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SYNTHESIS OF *O*-(4-FLUOROBENZOYL) ACETAMINOPHEN AND ANALGESIC ACTIVITY TEST IN MICE *(MUS MUSCULUS)*

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ABSTRACT

Acetaminophen is an analgesic drug that is often used. Acetaminophen can cause hepatotoxicity if it is consumed for a long period of time or consumed in high dose. Hepatotoxicity is caused by the formation of acetaminophen's metabolite (NAPQI) which bind the liver cell at the ortho position of the hydroxyl group. The purpose of this study is to synthesize acetaminophen derivate *O*-(4-fluorobenzoyl)acetaminophen and evaluate its analgesic activity. Synthesis *O*-(4-fluorobenzoyl)acetaminophen was carried out by reacting acetaminophen with 4-fluorobenzoyl chloride. The TLC and melting point test indicated the purity of the compound. Structure identification was done based on UV-Vis, FT-IR, and 1H-NMR spectra. The synthesized compound was tested for analgesic activity by hot plate method in mice (*Mus musculus*) and the result showed that *O*-(4-fluorobenzoyl)acetaminophen has higher analgesic activity than acetaminophen.

KEYWORDS: Synthesis; O-(4-fluorobenzoyl) Acetaminophen, Analgesic Activity.

INTRODUCTION

Pain is a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive and social components.[1] The sensation of pain that occurs encourage the individual concerned to seek treatment, among others, by taking painkillers (analgesic). Analgesics are classes of drugs that can suppress or reduce pain without losing consciousness.[2] Analgesia will change the perception and interpretation of pain by depressing the central nervous system in the thalamus and cerebral cortex.[3]

In Indonesia, one of the most common analgesics to relieve pain is acetaminophen, Acetaminophen is an active metabolite of acetylenic and phenacetine discovered accidentally in the 1800s.[4] Acetaminophen has the same effect as aspirin in inhibiting prostaglandin synthesis in the brain but its activity is very small as a peripheral prostaglandin inhibitor.[5]

Acetaminophen is not recommended for long treatment because in vivo use will be metabolized into a reactive form of imidoquinone and able to bind the liver tissue causing hepatotoxicity.[6,7]

A small amount of the drug is metabolized via Cytochrome P-450 undergoes *N*-hydroxylation to form *N*-acetyl-*p*-aminobenzoquinone (NAPQI) or *N*-acetylimidoquinone. Under normal circumstances, NAPQI combines with sulfhydryl groups in hepatic glutathione to decrease by more than 70% thus the reactive quinine interacts with the nucleophilic functional group, especially the -SH group in the hepatic protein, resulting in the formation of covalent bonds that cause hepatic necrosis. An overdose of acetaminophen may cause hepatic necrosis, tubular renal necrosis and hypoglycemic coma.[8]

In this study, to increase of the analgesic effect of acetaminophen, it has been modified the structure of acetaminophen by synthesizing the derivative *O*-(4-fluorobenzoyl)acetaminophen (Figure 1).

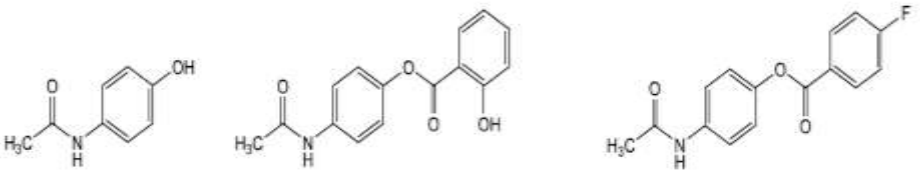

Figure. 1: The structure of acetaminophen derivatives.

To reduce toxicity and increase the activity of acetaminophen can be done by modification of the structure, namely the alteration or addition of functional groups contained in acetaminophen. Changes may be made to an amino group or phenolic hydroxyl group, or to both amino and phenolic hydroxyl groups because it has nucleophilic properties. The modification of the structure of a compound is based on the rational choice of the group substituent, in order to obtain new compounds with higher analgesic activity.

An example of the modification of acetaminophen has been performed is a phenetsal which is a salicylate ester of acetaminophen (Figure 1). Phenetsal has a lower toxicity and greater analgesic activity.[6,7] The phenetsal is used as a reference in this study because it has been shown to increase analgesic activity and able to reduce toxicity.

