International Islamic Medical Journal
Journal Homepage ://journal2.unusa.ac.id/index.php/IIMJ

The Effect of Honey Administration on The Histopathology of The Duodenum of Wistar Rats as a Inhibition of The Toxic Effects of Borax (Sodium tetraborate)

Dian Noviana[1], Mustika Chasanatusy Syarifah[1*], Retna Gumilang[1], Tri Wahyuni Bintarti[1]

[1]Medical Faculty, Universitas Nahdlatul Ulama Surabaya, Indonesia
*Corresponding author: mustika4n6@unusa.ac.id
DOI : 10.33086/iimj.v4i2.4445

ARTICLE INFO

Keywords:
Borax, Free Radicals, Oxidative Stress, Honey, Duodenum

Submitted: June 8th 2023
Reviewed: June 23th 2023
Accepted: July 11th 2023

ABSTRACT

Background: Borax can result in oxidative stress and cause the onset of gastrointestinal ulcerations that will dampen the duodenal villi to become shorter and can even disappear. Cell damage due to oxidative stress can be prevented by administering antioxidants. Antioxidants will inhibit the onset of chain reactions in the formation of free radicals by complementing the existing electron deficiency. Honey is one of the natural ingredients that is rich in antioxidants and part of thibbun Nabawi as one of the inhibitors of the toxic effects of borax.
Objective : The purpose of this study is to to analyze the effect of honey administration on the histopathological description of submucosa and epithelial mucosa of duodenal wistar rats as an inhibitor of the toxic effects of borax (Sodium tetraborate).
Methods: This study used the true experimental method with Post Test Only Control Group Design using 25 mice which will be divided into five groups, namely K (negative control), P (positive control), M1 (borax and honey dose 1), M2 (borax and honey dose 2), M3 (borax and honey dose 3). This study was conducted for 22 days then. Took the duodenal organ on all samples and then made histological preparations with HE staining. Observations were made with an Olympus microscope to see submucosal edema and damage to the epithelium of the duodenal mucosa.
Results: The results of the study found that borax 26 mg / head / day had an influence on the histopathological picture of duodenal borax. In addition, the administration of honey dose 75 mg / Kg BB provides a toxic inhibitory effect of borax in the duodenum best among other treatment groups. However, statistically there was no effect of honey administration on the histopathological picture of the duodenum of wistar rats as an inhibitor of the toxic effect of borax (Sodium Tetraborate) with $p>0.05$.
Conclusion: Statistically it can be concluded that there is no effect of the administration of honey on the histopathology picture of the duodenum of wistar rats as an inhibitor of the toxic effects of borax (Sodium tetraborate).

Introduction

In modern times, manufacturers continue to develop food processing techniques in order to meet public demand. One effort have widely applied is the addition of additives to food products. Adding these additives generally aims to extend the durability of food so that food can be stored for a more extended period. (Amalia et al., 2017).

BPOM (Indonesian Food and Drug Administration Monitoring) intensifies Food Control in all regencies/cities throughout Indonesia. Sampling was carried out on iftar/takjil snacks for later testing. The test result found that food samples contained hazardous ingredients, namely 0.73% rhodamine-B, 0.59% borax, and 0.45% formalin (BPOM RI, 2021). Following the Regulation of the Minister of Health No.033/MenKes/Per/XI/2012 concerning Food Additives, stipulating that borax is prohibited from being used as a food additive because borax is a hazardous and toxic substance. However, substance abuse is still common (Permenkes, 2012).

Borax is a source of radicals that can cause oxidative stress, which can cause cell damage. Antioxidants can prevent cell damage due to oxidative stress (Puspadewi, 2012). Antioxidants will inhibit the emergence of a chain reaction in the formation of free radicals by completing the existing electron deficiency so that these free radicals can be stable and prevent oxidative stress (Amalia et al., 2017)

The use of honey as medicine has been carried out since ancient times and has even existed since the time of the Prophet Muhammad. This method of treatment is known as Thibbun Nabawi. Thibbun Nabawi is a method of treatment that refers to all the sayings, teachings, and actions of the Prophet Muhammad related to the health sector, both in terms of prevention and treatment cure of a disease (Fatahilah, 2016). Honey has many ingredients that are very beneficial, such as minerals, antioxidants, vitamins, and oligosaccharides. (Cahyani, 2015). A study conducted by Anggraeni (2018) stated that giving honey to the treatment group at doses of 25 mg/Kg BW, 50 mg/Kg BW, and 75 mg/Kg BW could improve the histopathology of the duodenum of Wistar rats due to free radicals generated by lead acetate.

