Effect of Rhodamine b Against The Number of Primary Follicles in White Rats (Rattus norvegicus)

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Abstract: Rhodamine b is misused as a food coloring. Excessive use is certainly harmful to health. The purpose of this study proves that Rhodamine b can reduce the number of primary follicles in the ovaries of white rats. The design of this study is true experimental with Post Test only control group design. Sample in the research used were female white rats (Rattus norvegicus) aged 2-3 months with a bodyweight of 150-250 grams in the amount of 10 animals which were divided into 2 groups: negative control group, positive control group. Rhodamine b is given for 36 days at a dose of 18 mg / 200 gr orally. The number of primary follicles is calculated by HE staining. Data were analyzed and conducted One Way Anova test. The results showed that the administration of Rhodamine b significantly reduced the number of primary follicles in the ovaries of white rats. This is because rhodamine b is capable of binding with halogen compounds which are reactive so that it can damage cells. Based on the results of the analysis showed that Rhodamine b can reduce the number of primary follicles in the ovaries of the white rat.

Keywords: The number of primary follicles, Rhodamine b, White rats, Effect

1. Introduction

Rhodamine b is a greenish crystalline powder-shaped synthetic color, when water soluble in high concentrations of purplish red and at low concentrations of bright red [1]. Rhodamine b is a colorant not for food, including the xenobiotic group of carcinogens in the body and can increase free radicals. It contains chlorine compounds (Cl⁻), the CH₃-CH₃, the aromatic microcarbon policyclic (PAH) Activates the cytochrome enzyme P-450 as well as the very redox structure of the quinon and causes the formation of Reactive Oxygen species (ROS) [2]. Halogen compounds have high reactivity in achieving stability, so they can attack other adjacent molecules to find the pair of electrons so that they can damage the molecular shape itself, making it very dangerous if In the body in bulk. Rhodamine b has a chemical structure that binds to the compound chlorine (Cl). Chlorine compounds include halogen and radical compounds. Halogen compounds have high reactivity in achieving stability, so they can attack other adjacent molecules to locate the pair of electrons, which can damage the molecular shape itself. Therefore, the halogen compounds are very dangerous if they are present in the body in numerous quantities. The radical activity of chlorine compounds can cause macromolecules such as carbohydrates, proteins, fats and nucleic acids to suffer damage and induce toxic triggers of cancer in humans [3]. Because of its toxic nature, Rhodamine b is dangerous if eaten by humans and animals, can cause irritation to the skin, eyes and respiratory tract [4]. Exposure to Rhodamine b can slow down the cycle length of the oestrus on the squeaky adult females [5]. Thus, it can cause folliculogenesis disorder so that the follicles do not develop and have atresia [6]
2. Literature review
Rhodamine b has a molecular formula (C28H31N2O3Cl) with a molecular weight of 479 g/mol. Rhodamine b has a variety of other names, namely: Tetra ethyl Rhodamine, Rheonine B, D & C, Food Red 15, ADC Rhodamine B, Aizan Rhodamone and Brilliant Pink B. Chemical name Rhodamine b is N – [9-(Carboxyphenyl) – 6-(Diethylamino)-3-Xanten – 3-ylidene] – N-Ethyleyhanaminium chloride [7]. Rhodamine b belongs to the family of chemical compounds used as textile dyes such as Rhodamin 6G, Rhodamine b and others [8]. Rhodamin is generally toxic and soluble in water, methanol and ethanol [9]. At first Rhodamine b is used as a dye in the textile industry, one of the most important xanthene base dyes, famous for its good stability [10]. Rhodamine b is an organochlorin derivative. Organochlorin is a chemical containing chlorine and carbon. Organochlorin is chemically classified as an insecticide that has low toxicity but can persist in the environment and can accumulate in tissues through food. Organochlorin in the body can induce the P-450 cytochrome system and decrease the antioxidant enzymes in the liver, oxidizing the xenobiotes to produce ROS [11].

Cytochrome enzyme P-450 dependent oxidase has a role in biotransformation and detoxification of intermediate xenobiotic and metabolic compounds. Such metabolism produces peroxide compounds or reactive oxygen compounds, in fact hydrogen peroxide is harmless under normal conditions but may form highly hazardous hydroxyl radicals and result in damage to the cell structure if it binds to metals [12]. Cytochrome enzyme P-450 is a heme protein as a catalyst for oxidizing steroid metabolism trajectory, fatty acids, xenobiotes, medicinal, toxic and carcinogenic. Metabolism with P-450 can cause toxic metabolites that damage the cells. Activation of oxygen by the P-450 cytochrome in addition to being required as catalytic can also cause the occurrence of ROS [2].

Women experience menstruation regularly. The cycle is governed by the hypothalamus. The hypothalamus produces Gonadotropin releasing hormone (GnRH), which works on the anterior lobes (adenopituitary) or pituitary gland, which will further secreting gonadotropin hormones. Gonadotropin hormones consist of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), regulating the occurrence of cycle changes in the ovaries [13]. There is a follicle reserve that grows and is maintained by the supply of primordial follicles during puberty. Each month, 15 to 20 primary stage follicles (pre antral) are stimulated to grow under the influence of the FSH hormone and begin to achieve maturity marked by the primary oocyte begins to enlarge, while the follicular cells that surround it (from the Gepeng cell) turn into a cuboite cell and proliferate form a terraced epithelium i.e The ROS has an important role in the regulation of signaling tranduksi on folliculogenesis, oocyte maturation, corpus luteum, uterine function, embryogenesis, implantation of embryonic and development of Fetoplacenta [14]. Excessive ROS can cause oxidative stress so as to damage the molecules and oocyte cell structures as well as granulosic inside the follicle [15].