The synthesis design in this study was to acylate the –OH group of acetaminophen with 4-fluorobenzoyl chloride using a modified Schotten-Baumann reaction. The reaction that occurs is nucleophilic substitution and the –OH group of acetaminophen acts as a nucleophile. The implementation of the synthesis uses a basic solvent (triethylamine) and it also acts as an HCl catcher. In this research, the analgesic activity of *O*-(4- fluorobenzoyl) acetaminophen determining by hot plate method in mice *(Mus musculus*).

MATERIALS AND METHODS

1. Materials

Acetaminophen p.g (Interbat); 4-Fluorobenzoyl chloride p.s (Aldrich); Triethylamine (E.Merck); Tetrahydrofuran (E.Merck); Acetone p.a (E.Merck); Ethyl acetate p.a (E.Merck); Chloroform p.a (E.Merck); Methanol p.a (E.Merck); Sodium carboxymethylcellulose p.a (Interbat); Absolute ethanol p.a (E.Merck); Silica Gel Chromatography Plate 60 GF254 (E.Merck); Distilled water; *n*-hexane p.a (Riedel de Han).

2. Synthesis equipment

Glassware; Analytical balance (Ohaus); Mice scales (AND HL 100); Mel-Temp Electrothermal; Spuit injection disposable syringe (Terumo); Hot plate-magnetic stirrer (Labinco L32); Thin layer chromatography vessels; UV-Vis Spectrophotometer (Lambda EZ 201); FT-IR Spectrophotometer (Shimadzu FT/IR); Core magnetic resonance spectrophotometer (1H-NMR) (Hitachi FT-NMR R-1900); UV Lamp-254 (Topcon); Oven; and Stopwatch.

3. Synthesis of active compound *O*-(4-fluorobenzoyl) acetaminophen

Synthesis *O*-(4-fluorobenzoyl)acetaminophen was carried out by acylation reaction, and its process has been done with the elected Schoten Baumann method by reacting acetaminophen (0,012 mol) with 4-fluorobenzoyl chloride (0,014) mol, using tetrahydrofuran as the reaction solvent, and for an alkaline atmosphere using trimethylamine solution. Mixing the reaction is carried out at a cool temperature for 60 minutes, then the mixture of the compounds was

refluxed for 7 hours. After that process, the mixtures of the compounds were separated and added to a saturated solution of sodium bicarbonate to form crystals.

4. The Purity Test of the Synthesized Compound

a. Thin Layer Chromatography (TLC)

The purity of the compounds synthesized was indicated by TLC using three different polarity types of the solvent (*n*-hexane: ethyl acetate: ethanol = 5: 4: 1; chloroform: ethanol = 7: 3; and *n*-hexane: acetone = 5: 5).

b. Melting Point Determination

The melting point of the compounds synthesized can be determined by Mel-Temp Electrothermal.

5. Structure Identification of the Synthesized Compound

Identification of the molecular structure of the compounds has been synthesized using UV-Vis spectrophotometer, an Infra-red spectrophotometer (IR), and 1H-NMR spectrometer.

6. Analgesic Activity Test of Compound Synthesized

The analgesic activity test of the compound synthesized was determined by hot plate method in mice (*Mus musculus*), which is suitable for viewing central analgesic activity. Hot plate method has several advantages, including fast and easy, does not cause tissue damage in experimental animal, and the results reproducible. Hot plate test using a metal board that is heated at a constant temperature of 55 ± 1.0°C.

a. Preparation of Experimental Animal

The experimental animal used was white mice (*Mus musculus*) aged 2-3 months, weighing between 20-30 Grams, without physical disabilities, acquired from the Veteriner Farma Center, Jl. Achmad Yani, Surabaya. Before the mice being treated, environment adaptation was done for 2 weeks, then the mice were administered overnight before the trial. Mice were divided into several treatment groups, namely test group, control group and comparison group, each group consisted of 10 mice.

b. Settings of Dose

The doses were administered using Body Surface Area (BSA) and Human Effective Dose (HED) calculations. The doses of acetaminophen and test compounds administered in

experimental animal matched with the weights of each animal were 100 mg/kgBW. While for the control group using suspense CMC-Na 0.5% w/v with a dose of 50 mg/KgBW.

c. Implementation of Activity Test

Each group of the experimental animal was weighed and numbered. Furthermore, the animal was placed on a hot plate that has set the temperature of 55 ± 1.0°C and observed the latency time, which is the time interval when the mouse is placed on the hot plate with the response time of the mice in the form of lifting or licking the back foot, stomping the back foot or jumping. Three minutes later, the mice were given compound by per oral and the dose was adjusted to the weight of the mouse. The test group will be given the compound of the synthesis, the reference group gave acetaminophen, while the control group is given only CMC-Na 0.5% b/v. The latency time was again observed at 30, 60, 90 and 120 minutes after the compound was administered. Latency time is 11 seconds, it was determined as a cut off to avoid tissue damage in mice.

