Toxic Effect Of The Compound {1,3 Bis (*P*-Hydroxyphenyl) Urea} On Triiodothyronine (T3) Hormone Levels In Pregnant White Rats (*Rattus Norvegicus* L.)

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Abstract.
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs for pregnant women to treat fever, pain and inflammation. Specific NSAIDs can displace thyroid hormone from its protein binding sites, causing thyroid hormone measurement problems. {1,3 bis (p-Hydroxyphenyl)urea} is a modified p-aminophenol compound with potent analgesic and anti-inflammatory activity and less toxicity. This study is a follow-up to previous research to observe the toxic effect on triiodothyronine hormone levels after administration of {1,3 bis(p-Hydroxyphenyl)urea} compound in pregnant white rats. The toxic effect test was carried out by giving the test preparation to pregnant rats, which had been divided into five groups, namely the normal control group (CMC-Na 0.5%), the positive control (Gabapentin 50 mg/kg BW), Compound {1.3 bis (p- Hydroxyphenyl)urea} at a dose of 50 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW. Mice were given the test preparation every day from the 6th to the 15th day of pregnancy. Blood was taken on the 16th day, and T3 hormone levels were measured using the ELISA method. The results showed that the T3 hormone levels in the {1,3 bis(p-Hydroxyphenyl)urea group had no significant difference from the normal control group, so it was concluded that {1,3 bis(p-Hydroxyphenyl)urea) did not have a significant effect on the hormone T3.

Keywords: *{1,3 bis (p-Hydroxyphenyl)urea}, toxic effect, triiodoyhyronine, teratogenic*.

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs for pregnant women to treat fever, pain and inflammation. The indications for NSAID use during pregnancy are inflammatory bowel disease and chronic rheumatic diseases such as rheumatoid arthritis. NSAIDs work by inhibiting the cyclooxygenase 1 and 2 enzymes so that the production of prostaglandins (PGE2) and prostacyclin (PGI2), which are inflammatory mediators that result in decreased vasoconstriction of blood vessels. These NSAIDs can impact the emergence of several side effects and complications, such as impaired kidney function, oedema, hypertension, and bleeding in the gastrointestinal tract [1]. Certain non-steroidal anti-inflammatory drugs (NSAIDs) can displace thyroid hormone from its protein binding sites, causing thyroid hormone measurement problems. Several studies have examined the effect of NSAID administration on thyroid hormone levels [2, 3]. Studies have proven that diclofenac and celecoxib in silico and in vitro tests can bind to the β thyroid hormone receptor. This drug will prevent thyroid hormone from binding to receptors. These results were examined using arteries and showed an increase in thyroid hormone (T3) in the presence of diclofenac and celecoxib drugs.

This evidence suggests that the side effects of diclofenac and celecoxib may be partially attributed to interactions with thyroid hormone signalling that may contribute to cardiovascular side effects [4]. Under normal circumstances, circulating T4 and T3 are moderately bound to serum proteins. Only 0.02% of T4 and 0.3% of T3 circulate in the free form, which is responsible for the biological activity of circulating thyroid hormones. Various analgesic drugs displace thyroid hormone from its protein binding site and induce a transient increase in free hormone concentration. These drug-induced changes can lead to inappropriate diagnostic and therapeutic decisions if they need to be adequately understood [5, 6].Compound {1,3 bis (p-Hydroxyphenyl)urea} is one of the modified p-aminophenol compounds which has been registered with the

Directorate General of Intellectual Property Rights (IPR) by Dr. Hari Purnomo, M.S., Apt with the name HP 2009. This compound is thought to have more potent analgesic and anti-inflammatory activity and less hepatotoxic side effects than paracetamol because it has an atomic charge (-0.110) that binds liver cells, less positive than paracetamol (-0.107).