Duodenum is the first part of the small intestine, located under the stomach, shaped like a horseshoe (C shape). The duodenum absorbs all chemicals that enter the body and undergoes a process of absorption, distribution, metabolism, and excretion pharmacokinetically (Cahyani, 2015). Boric acid in borax will enter the digestive system and be absorbed by the intestinal villi. If the intestinal villi continuously absorb borax, it can result in increasingly

aggravating oxidative stress and cause gastrointestinal ulceration, which will impact the villi of the duodenum, jejunum, and ileum to become shorter and may even disappear (Purnama et al., 2013).

Based on the background above, the authors are interested in conducting research on the effect of giving honey on histopathological features of the duodenum of wistar rats as an inhibitor of the toxic effects of borax (Sodium tetraborate).

Objective

The purpose of this study is to analyze the effect of honey administration on the histopathological description of submucosa and epithelial mucosa of duodenal wistar rats as an inhibitor of the toxic effects of borax (Sodium tetraborate).

Significance of Research

This study are expected to be used as an inhibitor of the toxic effects of borax (sodium tetraborate). Then as the development of knowledge in forensic toxicology medicine and honey treatment according to the Prophet's method (Thibbun Nabawi) and to support existing theories.

Methods

This study used the true experimental method with Post Test Only Control Group Design using 25 mice which will be divided into five groups, namely K (negative control), P (positive control), M1 (borax and honey dose 1), M2 (borax and honey dose 2), M3 (borax and honey dose 3). This study was conducted for the next 22 days. Took the duodenal organ on all samples and then made histological preparations with HE staining. Observations were made with an Olympus microscope to see submucosal edema and damage to the epithelium of the duodenal mucosa.

Result

The negative control group of rats (Grup K) was only given eat and drink as usual; Group P as the positive rat control group, was given oral exposure to borax 26 mg/head/day; Group M1 as the first treatment group was given oral exposure to borax 26 mg/head/day and honey at a dose of 25 mg/Kg BW orally/day; Group M2 as the second treatment group was given oral exposure to borax 26 mg/head/day and honey at a dose of 50 mg/Kg BW per orally/day; Group M3 as the third treatment group was given oral exposure to borax 26 mg/head/day and honey at a dose of 75 mg/Kg BW orally/day.

Furthermore, after 14 days of treatment, all samples were taken from the duodenal organs. Histological preparations were made and observed under a microscope through 100x magnification to see duodenal submucosal edema and 400x magnification to see damage to the

duodenal mucosal epithelium. Then do the scoring on the level of cell damage. After all the data is obtained, the data is tabulated and then analyzed.

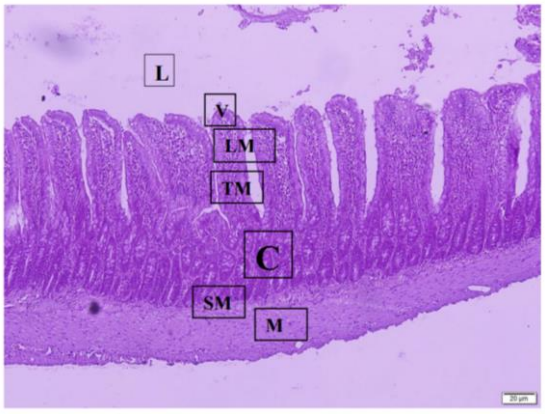

Figure 1. Histopathology of the rat duodenum with HE staining at 100x magnification was normal, the submucosa was normal, and the mucosal epithelium was not damaged. Both pathological changes did not occur.