RESULT AND DISCUSSION

Based on the analysis using *ChemBioDraw* program, acetaminophen has logP = 0,28 and MR = 40,25 [$cm3mol$], and the value of logP for *O*-(4-fluorobenzoyl)acetaminophen = 2,58 dan MR = 70,17 [$cm3mol$]. Increasing the logP value will improve the lipophilic properties of the compound that it will increase the penetration into the biological membrane. Thus the number of active molecules that will interact with the receptor is increased and it is predicted that his analgesic activity is greater.

In this research, synthesis of *O*-(4-fluorobenzoyl) acetaminophen has been done by acylation, which is synthesized in an alkaline atmosphere using trimethylamine solution between acetaminophen and 4-fluorobenzoyl chloride. In the acylation reaction, there is the substitution of the phenolic hydroxyl group of acetaminophen. In this case, the phenolic hydroxyl group of acetaminophen acts as a nucleophile attacking a carbon atom of an electrophilic 4-fluorobenzoyl chloride carbonyl group due to electron deficiency. During the reaction HCl is released which can disrupt the course of the reaction thus triethylamine solution is used to bind HCl to form a water-soluble salt. In this study, 4-fluorobenzoyl chloride reagents were dissolved in tetrahydrofuran and dropped slowly into acetaminophen which has been dissolved in tetrahydrofuran and has been basified with trimethylamine solution.

Synthesis results in the form of white needle-shaped crystals with a yield of 25%, it shows that the method of Schotten-Bauman was the selected method of the synthesis process to produce the *O*-(4-fluorobenzoyl) acetaminophen.

To determine the purity of the synthesized compound, thin-layer chromatography test was conducted by using 3 different types of solvent (*n*-hexane: ethyl acetate: ethanol = 5: 4: 1; chloroform: ethanol = 7: 3; and *n*-hexane: acetone = 5: 5). The result which was obtained found that the synthesized compound gave a single spot with Rf value which was different with the original compound, acetaminophen. The above shows that the desire compounds synthesized have been formed and relatively pure compounds also have different from the original compound. At the melting point analysis of test compound that has been synthesized, it has a melting point (190°C) and possessed a difference with the parent compound which has the melting point (164°C). In this test, it has been proven that the compound which has synthesized has been formed and has a relative purity because there were no other impurities in it.

To prove that the synthesized compound has been formed, then the characterization of the structure of synthesized compound was done by spectrophotometer. Compound was synthesized, λmaks (nm) in UV-Vis = 246,5 (sh); IR (KBr pellet), 3303 $cm{-}1$ (secondary NH), 1664 $cm{-}1$ (C = O amide), 1606 $cm{-}1$, 1544 $cm{-}1$ (C = C aromatic), 1733 $cm{-}1$ (C = O esters), 1205 $cm{-}1$ and 1083 $cm{-}1$ (O ether); 1H-NMR (solvent DMSO-d6), 2.01, s, (-CH3), 7.11-7.18, d, (-F), 7.36-7.42, d, (-O), 7.54-7.62, d, (-NH), 8.09-8.17, d, (=O), 10.01, s, (NH). The parent compound acetaminophen, λmaks (nm) in UV-Vis = 245,0 (sh); IR (KBr pellet), 3325 $cm{-}1$ (NH Primary), 3161 $cm{-}1$ (O-H), 1654 $cm{-}1$ (C = O amide), 1610 $cm{-}1$, 7.

1564 cm_{-1} (C = C aromatic); 1H-NMR (solvent DMSO-d_6), 1.93, s, (-CH_3), 6.62-6.64, d, (- OH), 7.28-7.30, d, (-NH), 9.09, s, (-OH), 9.61, s, (NH).