In vivo test results proved that the compound {1,3 bis (p-Hydroxyphenyl)urea} had potential as an analgesic based on the inhibition of the cyclooxygenase (COX-2) enzyme [7], whereas in silico tests on COX-1 and TNF-α showed that {1,3 bis (p-Hydroxyphenyl)urea} had higher activity in binding COX-1 (1CQE) and TNF-α (2AZ5) than the control, dexamethasone and diclofenac. This compound has the potential to be developed as an anti-inflammatory agent [8, 9]. The compound {1,3 bis (p-Hydroxyphenyl)urea} has anti-inflammatory activity by reducing the percentage of inflammation, the number of neutrophils and the amount of expression of COX-2, TNFα, IL-1β and IL-6 [10, 11]. Whereas in the toxicity test, it was found that the compound {1,3 bis (p-Hydroxyphenyl)urea} did not cause toxic symptoms up to a dose of 5000 mg/kg BW, so it was classified as practically non-toxic in the acute toxicity test and did not cause toxic effects up to a dose of 1000 mg/kg BW in the toxicity test subchronic [10]. This research is a continuation of our previous study, which was conducted to observe the toxic effect on triiodothyronine hormone levels after administration of {1,3 bis(p-Hydroxyphenyl)urea} compounds during the organogenesis period in pregnant white rats.

II. METHODS

2.1 Materials and Tools

The materials used in this study were {1.3 bis(p-Hydroxyphenyl)urea} suspension, 0.9% NaCl, 0.5% CMC Na, 0.1% methylene blue powder, distilled water, Ketamine injection and Gabapentin. Microscopes, microplate reader surgical instruments, CALBIOTECH ELISA kits and laboratory glassware were also used in this study.

2.2 Ethical Clearance

This study was conducted based on guidelines and approval from the Animal Research Ethics Committee (AREC) of the Faculty of Mathematics and Natural Sciences, the University of Sumatera Utara, with approval number 0530/KEPH-FMIPA/2022, 16 June 2022.

2.3 Preparation of Test Animals

The test animals used in the teratogenic test were pregnant rats of the Wistar strain (Rattus norvegicus L.) (150-200 g) with an estimated age of around two months. The 25 rats were divided into five groups, and each consisted of 5 rats. The test animals were obtained from the Pharmacology Laboratory of the USU Faculty of Pharmacy. Before testing, rats were acclimatized for 7-14 days [12]. Mice were housed in a room with controlled temperature and access to water and food. Two weeks before testing, experimental animals must be treated as well as possible in well-ventilated cages and always kept clean. In teratogenic trials, each group of animals was separated and handled separately. Mice were separated, each one tail per cage. All animal procedures and treatments were carried out at room temperature (20-22◦ C), and special care was taken to avoid environmental disturbances that might affect the animal's response.

2.4 Blood Serum Collection of Pregnant White Rats

Mice were anaesthetized with ketamine injection. Blood collection was carried out using a syringe directly from the rat's heart as much as 1 mL. The blood obtained was then put in a vacutainer tube and tilted at an angle of 45° for approximately three hours. After that, it was centrifuged at 3000 rpm for 15 minutes. The supernatant formed was separated and centrifuged for 15 minutes at the same speed of 3000 rpm. After the second centrifugation, the supernatant (serum) was separated, transferred to a new Eppendorf tube, and then stored in the freezer [13].

2.5 Statistical Analysis

Data were analyzed using one-way ANOVA and Tukey post-hoc test.