Abbreviation:

L	Lumen
TM	Tunika Mukosa
V	Vili
LM	Lamina Propis
S	Submukosa
M	Muskularis
C	Crypt

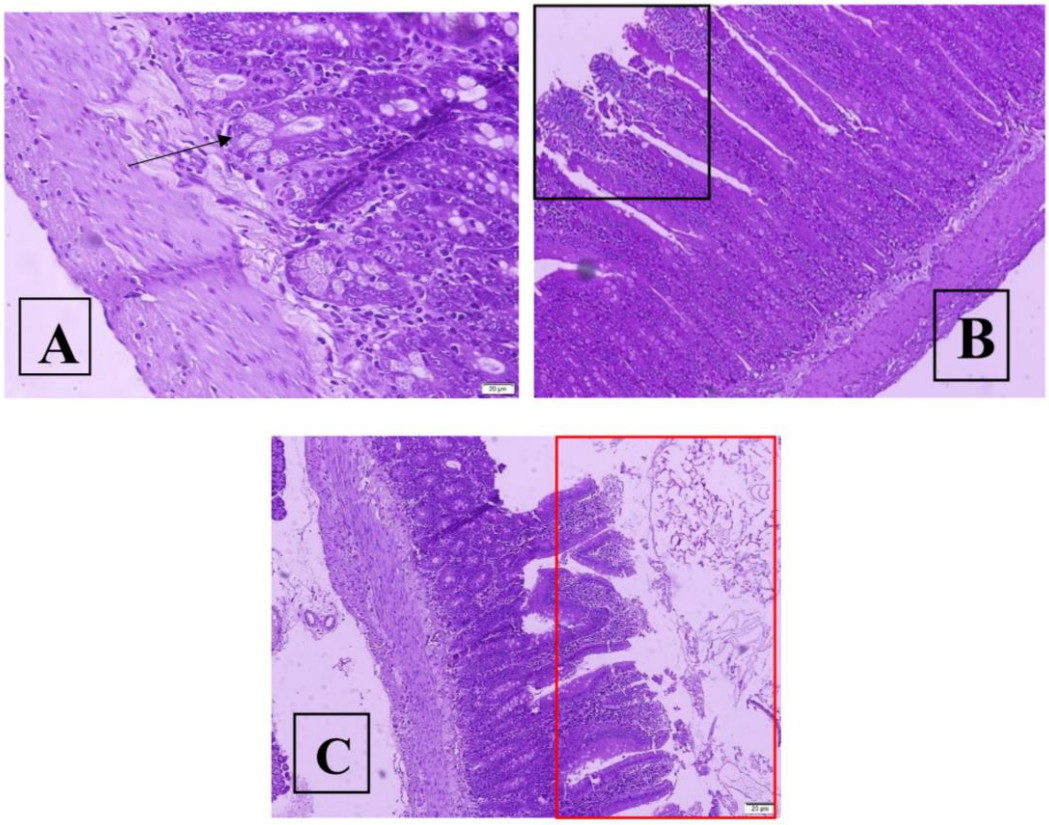

Figure 2. Duodenum histopathology with HE staining. The submucosa is edematous (A), and the mucosal epithelium is damaged (B and C).

Description:

Black arrow:	Submucosal edema
Black square:	Epithelial desquamation
Red square:	Epithelial erosion

Table 1. Results And Mean Scores of Submucosal Edema And Mucosal Damage White Rat Duodenum

Submucosal Edema			
Sample	Mean	N	Standard Deviation
Negative Control	0,8	5	0,447213595
Positive Control	0,4	5	0,547722558
Treatment 1	0,4	5	0,547722558
Treatment 2	0,4	5	0,547722558
Treatment 3	0	5	0
Mucosal Epithelial Damage			
Sampel	Mean	N	Standard Deviation
Negative Control	0,8	5	1,095445115
Positive Control	0,4	5	0,894427191
Treatment 1	0,4	5	0,547722558
Treatment 2	0,8	5	0,447213595
Treatment 3	0	5	0

