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The Effects of Borax ($NA_2B_4O_710H_2O$) on Histopathology of Wistar Rats' Cerebrum (*Rattus norvegicus*)

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ABSTRACT

The present study aimed to determine the effects of borax ($Na_2B_4O_7.10H_2O$) addition on the changes of histological cerebrum imaging in the brains of white mice (*Rattus norvegicus*). The current research was an experimental study with randomization of 24 white mice that were divided into four treatment groups with five replications. Borax was dissolved for each treatment with a dose of 19 mg/mouse/day, 26 mg/mouse/day, and 37 mg/mouse/day, and it was administered orally for 14 days. Then, it was analyzed statistically using the Kruskal-Wallis test. The statistical analysis results suggested that there were significantly different results in each treatment group. The control treatment with an administration dose of 26 mg/rat/day had a significantly different result in the worst cloudy swelling degeneration of cerebrum in histopathology imaging on Wistar rats (*Rattus norvegicus*). Using the Mann-Whitney test, it was found that the dose of borax at 37 mg/rat/day led to significant difference, compared to the other treatment groups, which means that 37 mg/rat/day of borax caused the worst pyramidal cell necrosis in histopathology imaging of the cerebrum on white mice. Borax exposure on Wistar rats (*Rattus norvegicus*) can cause cloudy swelling at a dose of 26mg/head/day, and pyramidal cell necrosis at a dose of 37 mg/head/day.

Keywords: Borax, Cerebrum, Cloudy swelling, Necrosis

INTRODUCTION

Borax is a component of many detergents, cosmetics, and enamel glazes. It is used to make buffer solutions in biochemistry, as a fire retardant, as an anti-fungal compound, in the manufacture of fiberglass, as a flux in metallurgy, neutron-capture shields for radioactive sources, a texturing agent in cooking, as a cross-linking agent in slime, as an alkali in photographic developers, as a precursor for other boron compounds, and is useful as an insecticide (similarly to boric acid, Levy and Lisensky, 1978). The use of borax on a large scale as a food preservative can be profitable in terms of production since borax can be purchased at a relatively cheap price. However, the negative impact resulting from excessive use can cause health problems. Borax is used excessively as preservatives in the food-making process of some foods, such as in *bakso*, noodles, and dumplings (Sugiyatmi, 2015). In a small amount, borax can create a chewy effect in the food, making the food stickier, long-lasting, and feel tasty. Compared to other preservatives, borax can maintain the food texture, which results in the longer maintenance of food without any change in chewiness and appearance. Borax can also maintain acidity causing the food not to spoil quickly.

The use of borax on a large scale as a food preservative can certainly be profitable in production since borax can be purchased at a relatively cheap price, compared to other harmless preservatives. In fact, even non-hazardous preservatives must be used in the right dosage. Borax even needs more cautious treatment since people cannot estimate the dosage used as a food preservative (Meacham et al., 2010).

The direct danger caused by the excessive use of borax or the residual left can have a systemic impact on the body (Dourson et al., 1998). Visible effects of borax include skin hardening or dead skin. The more dangerous effects of borax include making damage to the liver, stomach, small intestine, large intestine, testicular, and ovarian organ infertility, and it stimulates cancer cell growth (Nasution, 2009). Borax attacks cells, especially in the mitochondria, and it is accumulated in the cytoplasm and can disturb the cells' metabolism function. The study aimed to determine the effects of borax ($Na_2B_4O_7.10H_2O$) addition on the changes of the histological imaging of cerebrum in Wistar rats' brains (*Rattus norvegicus*).

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MATERIALS AND METHODS

Ethical approval

All experimental protocols and procedures were approved by the Institutional Animal Care of Indonesia. The present research was conducted in experimental animal cages in the Faculty of Veterinary of Universitas Airlangga in Indonesia. The making of histopathological preparations of mice brains was carried out at the Veterinary Pathology Laboratory of the Faculty of Veterinary of Universitas Airlangga.

Research material

The experimental animals used in the current research were 24 healthy male Wistar rats (*Rattus norvegicus*) aged 1.5 months old and weighed approximately 100 grams. The rats were obtained from the Faculty of Medicine, Universitas Airlangga, Indonesia. The equipment used in the current study included 40 cm × 25 cm × 12 cm plastic cages, wire mesh for cage cover, feeding and drinking places, digital scales, feeding needles, surgical scissors, sterile scalpel, sterile tweezer, object glass, cover glass, tray as a container, small pot, lid as organ storage, Bunsen burner, oven, aluminum foil, microtome, staining jar, refrigerator, camera, and Eclipse C® 140 with NIS application (Nikon imaging system) BR40. The materials of the current study consisted of borax ($Na_2B_4O_7.10H_2O$) obtained from a chemical store on Jalan Tidar (Surabaya), sterile aqua dest as a borax solvent, 511 pellet chicken feed from PT Charoen Pokphand (Surabaya), drinking water, husk as the base for an individual cage, sterile cotton, ketamine, and 10% formalin.