III. RESULT AND DISCUSSION

Levels of the hormone triiodothyronine (T3) is one of the thyroid hormones used to evaluate a compound's toxic effects on pregnant rats [12]. T3 is a biologically active hormone with a metabolic potential three times that of T4, and T4 is considered a precursor or prohormone, which, when needed, will be broken down in the tissues to form T3 [14]. Thyroid hormone disorders in pregnant women will disrupt blood flow from the mother to the placenta resulting in impaired growth and development of the fetus. Table 1 shows that the observation of T3 hormone levels in the compound test group {1.3 bis (p-Hydroxyphenyl)urea} doses of 1000 mg/kg BW, 500 mg/kg BW and 50 mg/kg BW did not have a significant difference ($p>0.05$) compared to with the normal control group. Meanwhile, there was a significant difference ($p<0.05$) between the {1.3 bis(p-Hydroxyphenyl)urea) group and the positive control group. The levels of the T3 hormone compound group {1,3 bis (p-Hydroxyphenyl)urea obtained were still within normal limits. For the positive control group, there was a slight decrease in T3 hormone levels in pregnant rats, which means that gabapentin 50 mg/kg has the potential to induce hypothyroidism. Patients receiving gabapentin therapy can cause a decrease in thyroid hormone in the blood [15].

Table 1.T3 hormone levels

Group	Number of pregnant rats	T3 Levels (ng/mL)
		Mean ± SEM
CMC Sodium 0.5%	5	2.24 ± 0.11
Gabapentin 50 mg/Kg BW	5	1.86 ± 0.02*
50 mg/Kg BW	5	2.23 ± 0.17
500 mg/Kg BW	5	2.42 ± 0.09
1000 mg/Kg BW	5	2.54 ± 0.04

Information : SEM : Standard Error of Means

* : significantly different from the normal control group ($p <0.05$)

The hormone triiodothyronine (T3) is a thyroid hormone used to evaluate a compound's toxic effects on pregnant rats. T3 is a biologically active hormone with a metabolic potential three times that of T4, and T4 is considered a precursor or prohormone, which, when needed, will be broken down in the tissues to form T3. The hormones T4 and T3 are synthesized in the thyroid follicles. The TSH hormone stimulates the synthesis and release of T3 and T4, as well as the uptake of iodide, which is essential for thyroid hormone synthesis. Although T4 is synthesized significantly, it is converted to the more potent T3 by deiodination in peripheral tissues. During normal pregnancy, circulating levels of thyroid-binding globulin increase; consequently, total T3 and T4 also increase [16]. Normal levels of the T3 hormone range from 0.6-1.81 ng/mL [17].The mechanism of the compound {1,3 bis (p-Hydroxyphenyl)urea in increasing the T3 hormone is still unknown.

However, studies on thyroid hormone effects in pregnant rats using p-aminophenol derivatives have been investigated. High doses of paracetamol cause cytoplasmic vacuolization and damage to the follicular and colloid structures, causing a decrease in stimulating thyroid hormone (TSH) levels which will lead to an increase in T3 and T4 hormone levels. The mechanism by which paracetamol causes an effect on thyroid hormones is by inhibiting cyclic adenosine monophosphate (cAMP) [18].Studies on the effects of p-aminophenol derivatives are still limited. However, many studies have used other analgesic and anti-inflammatory drugs such as mefenamic acid, salicylic acid and diclofenac sodium. Based on several studies, these analgesic and anti-inflammatory drugs affect thyroid hormones, namely increasing levels of the hormones triiodothyronine (T3) and thyroxine (T4) by preventing the binding of thyroid hormones from protein binding sites resulting in increased levels of T3 and T4 hormones [19, 20]. In this study, the levels of the hormone T3 in pregnant rats that were given the compound {1,3 bis (p-Hydroxyphenyl)urea slightly increased with increasing doses of the compound {1,3 bis (p-Hydroxyphenyl)urea.

IV. CONCLUSION

In this study, the compound {1,3 bis (p-Hydroxyphenyl)urea caused an increase in T3 hormone levels as the dose of compound {1,3 bis (p-Hydroxyphenyl)urea) increased. The T3 hormone levels in the {1,3 bis(p-Hydroxyphenyl)urea group did not differ significantly from the normal control group, meaning

that the {1,3 bis(p-Hydroxyphenyl)urea compound had no significant effect on the T3 hormone. However, further studies of the fetus are needed by observing the reproductive appearance of the mother, external malformations and skeletal malformations.

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