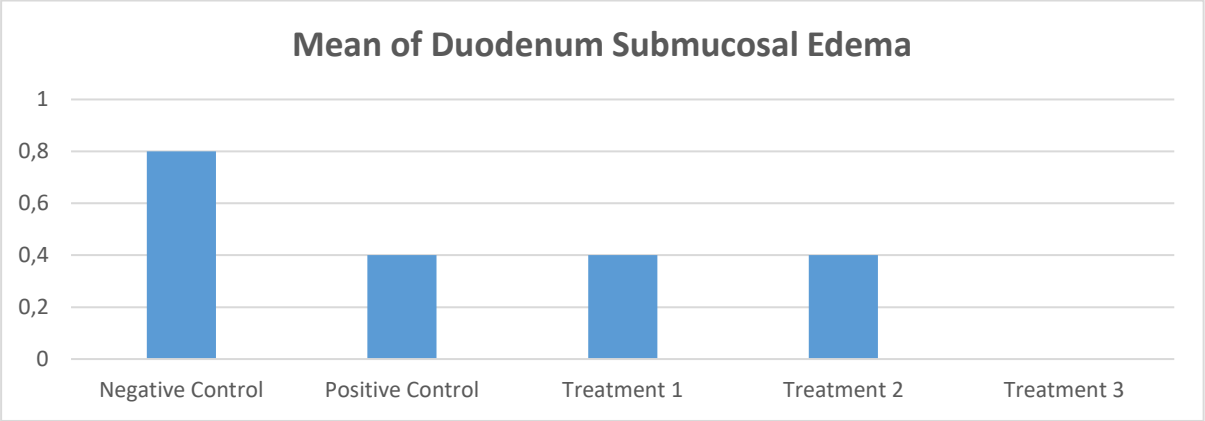

Fig 3. Average Duodenal Submucosal Edema of White Rats.

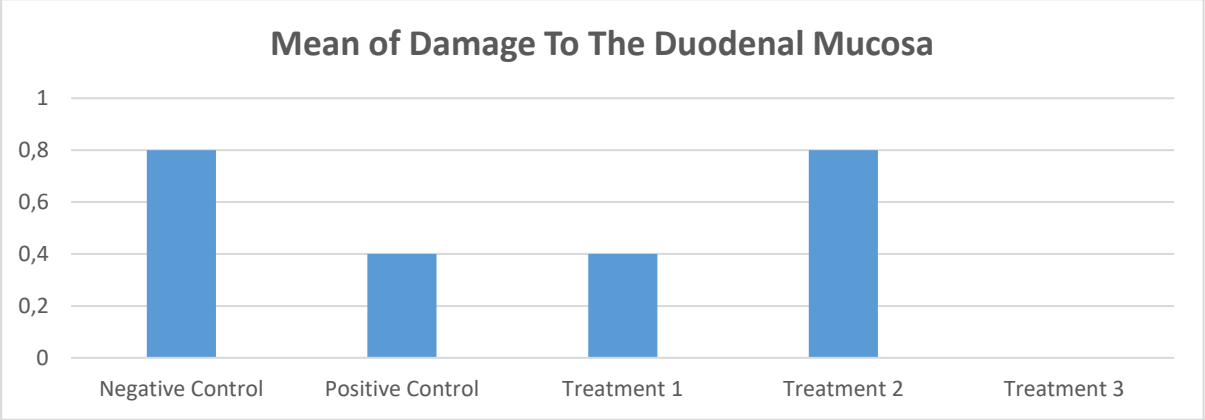

Fig 4. Average Damage to The Duodenal Mucosa of White Rats.

Figure 5.3 shows that the negative control has the highest mean value of submucosal edema among the other groups, which is equal to 0.8. Treatment One and Treatment 2 were in the second position for the positive control group with the same value of 0.4. In the treatment group, Group 3 is the lowest average submucosal edema among the other treatment groups, which is equal to 0.

In Figure 5.4, it can be seen that the negative control and treatment 2 have the highest average value of mucosal damage among the other groups, which is equal to 0.8. The positive control group and treatment 1 are in the second position with the same value of 0.4. In the treatment group, 3 has the lowest average value of mucosal damage among the other treatment groups, equal to 0.