Research design

The current study employed a laboratory experimental method. The research design used was a completely randomized design of various borax doses of 19 mg/rat/day, 26 mg/rat/day, and 37 mg/rat/day (Wagner and Wolff, 1976). The treatment groups were defined as P0 including Wistar rats treated with 0.5 ml/rat/day sterile aqua dest, P1 entailing Wistar rats treated with 0.5 ml/rat/day sterile aquadest + 19 mg/mouse/day borax, P2 having Wistar rats treated with 0.5 ml/rat/day sterile aquadest + 26 mg/mouse/day borax, and P3 including Wistar rats treated with 0.5 ml/rat/day sterile aquadest + 37 mg/mouse/day borax.

On day 15 of the experiment, the rats were intramuscularly anesthetized using ketamine. After that, fixation and dissection were conducted to separate the cerebrum from the brain organ. Furthermore, the pathology of anatomy was observed and histopathology preparation of the cerebrum was performed. The observed changes on the histopathologic imaging included cloudy swelling and pyramidal cell necrosis aspects (Sairazi et al., 2017). The damage level scoring (cloudy swelling and necrosis degenerations) in one visual field can be seen in Table 1 as follows (Purnima et al., 2013):

Table 1. Histopathological scoring of Borax exposure in the cerebrum of rats

Score	Degeneration	Necrosis
0	If the degenerative cloudy swelling of the pyramidal cell was not found at a third of the observed visual field of the cerebrum.	If the pyramidal cell necrosis was not found at a third of the observed the visual field of the cerebrum.
1	If the cloudy swelling degeneration of the pyramidal cell was found at a third of the observed visual field of the cerebrum.	If the pyramidal cell necrosis was found at a third of the observed visual field of cerebrum.
2	If the cloudy swelling degeneration of the pyramidal cell was found at a third until two-third of the observed visual field of the cerebrum.	If the pyramidal cell necrosis was found at a third until two-third of the observed power field of the cerebrum.
3	If the cloudy swelling degeneration of the Pyramidal cell was found at two-third until the whole of the observed visual field of the cerebrum.	If the pyramidal cell necrosis was found at two-third until the whole of the observed power field of the cerebrum.

Data analysis

The present study was analyzed using the Kruskal-Wallis test ($p < 0.05$). If significant differences were found, it would be continued with the Mann-Whitney test (Purnima et al., 2013).

RESULTS

The results of statistical analysis with the Kruskal-Wallis test suggested that there was a significantly different cloudy swelling degeneration in each treatment group ($p < 0.05$). Based on the results of statistical analysis with the Kruskal-

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Wallis test, it was found that there were significantly different results in each treatment group ($p < 0.05$). In the Mann-Whitney test, it was found that P0, P1, and P3 were not significantly different statistically; values of P0 and P2 were significantly different; values of P1, P2, and P3 were not significantly different ($p > 0.05$); and values of P2 and P3 were not significantly different either ($p > 0.05$). In Table 2, it was identified that most cloudy swelling degeneration occurred in P2, whereas, the pyramidal cell that had cloudy swelling degeneration was hardly found in P3, whom the highest dose was used since more than two-thirds of the whole of the pyramidal cell had necrosis in one visual field of the Wistar rats.

Meanwhile, the results of the statistical analysis of the Kruskal-Wallis test indicated that there were significantly different results of necrosis in the pyramidal cell of Wistar rats' cerebrum in each treatment group. Regarding the Mann-Whitney test, P3 was significantly different ($p<0.05$), compared to the whole treatment groups. An insignificant difference ($p > 0.05$) only occurred in P1 and P2.

Table 2. Histopathological Imaging Analysis of the Cloudy Swelling Level and Necrosis of the pyramidal Cell of Cerebrum in each treatment group of Wistar rat (*Rattus norvegicus*)

Treatment	Cloudy Swelling Degeneration Mean ± SD	Necrosis Mean ± SD
P0: Control of 0.5ml/rat/day sterile aquadest	$0.10^{a} \pm 0$	$1^{a} \pm 0$
P1: 0.5 sterile aquadest + 19 mg/rat/day borax	$0.33^{abc} \pm 0.516$	$1.5^{b} \pm 0.54$
P2: 0.5 sterile aquadest + 26 mg/rat/day borax	$0.83^{c} \pm 0.408$	$1.5^{bc} \pm 0.548$
P3: 0.5 sterile aquadest + 37 mg/rat/day borax	$0.38^{acd} \pm 0.495$	$1.63^{d} \pm 0.71$

Different superscript in the same column indicates that there were significant differences ($p < 0.05$), SD: Standard deviation

DISCUSSION

Brain damage caused by borax exposure can pass through various pathophysiological ways. Borax which is orally got into the stomach would react with hydrochloric acid (HCl) causing faster synthesis to become boric acid (Sugiyatmi, 2015). Furthermore, the boric acid would be absorbed by the intestines, then it gets into the blood circulation until reaching the organs, and causes some damages including brain damage.

