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The effect of Rhodamine B on the cerebellum and brainstem tissue of *Rattus norvegicus*

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Abstract

Background: Rhodamine B is a component of xenobiotic substance metabolized by cytochrome P450 in the body to produce free radicals, which affects the activity of Superoxidase Dismutase (SOD), thereby, leading to oxidative stress, injury, increase in cell apoptosis and brainstem. This study aims to determine the effect of Rhodamine B on BAX and BCL-2 in the cerebellum and brainstem tissue of *Rattus norvegicus*.

Design and Methods: The True Experimental Design was used to carry out a post-test examination on the control group of twenty-eight Wistar female *Rattus norvegicus* mice between the ages of 10-12 weeks. Then, samples were categorized into 4 groups in body weight doses of 4.5 mg/200g, 9 mg/200g, and 18 mg/200g. They were administrated with Rhodamine B personde for 36 days.

Results: The results showed that Rhodamine B had a direct and indirect effect on BAX and BCL-2 expressions, respectively, in the cerebellum tissue and wistar strain of *Rattus norvegicus*. In addition, the positive path coefficient of BAX expression has a positive effect on BCL-2. This means that an increase in BAX has a direct impact on decreasing BCL-2 expression in cerebellum tissue and brainstem of *Rattus norvegicus* wistar strain along with an increased dose of Rhodamine B.

Conclusions: In conclusion, Rhodamine B tends to increase BAX expression which directly decreases BCL-2 in Cerebellum tissue and Brainstem in *Rattus norvegicus* along with increasing doses.

Introduction

A textile dye, Rhodamine B is toxic to the human body. It often enters the body when mixed with food, thereby causing oxidative stress on cells and tissues. The use of Rhodamine B in food for a long time leads to liver dysfunction or cancer, and when exposed to large amounts over a short period, it results in acute poisoning.[1] According to the World Health Organization, foodborne diseases are infections or poisoning caused by microbes that enter the body through food consumed. Based on data from the Directorate of Environmental Health and Public Health Emergency Operation Center (PHEOC) in 2017, there was a total of 163 outbreaks of food poisoning, and a Case Fatality Rate (CFR) at 0.1%. The outbreak of food poisoning was tagged second by the KLB report of the PHEOC, after the diphtheria outbreak. This shows that food poisoning is still a public health problem that needs to be prioritized.[2]

Peroxydisulfate (PS) is activated with dyes such as Rhodamine B (RhB), eosin Y (EY), and methylene blue (MB) under irradiated (Vis) visible light. This leads to the simultaneous degradation of dyes and antibiotics, such as tetracycline hydrochloride through the radical and nonradical pathways. The experimental results show that the radical reaction is the main route, caused by PS reduction by electron photogeneration of the dye.[3]

Rhodamine B is included in xenobiotic substances that are metabolized by cytochrome P450 in the body to produce free radicals such as ROS (reactive oxygen *species*). This affects the activity of Superoxidase Dismutase (SOD), which leads to oxidative stress, cell injury, and increases apoptosis in cerebellum tissue and brainstem. SOD enzymes are the most critical endogenous antioxidants and have the ability to improve the effects of oxidative stress. It catalyzes the reaction of superoxide to hydrogen peroxide and oxygen. Furthermore, it plays an important role in protecting the body cells and preventing inflammation caused by free radicals.[4]

The cerebellum is located in the posterior cranial fossa behind the pons and medulla oblongata, separated by the fourth ventricle. It plays an important role in the coordination and accuracy of motor functions. Its degeneration leads to various abnormalities such as walking difficulties, body tremors, and limb cramps. The brainstem is located at the base of the brain and connects the subcortical structures to the spinal cord. It is related to a variety of vital functions, such as the sleep-wake cycle, awareness, respiratory/cardiovascular control, accommodating the cranial nerve core, and facilitating communication between the cerebrum, spinal cord, and cerebellum through nerve channels.[5] Discrete circuits support control of the dynamics of saccade eye movements in the cerebellum and brain stem. There are two brain

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Significance for public health

Rhodamine B is a component of xenobiotic substance metabolized by cytochrome P450 in the body to produce free radicals, which affects the activity of Superoxidase Dismutase (SOD), thereby, leading to oxidative stress, injury, increase in cell apoptosis. Oxidative stress can cause damage and death of specialized cells in the cerebellum and brainstem tissue. This study investigates the effect of Rhodamine B on the expression of BAX and BCL-2 in the cerebellum and brainstem tissue of Rattus norvegicus.