The results of the study found that borax 26 mg/head/day had an influence on the histopathological picture of duodenal borax. In addition, the administration of honey dose 75 mg / Kg BB provides a toxic inhibitory effect of borax in the duodenum best among other treatment groups. However, statistically there was no effect of honey administration on the histopathological picture of the duodenum of wistar rats as an inhibitor of the toxic effect of borax (Sodium Tetraborate) with $p>0.05$.

Discussion

Histological Picture of Wistar Rat Duodenum with Borax Administration

The research results show that administering borax as much as 26 mg/rat/day orally in Wistar rats shows the presence of submucosal edema and necrosis of the duodenal mucosa in the rats studied. This result follows a study conducted by Elziyad (2013), which stated that a dose of borax of 37 mg/head/day had a more severe effect on duodenal submucosal edema, 57%, and duodenal mucosal necrosis, 45% more severe than a dose of 26 mg/rat/day. It can be concluded that borax has an effect on edema and necrosis in the cells of the rat duodenum.

The result also follows the theory that using borax-containing borax acid as an active ingredient can go through the digestive system and then be absorbed by the intestinal villi. If the intestinal villi continuously absorb borax, it can cause oxidative stress, which will have an impact on the villi of the duodenum, jejunum, and ileum to become shorter and may even disappear (Purnama et al., 2013). The use of borax can interfere with mitochondrial function by causing the failure of energy synthesis so that ATP also fails to form.

The failure of energy synthesis is caused by the formation of bonds between NAD+ coenzymes (H+ ions) and boric acid, resulting in an obstacle to the sodium-

potassium pump, which maintains intracellular stability (Elziyad, 2013). Manifestation of inhibition carried out by boric acid will cause the failure of energy synthesis and impact cell damage. The process of glycolysis produces 2 moles of ATP needed for the smooth running of sodium (Na+) and potassium (K+). The inhibited glycolysis process cannot produce sufficient ATP, so Na+ will attract water, as a result of which there will be an accumulation of water in cells followed by hydrophilic degeneration (Amalia et al., 2017).

This study also found that the negative control group of rats that were not given any treatment experienced submucosal edema and damage to the duodenal mucosal epithelium. This result can be caused by many factors, namely, excessive physical activity in rats or stress due to environmental influences. The findings follow the research of Pujaswarini et al. (2019), which states that all physical activity, both light and strenuous physical activity, can increase levels of ROS (Reactive oxygen species), which in turn will have an impact on increasing free radicals in the body. The negative control group was not given honey, so the endogenous antioxidants in the mice's bodies had to work alone against these free radicals. The imbalance between the amount of antioxidants and the high number of free radicals in the body will lead to oxidative stress.

Duodenum Histology with Borax and Honey Administration

Based on this research, giving borax and honey to 'Treatment 1' rats at a dose of 25 mg/Kg BW showed a decrease in duodenal mucosal epithelial necrosis compared to positive control rats. Whereas in 'Treatment 2' with a dose of 50 mg/Kg BW turned out to have a more significant effect on mucosal epithelial necrosis when compared to all other treatments. The same thing could cause this as in the negative control rat group, where the activity of the rats could not be controlled by the researchers or due to enzymes forming endogenous free radicals in the body, which could cause levels of free radicals in the body of the 'Treatment 2' rats to be higher when compared to the treatment group. So the other dose of honey as an antioxidant becomes inadequate and still causes submucosal edema and necrosis of the duodenal mucosal epithelium (Sirait, 2016).

The results of the research show that the administration of honey at a dose of 75 mg/Kg BW per oral/day could inhibit the toxicity of borax at a dose of 26 mg/head/day so that no submucosal edema or duodenal mucosal necrosis was seen in Wistar rats better than other doses of honey. This shows that the best dose of honey at a

75 mg/Kg BW dose protects the duodenum from borax toxicity. These findings support research by Anggraeni (2018), which states that giving honey can reduce MDA levels in rats. MDA levels are lipid peroxidation products that can be used as a marker to measure free radical levels. Honey contains flavonoids that have benefits as endogenous antioxidants; these substances will inhibit lipid peroxidation reactions due to free radicals and neutralize free radicals in the body (Anggraeni, 2018).

