Electronic Journal of Education, Social Economics and Technology
Vol. 5, No. 2, (2024), pp. 203-207
ISSN 2723-6250 (online)
DOI: https://doi.org/10.33122/ejeset.v5i2.180

SAINTIS publishing

Research Article

Toxicity Test of the Combination of N-Hexane Extract of Black Pepper *(Piper nigrum L.)* and Yogurt on the Digestive Organs of Mice *(Mus musculus)*

Nurul Inayah, Enni Mutiati*, Halimatus Sa'diyah

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Annuqayah, Sumenep, Jawa Timur, Indonesia
*Corresponding Author: enni.mutiati@gmail.com | Phone Number: +6287846813777

ABSTRACT

Black pepper (Piper nigrum L) is a spice that is widely used in cooking by people in Indonesia, earning the nickname "King of Spice". The chemical content in Black Pepper (Piper nigrum L) is piperine. Piperine can activate Transient Receptor Potential Vanilloid Type 1 (TRPV1), causing heartburn, heat and increased stomach acid. Therefore, researchers combined black pepper (Piper ningrum L.) with yogurt, because it is known that yogurt can suppress stomach acid and the growth of bad bacteria in the intestines. The aim of this research was to determine the potential toxicity of the combination of black pepper extract (Piper ningrum L.) and yogurt on the digestive organs and the duration of death of mice (Mus musculus). In research that has been carried out using RAL with 5 treatments and 4 replications. The samples used were female mice (Mus musculus). Next, descriptive analysis was carried out. The results of the research that has been carried out show that the concentration of 25 mg/kg bw in the combination of Black Pepper (Piper ningrum L.) extract and yogurt on the anatomy of the stomach and intestines is closer to the positive control (P1) and the combination of Black Pepper (Piper ningrum L.) extract and yogurt with a concentration of 75 mg/kg bw on stomach and intestinal anatomy was closer to the negative control (P0).

Keywords: Toxicity; Black Pepper; Yogurt; Digestive Organs

1. INTRODUCTION

Black pepper (*Piper will be removed* L.) used as a spice and traditional medicine by the Indonesian people(Jihadi, Duck, 2016) . Black pepper (*Piper will be removed* L.) is a type of spice that gets the nickname King Of Spice, because it is a world trade item (Hammouti, dkk:2019). Contents in Black Pepper (*Piper will be removed* L.) among them are piperin, egg whites, saponins, flavonoids, essential oils, cavisin, resins, amylum, piperiline, piperoleine, dihydrocarveol, canyo-fillene oxide, caryotone, poperanine, piperonal, tran piocarrol, and pepper oil (Hermit, 2016). Piperin acts as an antioxidant which is a spicy substance from black pepper (*Piper will be removed* L.) ((Hammouti, dkk:2019). The spicy substance piperine activates the Transient Receptor Potential Vanilloid Type 1 (TRPV1), causing heartburn, heat, and increased stomach acid (Namara, 2005). Increased stomach acid causes gastritis. Gastritis is a health inflammation caused by irritation and infection of the gastric mucosa and submucosa (Srinivasan; dkk,2007). Therefore, the researchers combined black pepper (*Piper will be removed* L.) yogurt, because it is known that yogurt can suppress stomach acid and the growth of bad bacteria in the intestines. In addition, the combination will increase the antioxidant action in black pepper (*Piper will be removed* L) (Shori, 2022). Therefore, it is necessary that this research be carried out so that it can be used as a solution for the community to suppress stomach acid (Banerjee,2017) and treat diarrhea and intestinal infections. The purpose of this study is to determine the impact of the use of a combination of black pepper extract (*Piper nigrum* L) and yogurt against the digestive organs.