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regions involved in magnetic resonance morphometry (MR), namely the ROS and SOD.[6] The ROS is produced due to exposure to Rhodamine B and affects the activity of Superoxidase Dismutase (SOD), which leads to oxidative stress and cell injury. Damage to the cerebellum and brainstem leads to functional abnormalities such as impaired coordination and accuracy of motor function and learning, sleep-wake cycle disorders, awareness, respiratory/cardiovascular control, impaired in coordinating communication between the cerebrum, spinal cord, and cerebellum. This study focused on the effect of Rhodamine B on the expression of BAX and BCL-2 in the cerebellum and brainstem tissue of *Rattus norvegicus*.

Design and Methods

Animal

A female healthy Rattus norvegicus Wistar between the ages of 10-12 weeks found in the experimental animal raising unit (UPHP) was used to carry out this research. Mice are laboratory animals commonly used as a research model before being treated in humans.

Rhodamine B treatment

The experimental animals were all adapted first at room temperature of 22-25°C for 11 days at the UB Pharmacology Laboratory, Faculty of Medicine. Before treatment, the rats were synchronized with the estrous cycle using the Whitten method for 5 days and grouped into the control (standard food ad libitum), group I (standard food and Rhodamine B dose 150 ppm (4.5 mg/ 200 gBW), group II (standard food and Rhodamine B dose 300 ppm (9 mg/ 200 gBW)), and group III (standard food and Rhodamine B 600 ppm dose (18 mg/ 200 gBW)). Rhodamine B was administered per sonde for 36 days.

Euthanasia

After 36 days of exposure to Rhodamine B, all rats were anesthetized using inhaled chloroform, and those turned off at the hippocampus tissue, underwent surgery on the brains using a 10% formalin solution.

Analysis of BAX and BCL-2

Analysis of BAX and BCL-2 expression in hippocampus tissue using immunohistochemical staining was conducted and observed with a microscope. A brown hippocampus tissue shows the presence of BAX and BCL-2 expression, while purple, it shows none. The calculation of BAX and BCL-2 expressions based on weak color intensity are as follows (1), medium (2), strong (3), very strong (4) using the help of OliVIA software. Data results are then processed using SPSS for Windows software.

Results

The structural coefficient of determination

Before analyzing the structural model, it is necessary to calculate its determination coefficient value for testing the goodness of fit model by obtaining the predictive value of relevance. This coefficient is used to determine the ability of the structural model to explain the data that has been obtained. The calculated results from Table 1 show the predictive-relevance (Q2) value of 0.741 or 74.1%, and this indicates that the model explains the information contained in the data of 74.1%. The remaining 25.9% is analyzed by other variables not contained in the model, as well as errors.

Testing of direct influence

Table 2 describes the results of testing the direct effect of the structural model, the coefficient of Rhodamine B on BAX expression obtained was 0.839, with a P-value of 0.000. Furthermore, Rhodamine B shows a significant direct effect when P-values less than 0.05 (P<0.05). The positive path coefficient implies that Rhodamine B has a positive influence on BAX expressions, with an increase due to the higher dose. Based on the results of testing the direct effect of structural models, the path of influence of Rhodamine B on BCL-2 Expression obtained a coefficient of -0.774 with a p-value of 0.000. Rhodamine B has a significant direct effect on BCL-2 expression when the p-values less than 0.05 (P<0.05). The negative value path coefficient implies that Rhodamine B has a negative influence on BCL-2 expression. A higher dose of directly decreases the BCL-2 expression. On the influence path of BAX Expression on BCL-2 Expression, the coefficient is 0.184, with a P-value of 0.003. P The BAX expression had a significant direct effect on BCL-2 expression when the P<0.05. The positive path coefficient means that BAX expression has a positive influence on BCL-2 expression, and an increase directly decreases the expression.

Testing of indirect effects

Table 3 shows the results of testing the indirect influence of Rhodamine B on BCL-2 expression, the path coefficient was 0.142, with a P-value of 0.006 (P<0.05). From the testing, it was proven that Rhodamine B had a significant indirect effect on BCL-2 expression. Furthermore, it also has a direct/indirect influence on BAX expression and BCL-2, respectively. Increased Rhodamine B will have a direct impact on BAX Expression and indirectly reduces BCL-2.

Discussions

The results showed that Rhodamine B has a direct and indirect effect on BAX and BCL-2 expressions in the cerebellum tissue and

Table 1. R² and Q² structural model.

Intermediary variables and bound variables	R-Square	Q-Square
Expression of BAX	0.976	0.741
Expression of BCL-2	0.600	-

Table 2. Testing of direct influence.

