body weight (0.2 ml)
b. Positive control group K (+): mice were given ibuprofen tablets 1.04 mg / 20 grams of mouse body weight (0.2 ml)
c. Treatment group 1 (P1): mice were given N-4t-butylbenzoyl acetate compound at a dose of 0.52 mg, as much as 0.2 ml / 20 grams of mouse body weight.
d. Treatment group 2 (P2): mice were given N-4t-butylbenzoyl acetate compound at a dose of 1.04 mg as much as 0.2 ml for every 20 grams of mouse body weight.
e. Treatment group 3 (P3): mice were given N-4t-butylbenzoyl acetate compound at a dose of 1.56 mg as much as 0.2 ml per 20 grams of mouse body weight.

Expected within 30 minutes. After that, 1% acetic acid injection was carried out and observations were made every 10 minutes on a time scale of 180 minutes for all treatments.

Calculation of % Writhing Protection and % Analgesic Effectiveness

The magnitude of the inhibition of the amount of writhing is calculated using the Henderson and Forsaith equation with the formula:

$$\%\ Writhing\ Protection = 100 - \left[\left(\frac{P}{K}\right) x100\right]$$

Description:
P = Cumulative number of mice wriggling after drug administration
K = Cumulative number of mice wriggling in the negative control
(Gupta et al., 2015)

% Analgesic Effectiveness

$$\%\ analgesic\ effectiveness = \frac{\%\ Writhing\ protection\ drug\ administration}{\%Writhing\ protection\ positive\ control}\ x\ 100\%$$

(Puteri et al., 2024

Data Analysis

The research data obtained regarding the number of wriggles were tested for normality using the Shapiro-Wilk test. Data is considered normally distributed if p > 0.05. Furthermore, a homogeneity test (Levene's test) was carried out, where a p-value> 0.05 indicates that the data obtained is homogeneous. Then, a statistical analysis was carried out using the ANOVA (Analysis of Variance) method with a 95% confidence level. Continued with the Mann-Whitney U Test to identify treatment groups that differ significantly from other groups

Result and Discussion

In testing the analgesic power of the N-4t-butylbenzoyl Acetate compound, the animals used were male mice (Mus musculus) strain Balb-C, which have higher sensitivity compared to mice from other strains (DDY and Swiss Wistar). Male mice strain Balb-C (Mus musculus) in this study were triggered to experience pain by using acetic acid as a pain stimulant (Firmansyah et al., 2022). The sterile 1% v/v acetic acid solution used functions as a stimulant of prostaglandin synthesis and causes pain. Based on the Witkin Method (writhing test/writhing method), the type of pain inducer used was a 1% sterile acetic acid solution with a dose of 75 mg/kgBW, which had caused a pain effect (Hajiallilo & Abbasi-Maleki, 2021). Administration was carried out through the intraperitoneal (i.p) route to avoid the decomposition of acetic acid when passing through the physiological tissue in certain organs. The acetic acid solution is expected to damage body tissue if given through other routes. This solution is injected intraperitoneally (i.p.), that is, injected directly into the abdominal cavity to obtain rapid absorption, so that a sterile solution of 1% v/v acetic acid is prepared in the form of a sterile preparation.

Several methods used to test analgesic ability include the chemical induction method, electrical method, heat method, and mechanical method. In this study, the method used was chemical induction (Siegmund test), where mice were given pain induction with 1% v/v acetic acid solution intraperitoneally (i.p), which then produced a pain response in the form of writhing (Suhendy et al., 2020; Damayanti et al., 2021). Writhing can be observed through abdominal contractions characterized by a stiff stomach and legs bent backwards. This method was chosen because it has advantages such as not taking long, being easy to do and observe, and fast workmanship by simply injecting a sterile acetic acid solution as a pain trigger. However, this method also has a weakness, namely that it is only appropriate for testing pain against peripheral analgesics. The analgesic ability test of the N-4t-butylbenzoyl Acetate compound in male mice (Mus musculus) can be observed through the average movement that appears for 180 minutes with a 10-minute interval (Figure 1).

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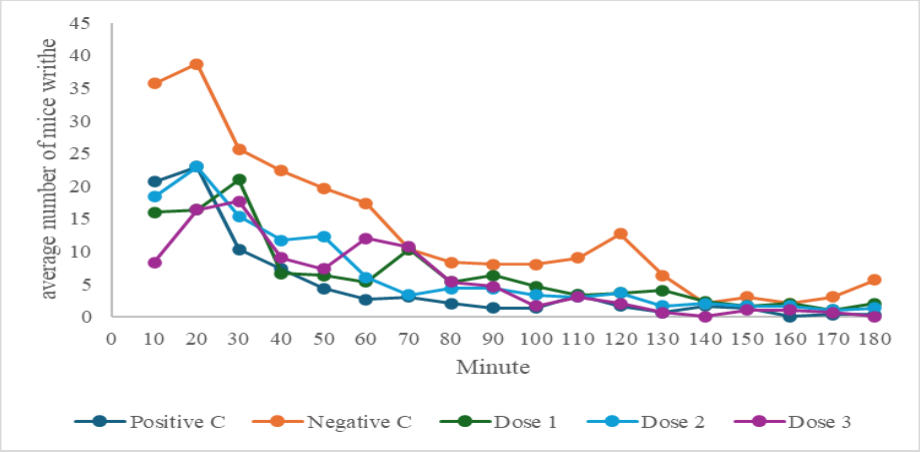

Figure 1. Average curve of mouse writhing every 10 minutes for 180 minutes.