Ovulation is important in reproduction, with the increase in LH as a promoter on physiological changes, a mature ovum release occurs. After a large surge of LH also as a precursor in generating ROS, the reduction in the levels of this precursor interferes with ovulation [16].

3. Research method
This research uses the design of pure experimentation (true experimental) with the post test only control group design method. This research is done by giving the treatment of the animal try namely White mouse (Rattus norvegicus). The white mouse in the study is divided into 2 treatment groups: (1) The Negative control group (KN) is a group of white rats that were not given treatment, (2) Positive control Group (KP) that is a group of white rats given treatment of Rhodamine b 18 mg/200.

This research was conducted in the laboratory of pharmacology of Brawijaya University as a place for maintenance of animal care and administration, laboratory of Anatomy pathology as place of cutting ovarian organ and examination of number of primary follicles. Rhodamine b is obtained from the Faculty of Pharmacology Laboratory of Brawijaya University with Merck ' Sigma-Aldrich Pte '.

The research process begins with the acclimatization of the 10 white mice that have fulfilled the criteria of inclusion and exclusion for 15 days. Then the white mouse performed synchronization phase Oestrus for 3 days. After that the white mouse is grouped into 2 groups. Performed treatment for
36 days. The re-synchronization in the white Mouse is performed back for 6 days before termination. Termination with Ketamin injection 0.2 mg/200 grams of rats intra muscular for surgical and organ harvesting. Further sampling is done to check the number of primary follicles by staining of Hematoxilin Eosin (HE) in ovarian tissue.

4. Results and Discussion

An ovarian tissue histopathology image is obtained using an Olympus microscope with a 400x magnification with the colorization of Hematoxilin Eosin.

Counting the number of follicles is done by counting the total number of primary follicles, observed in any field of view. The number of primary follicles is calculated in 8 viewpoints with 400x magnification. Marking the number of follicles has been calculated marked using the help of cell account software.

Test One Way Anova and based on the test obtained results there is a meaningful difference the average number of primary follicles of the two treatment groups, it is shown from the value \( p-value = 0.000 < \alpha \). Based on the total value of the number of primary follicles of the negative control group (2.2 ± 1.92) is higher compared to the positive control group (1 ± 0.84). This means that the white mouse that is being hammered Rhodamine b suffered a decrease in the number of primary follicles than the white mouse that does not have Rhodamine b. It is in accordance with research conducted by Febrina et al, (2013) that exposure to Rhodamine b in mice at a dose of 150 ppm, 300 ppm and 600 ppm orally during two oestrus cycles, proved to be slowing the length of the Oestrus cycle in the squeaky adult females. In addition to that oxidative stress can cause the occurrence of cell apoptosis granulosa thereby causing atresia in the follicle. It affects the folliculogenesis process so that the follicles do not develop which results in the occurrence of ovulation disorders in women [6].

Exposure to Rhodamine b in Wistar rats at a dose of 4.5 mg, 9 mg, 18 mg orally, which is inserted directly into the stomach through the mouth with prolonged exposure of 36 days, can affect the hypothalamus, endometrial and ovarian, but at a dose of 18 mg can rapidly increase the expression of BAX (proapoptosis) and decrease the expression of BCL-2 (antiapoptosis protein) in the hypothalamus tissue thereby lowering FSH and LH

The results of the One Way Anova test can be seen in the table below:

| Treatment group          | n | Ovary Primary Follicles | Mean | \( p-value \) |
|-------------------------|---|-------------------------|------|---------------|
| Negative Control (KN)   | 5 | 2,2±1,92                |      | 0,000 < \( \alpha \) |
| Positive Control (KP)   | 5 | 1±0,84                  |      |               |

Reactive oxygen species (ROS) that cause oxidative stress affect ovarian function. The ovarian process is governed by a variety of molecular signaling hormones that themselves Controlled by
several physiological regulators including reactive oxygen species (ROS). ROS is a produced in the ovarian follicles and the corpus luteum, and they play an important role in follicular development and survival. The effect of oxidative stress on female reproduction has been widely described [17].

The occurrence of a decrease in follicle count is not only caused by oxidative stress present in the ovaries, but can also be caused by the presence of oxidative stress that occurs in the hypothalamus dishaft thereby disrupting hormones that support follicular growth. Oxidative stress occurring in the hypothalamus shaft organs can cause interference in the production of reproductive hormones including GnRH, LH, FSH and estradiol [18]. It is well known that ROS is an important molecule involved in cell signaling regulation when maintained on physiological cellular concentration. For example, the resulting superoxide by NADPH oxidase (NOX) derived from cell metabolism [19]. However, excess production of ROS can induce oxidative damage [20]. All non-pathological cells are supplemented with complex antioxidant defense systems to offset the oxidant effect [21]. The role of ROS in gynecology and assisted reproduction has been widely studied in several years [22]. In humans, increased percentage of ROS produce granulose cells. Produce fewer oocytes taken and reduce the rate of implantation. However, environmental and lifestyle changes (psychological stress, smoking, alcohol consumption, and the environment. Pathological conditions can lead to accumulation of ROS that leads to oxidative stress. That can affect women's reproductive health [23].

5. Conclusion

The administration of Rhodamine b may decrease the number of primary follicles in white rat ovaries (Rattus norvegicus). From the results of this study showed that Rhodamine b as a chemical compound is able to lower the number of primary follicles of the ovaries so that it can be a contributor in the incidence of infertility disorder.

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