Direct influence path	Path coefficient	P-value
Rhodamine B -> BAX	0.839	0.000
Rhodamine B -> BCL-2	-0.774	0.000
BCL-2 -> BAX	0.184	0.003

Table 3. Testing of indirect effects.

Indirect effects	Coefficient of indirect influence	P-value
Rhodamine B ->expression BCL-2	0.142	0.006

Wistar strain *Rattus norvegicus* brainstem, respectively. An increase in Rhodamine B directly increases BAX expression and indirectly reduces BCL-2. The positive path coefficient means that BAX expression has a positive effect on BCL-2, while increased BAX expression decreases the BCL-2. Damage to the cerebellum tissue and brainstem leads to functional abnormalities, including impaired coordination and precision of motor function/learning, sleep-wake cycle disorders, awareness, respiratory/cardiovascular control, and impaired in coordinating communication between the cerebrum, spinal cord, and cerebellum.

In food processing, Rhodamine B causes various health problems such as nausea, vomiting, diarrhea, cancer, cardiovascular disease, kidney disease, liver dysfunction, hormonal imbalance, premature birth, decreased immunity, nervous system development disorders, mental health problems, and learning disabilities/cognitive dysfunction.[7] The research was previously conducted by Marpaung Kun Sri Budiasih (2017) to determine the effect of Rhodamine B and saccharin on the activity of Superoxide Dismutase (SOD) in the kidney of white mice (*Rattus norvegicus*). The results showed that the significant combination of Rhodamine B and saccharin (P<0.05) was able to reduce SOD activity by 47.11% compared to the negative control group. Kidney damage in the form of glomerular hypertrophy and tubules, narrowing of the Bowman space, and the presence of hemorrhage. Based on these results, it is concluded that the combination of Rhodamine B and saccharin as food additives are more toxic.[4] Apart from harming human health, Rhodamine B also provides benefits, as follows: 1) used in the early detection and apoptotic imaging of isothiocyanate silica-coated fluorescent nanoparticles (RBITC-DSFNPs). This bioprobe recognizes the early stage of apoptotic cells by binding between annexin V and phosphatidylserine on the outer membrane and by monitoring the increase in the number of early apoptotic cells along with extended induction times. This labeling method has a better photostability compared to fluorochromes such as Cy3 labeled annexin V and offered a promising approach for tumor-related research,[8] 2) The new design of eight half iridium (Ir) and ruthenium (Ru) sandwich compliments fluorescing by introducing Rhodamine derivatives into the N ^ N binding ligand features bioimaging and anticancer agents,[9] 3) Rhodamine-derived superparamagnetic maghemite nanoparticles (SAMN-R) shows relatively high absorption by mammalian cells, excellent ad long term stability in intracellular space of rhodamine fluorescence shells with specific pH. SAMN-R was previously tested on rat stem cells, rabbits and humans, and it is a strong cell marker, long-term stable investigation for precipitation of lysosomes, that do not affect nuclei, and with theranostic potential,[10] 4) Fluorescent properties of rhodamine coated nanorods were studied using spectrometry and confocal microscopy. The photoluminescence spectrum of rhodamine functioned by HMMSN nanorods shows that excitation has a maximum located at λmax ex = 560 nm and peak emission at λexc em = 580 nm according to excitation/fluorescence emission. This optical functionality is added to the silica surface without changing the structural, texture, or physical properties of blessing nanorods with additional fluorescent marking for biological imaging,[11] 5) The fluorescence brightness distribution of rhodamine B is used to investigate the amount and depth of drug delivery through interactive pressure on the hydrophobic delivery of rhodamine B to ex vivo heated arterial walls to determine optimal drug delivery conditions.[12]

Subsequent research on curative efforts in increasing hepatocyte cells exposed to Rhodamine B through herbal therapy of garlic water extract (Allium sativum) was used to determine the levels of SGOT, and SGPT and histopathological features of the rat liver (*Rattus norvegicus*) exposed to Rhodamine B. The results showed that the administration garlic extract at a dose of 1.5 mL / 0.2 kg was able to reduce SGPT activity, ie, for treatment A to E, 18.89%, 17.3%, 40.96% and SGOT 41.44%, 45.96%, 49.69%, and to increase hepatocyte cells in rats exposed to Rhodamine B. In summary, garlic water juice can be used as an herbal therapy in rats exposed to Rhodamine B.[1]

Conclusions

In conclusion, Rhodamine B has been verified to be able to increase BAX expression, which directly affects BCL-2 Cerebellum tissue and Brainstem on *Rattus norvegicus* as dose increases.

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