Boric acid attacks cells from the body's systemic process, especially mitochondria (Dourson et al., 1998). Normally, mitochondria function to produce energy for cell activities through the oxidative phosphorylation process (Nielsen, 1994; Pizzorno, 2015). Boric acid will inhibit H^+ ion that makes a co-enzyme bound between NAD^+ and H^+ ion. The H+ ion which bounds with boric acid will cause the failure of reduction-oxidation reaction in mitochondria (Litovitz et al., 1988). In addition, boric acid is bounded with proteins and lipids causing malfunction and forming Reactive Oxygen Species (ROS, Tanaka et al., 2016). The failure of reduction-oxidation reaction in mitochondria and the increase of ROS can cause a decrease in neuron cell viability caused by the inhibition of the electron transport chain. That process could disturb Adenosine triphosphate (ATP) formation that causes cell degeneration or even necrosis. The ATP is required for the fluency of sodium (NA^+) and potassium (K^+) pumps.

The mechanism of the sodium-potassium pump's disturbance starts with the shifting of sodium ion. Then, H_2O and calcium (Ca^+) get into the cell, while potassium and magnesium (Mg+) move out from the cell increasing the intracellular fluid influx, changes of the cell form, and acute cell swelling (Price and Wilson, 1986). The process of cloudy swelling degeneration occurs due to the failure of ATP formation as the implication of mitochondria function experiencing energy synthesis disturbance (Jamison, 1974). The failure of energy synthesis is caused by the boric acid and co-enzyme bond of NAD^+ and H^+ ion. Therefore, it inhibits the sodium-potassium pump to maintain intracellular stability. The cells which are supposed to release metabolic energy to pump sodium ion out of the cells cannot function properly. This situation increases sodium ion concentration inside the cells followed by water entrance causing cell inflammation and cloudy cytoplasm. If the influence of toxic substances can be removed, the cell would return to its normal situation (Nielsen, 1994).

Necrosis is the cell destruction caused by pathological damage, such as toxin exposure (Kumar, 2007). Some biochemical mechanisms of necrosis include ATP depletion, oxygen deprivation, or the formation of Reactivated Oxygen Species, the loss of calcium homeostasis, the defect in plasma membrane permeability, and mitochondrial damage (Heindel et al., 1992). The cerebrum pyramidal cell necrosis of the Wistar rats' brains with borax exposure started with boric acid and co-enzyme bound of NAD^+ and H^+ ion. It led to the failure of a reduction-oxidation reaction inside the mitochondria (Nielsen, 1994). The manifestations of failure in mitochondrial function would disturb glycolysis, energy synthesis, and ATP formation. The ATP is required to ease the sodium-potassium pump. If ATP formation is obstructed, water accumulation will occur inside the cytoplasm since sodium absorbs water. Next, cloudy swelling occurs in the cell. Extended and continuous effects of toxic substances make the cell unable to metabolize resulting in cell death or necrosis (Price and Wilson, 1986).

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Extended cell degeneration disruption and the relatively large effect of toxic substances in cells will surpass the interference compensation limit causing necrosis to take place. Necrosis is marked by the morphological changes of the cell nucleus, including pyknosis, karyorrhexis, and karyolysis. Pyknosis is the depreciation and compaction process of the cell nucleus causing it to be more basophilic with bluer cell color with H & E coloring. Microscopically, it was marked by the cell nucleus looking more compact and darker. Karyorrhexis is marked by the spread of broken nucleus and chromatin fragments formation, while karyolysis is marked by the nucleus dissolution (Kumar, 2007). Necrosis process also increases the bloodstream, and is followed by capillary vasodilation that occurs actively inside the microcirculation. Cell swelling around the capillary will press and cause lumen narrowing, causing the blood unable to stream smoothly, commonly referred to as congestion (Bezabeh et al., 2004).

In imaging pyramidal cells of the cerebrum caused by borax exposure, the cells experienced widespread necrosis that caused systemic damage or cell injury around it, followed by an inflammatory reaction. The capillary was blocked by cell inflammation-causing blood vessel dilatation, followed by increasing blood amount. In this stage, the erythrocytes would accumulate in the capillary as congestion. If the capillary is broken, it will be followed by the spread of erythrocytes around the cell, resulting in hyperemia (Bezabeh et al., 2004). Borax can also cause brain, heart, fat, and kidney disturbance. In addition, borax can cause comma, central nervous system disruption, depression, and even mortality (Murray, 1998). The exposure of 20.8 mg/kg BW of borax in Wistar rats for 14 days increased the dehydrogenase inside the cerebrum (Parks and Edwards, 2005). Histologically, degeneration, congestion edema in the brain, meninges, perivascular hemorrhage, and intravascular thrombosis may occur.

CONCLUSION

Borax addition can cause cerebrum cloudy swelling on Wistar rats (*Rattus norvegicus*) at the dose of 26 mg/rat/day. Meanwhile, borax addition at the dose of 37 mg/rat/day can cause necrosis in the pyramidal cells of Wistar rats' cerebrum.

DECLARATION

Authors' contribution

All authors contributed equally to conduct this study.

Competing interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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