The mechanism of flavonoids' action in inhibiting lipid peroxidation reactions is by donating one of their hydrogen atoms. Flavonoids can give an H+ (Hydrogen) atom from the OH- (Hydroxyl) group to free radicals and produce flavonoid phenoxyl radicals. This flavonoid phenoxyl radical will bind again with free radicals and then form a second phenoxyl radical. This second flavonoid phenoxyl radical is a compound with conjugated double bonds. It can balance the structure of its compounds by delocalizing electrons and providing benefits by eliminating the effects of free radicals in the body (Pratama, 2016). The effect of lost free radicals will also eliminate oxidative stress due to Borax administration so that submucosal edema and mucosal necrosis do not occur in the duodenum.

The duodenal submucosal edema test obtained a significance value of 0.171 from the Kruskal-Wallis test. In the Kruskal-Wallis test, damage to the duodenal mucosal epithelium had a significance value of 0.234. The value of these two significances is greater than α (0.05).

Conclusion

Statistically, it can be concluded that honey administration does not affect the histopathological appearance of the Wistar rat duodenum as an inhibitor of the toxic effects of borax (Sodium tetraborate).

References

Amalia, R., Wurlina., & Hestianah, E. P. 2017. Efek Pemberian Vitamin C Dan Vitamin E Terhadap Gambaran Histopatologi Duodenum Mencit Yang Dipapar Boraks. *Jurnal Veterina Medika*, 10(1): 23 – 30.

Anggraeni, D. 2018. Pengaruh Terapi Preventif Madu Hutan Sumbawa terhadap Kadar Malondialdehida (MDA) dan Histopatologi Duodenum Tikus (Rattus novergicus) yang Diinduksi Plumbum Asetat. *Skripsi*. FKH UB. Program Studi Kedokteran Hewan.

BPOM RI. 2021. *Intensifikasi Pengawasan Pangan Selama Bulan Ramadhan Hingga Menjelang Idul Fitri*. Sistem Keamanan Pangan Terpadu oleh Badan Pengawas Obat dan Makanan RI.

Cahyani, DI. 2017. Pengaruh Penambahan Teh Hijau terhadap Aktivitas Antioksidan dan Kadar Protein Minuman Fungsional Susu Kedelai dan Madu. *Skripsi*. FK Diponegoro. Program Studi Ilmu Gizi.

Elziyad, MT. dan Widjaja, NMR. dan Plumeriastuti, H. 2013. Pengaruh Boraks terhadap Gambaran Histopatologi Duodenum Tikus Putih (Rattus novergicus). *Julnal Veterina Medika,* 6(2):133 – 138.

Fatahilah, M. 2016. Klinik Pengobatan Thibbun Nabawi di Kota Pontianak. *Jurnal Online Mahasiswa,* 4(2):108-118.

Pratama, A. Y. 2016. Pengaruh Terapi Preventif Madu Hutan Riau terhadap Kadar MDA dan Kadar ALP pada Serum Hewan Model Tikus yang Diinduksi CCL4. *Skripsi*. FKH UB. Program Studi Kedokteran Hewan.

Pujaswarini, N. M. H., Berata, K., & Setiasih, N. L. E. 2019. Ekstrak Daun Kelor Memulihkan Perubahan Histopatologi dan Morfometri Duodenum Tikus Setelah Aktivitas Fisik Berlebih. *Jurnal Indonesia Medicus Veterinus,* 8(6): 739 – 749.

Purnama, M. T. E., Widjaja, N. M. R., & Plumeriatusi, H. 2013. Pengaruh Boraks terhadap Gambaran Histopatologi Duodenum Tikus Putih (Rattus novergicus). *Skripsi*. FKH UNAIR. Program Studi S1 Pendidikan Dokter Hewan.

Sirait, R.C., Tjahjono, K., & Setyawati, A. N. 2016. Pengaruh Pemberian Ekstrak Jintan Hitam (Nigella sativa) terhadap Kadar MDA Serum tikus Sparague Dawley Setelah Diberikan Paparan Asap Rokok. *Jurnal Kedokteran Diponegoro,* 5(4): 1603 – 1612.