Observation of activity every 10 minutes showed that at the 20th minute, almost all treatments showed a high amount of activity and then decreased again in the next 10 minutes until the 180th minute. This may be because at the 20th minute, the acetic acid used as an inducer began to decrease in effectiveness. Furthermore, the average activity of the negative control was the highest compared to the N-4t-butylbenzoyl acetate compound treatment group and the positive control (Ibuprofen). This is because CMC-Na has no pharmacological activity in reducing pain (Wahyuni et al., 2023; Damayanti et al., 2021). In the treatment group, the higher the dose of N-4t-butylbenzoyl acetate (dose 1.56mg of 0.2ml/20 grams of mouse body weight), resulted in the average writhing.

The results of the normality test of the number of mice activity data in all treatments using the Shapiro-Wilk method can be seen in Table 1, which shows that all treatment activity data are normally distributed (>0.05), except for the negative control (<0.05). Therefore, to determine whether there is a difference in the number of activities between treatment groups, a one-way ANOVA parametric statistical analysis was carried out with a 95% confidence level. The results of the One Way Anova analysis showed a sig. value of 0.192 (p>0.05), which indicates that there is no difference in the number of mice activities in all treatment groups (Anbarasi et al., 2022).

Table 1. Results of the Normality Test of Mice Writhing Data

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Perlakuan	Statistic	df	Sig.	Statistic	df	Sig.
Geliat	Kontrol Positif	.341	3	.	.847	3	.234
	Kontrol Negatif	.380	3	.	.763	3	.029
	Dosis 1	.207	3	.	.992	3	.832
	Dosis 2	.178	3	.	.999	3	.953
	Dosis 3	.385	3	.	.750	3	.000

a. Lilliefors Significance Correction

The results of the calculation of % protection against acetic acid pain induction for each treatment group can be seen in Table 2. In Table 2, it can be seen that the percentage of protection of the negative group is zero, because it is used as a standard of comparison. In the test group, the higher the dose given, the higher the %

protection. The higher the % protection value, the less movement occurs in mice. The compound N-4t-butylbenzoyl acetate can reduce pain stimuli due to acetic acid. Meanwhile, the highest protection is with positive control or administration of Ibuprofen.

Table 2. Results of calculating % protection against acetic acid pain

Groups	% Protection
Negative control	0
Positive control	64.09
Dose 1	50.21
Dose 2	50.35
Dose 3	57.36

Table 3. Results of the Mann-Whitney U Test on % Protection against acetic acid pain induction

Gropus	Groups	Sig.	Description
Negatif	Positif	0.317	TB
	Dose 1	0.317	TB
	Dose 2	0.317	TB
	Dose 3	0.317	TB
Positif	Dose 1	0.317	TB
	Dose 2	0.317	TB
	Dose 3	0.317	TB
Dose 1	Dose 2	0.317	TB
	Dose 3	0.317	TB
Dose 2	Dose 3	0.317	TB

TB = No Meaningful Difference

To identify differences between treatment groups, non-parametric statistical analysis of the Mann-Whitney U was performed, as shown in Table 3. Table 3 shows that the percentage of protection for all treatments did not show significant differences. Furthermore, Table 4 shows the results of the calculation of the percentage of analgesic effectiveness.

Table 4. Results of calculating the % effectiveness of analgesics in the treatment group for Ibuprofen

Groups	% Efektivitas Analgetik
Positive control	100
Dose 1	78.33
Dose 2	78.56
Dose 3	89.50

In Table 4 above, increasing the dose of N-4t-butylbenzoyl acetate compound results in an increase in the analgesic effect produced. However, the

greatest analgesic effect is observed with the administration of ibuprofen (positive control) (Nayoan & Syamsi, 2023). This phenomenon occurs based on the pharmacological principle related to the relationship between drug concentration and its effects, where at low doses, the response to the drug generally increases proportionally with increasing dose. When the dose is increased, the increase in response decreases until finally, with increasing dose, no further increase in response occurs. The response to low doses of the drug generally increases directly proportional to the amount of the dose. The effect of a drug is determined by the amount of the dose given. If the dose given is below the threshold limit, the effect will not be achieved. The response depends on the natural impact being measured. Increasing the dose may strengthen the effect at that level. The dose-effect reaction can vary depending on the sensitivity of the individual using the drug (Sulasmi et al., 2024; Chaachouay, 2025).

Conclusion

The number of mouse activities triggered by male mice of the Balb-C strain showed that the N-4t-butylbenzoyl acetate compound at a dose of 1.56 mg produced the highest number of activities, % protection, and % analgesic effectiveness compared to the other two doses. Based on statistical analysis, there was no significant difference in all treatments of the N-4t-butylbenzoyl acetate compound compared to ibuprofen (positive control).

Declaration of Competing Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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