Based on structural analysis using UV-Vis spectrophotometer, an infrared spectrophotometer (IR), and 1H-NMR spectrometer showed that there has been acylation at – OH group, thus it can be concluded that the synthesized compound formed is *O*-(4- fluorobenzoyl)acetaminophen with the chemical structure as following.

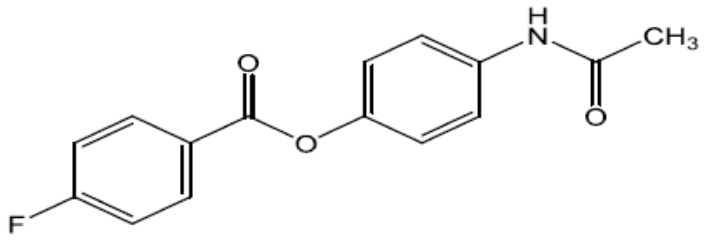

Figure. 2: Structure of *O*-(4-fluorobenzoyl) acetaminophen.

The compound of synthesis obtained in this study continued with analgesic activity test using hot plate method. The analgesic activity test aims to determine the biological activity of *O*-(4-fluorobenzoyl) acetaminophen in vivo. The hot plate method was chosen because it is suitable for viewing central analgesic activity.[9] In addition, analgesic test using hot plate method also has several advantages, including fast and easy, does not cause tissue damage in animals, and the results reproducible. Hot plate test using a metal board that was heated at a constant temperature of 55 ± 1.0 ° C. The observation is performed at the time of latency, is the time required for the mouse when placed on the hot plate to respond in the form of licking the hind legs, lifting the hind legs, stomping the back foot, or jumping.[10]

The results of observation of mice latency time and %MPE occurring as a result of pain response to hot stimulation from hot plate at the minute of 0, 30, 60, 90, and 120 minutes after giving 0,5% CMC-Na control, comparison of acetaminophen (100 mg/kgBB), and the *O*-(4-fluorobenzoyl)acetaminophen (100 mg/kgBB) test compound in mice can be illustrated as shown in Figure 3.

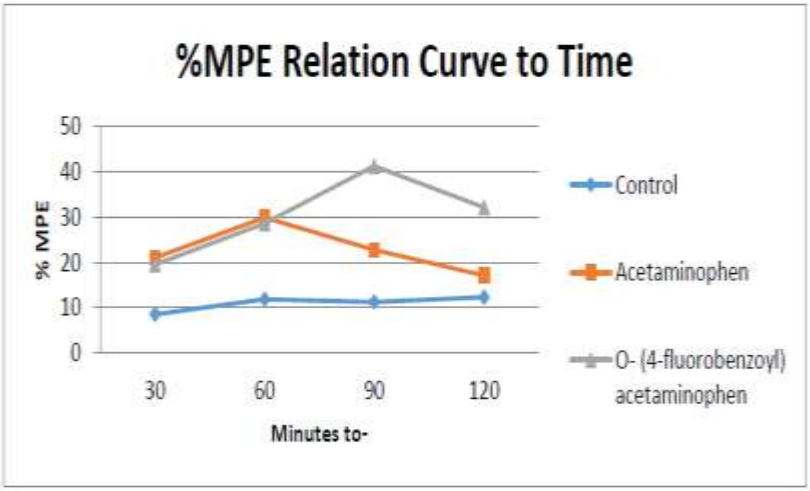

Figure. 3: %MPE Relation Curve to Time.

Figure 3 shows that acetaminophen has an analgesic peak time in the 60th minute, whereas the *O*-(4-fluorobenzoyl)acetaminophen compound had an analgesic peak time in the 90th minute. The analgesic activity possessed by the *O*-(4-fluorobenzoyl)acetaminophen compound is greater than the analgesic activity of acetaminophen. The *O*-(4-fluorobenzoyl)-acetaminophen compound has several advantages, such as having a larger lipophilic so that the penetration of the drug compound by the membrane gets better. In addition, the *O*-(4-fluorobenzoyl)acetaminophen compound is predicted to be non-hepatotoxic because there is no -OH group in its structure, not to be able to form of the *N*-acetyl-*p*-aminobenzoquinone so that it is likely to be an alternative candidate for a new NSAID drug.

CONCLUSION

O-(4-fluorobenzoyl) acetaminophen compound has been synthetized and has greater analgesic activity than acetaminophen *in vivo* in mice *(Mus musculus)* with hot plate test.

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