2. RESEARCH METHOD

The research that has been conducted uses a Complete Random Design (RAL) with five treatments and four replicates. The research design is as follows:

Table 1. Research design

Black Pepper Extract Concentration	U1	U2	U3	U4
Distilled Water, 0.5 ml (P0)				
Yogurt, 0.5 ml (P1)				
Black Pepper Extract 25 mg/kg BW + Y (P2)				
Black Pepper Extract 50 mg/kg BW + Y (P3)				
Black Pepper Extract 75 mg/kg BW + Y (P4)				

Notes:

LH: Black Pepper Extract

Y: Yogurt

The variables in this study include: Independent variables, namely Black Pepper extract (*Piper Ningrum* L.) and Yogurt. Bound variables, namely the digestive organs, especially the stomach and small intestine and the duration of death in mice (*Mus Musculus*). The control variable is female mice (*Mus Musculus*). The tools used in this study are a 1 ml gastric sounder, a maintenance cage, a balance, a Bunchner funnel, a rotary evaporator vacuum trap, filter paper, binocular microscope, a bottle, a sexy board, a dissecting set, a droppipette, a glass funnel, a hot plate, a glass mixer, an erlenmeyer 500ml, a 100 ml conducer jar, a 10 ml conducer cup, a 50 ml glass container, and a 500 ml glass beaker. The materials used are female mice of the Balb-C strain, pellets, water, Black Pepper (Piper nigrum L), yogurt, N-hexane, NaCl 0.9%, chloroform, cotton, and aquades water.

Research Procedure

Preparation for Treatment on Trial Animals

This study used female mice of the Balb-C strain with a body weight of ±25 grams and a ±3-month age. The animals were acclimatized for 2 weeks at room temperature 20-25oC, after that, the animals were given Black Pepper extract (Piper nigrum L) and yogurt orally once a day for 14 days with a dose of 0.5 ml aquades as a control (P0), yogurt (P1), 25 mg/kg BB combined black pepper extract and yogurt (P3), 50 mg/kg BB combined black pepper extract and yogurt (P4), 75 mg/kg BB combination of black pepper extract and yogurt (P5). On the 15th day, the animals were anesthetized, dissected, and their stomachs and intestines were observed. The rest of the experimental animals were given the usual treatment until the 28th day to test for subacute toxicity and dissected on the 29th day.

Preparation of Black Pepper Extract *(Piper nigrum L.)*

Black Pepper Powder (Piper nigrum L) is stratified using N-hexane. The resulting maserat is evaporated using a rotary evaporator to obtain a viscous extract.

Giving Treatment

Black Pepper Extract (Piper nigrum L) is administered to fertile female mice orally for 14 and 28 days according to the prescribed dosage and dissolved with a 0.5 ml yogurt solution.

Observation of Experimental Animals

The animal was observed and the day of its death was written. After surgery, damage was observed in the test animal in the stomach and small intestine. Toxicity behavior in the digestive organs was analyzed descriptively.

3. RESULTS AND DISCUSSION

Based on the results of the study for 14 days, there was no abnormal behavior and the feces in mice (*Mus musculus*) were not diluted (P3, P4, P5). The behavior in mice (*Mus musculus*) treatment (P3, P4, P5) was the same as the behavior in mice (*Mus musculus*) negative (P0) and positive (P1) controls, and there was no swelling in the stomach and intestines. This showed that the combination of Black Pepper extract (*Piper ningrum* L.) and yogurt did not cause acute toxicity to the stomach and small intestine in mice (*Mus musculus*), (See in **Figure 1**).

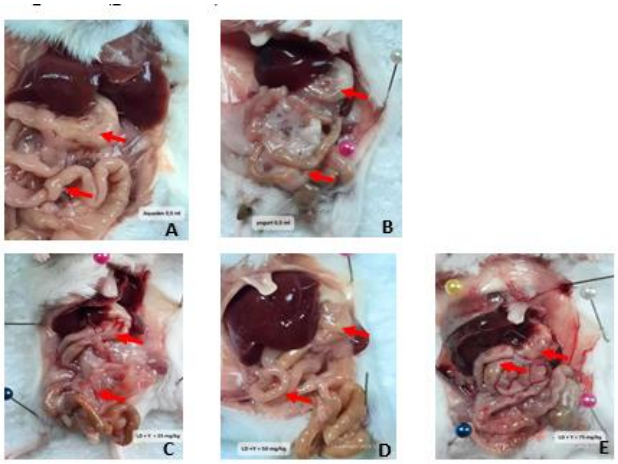

Figure 1. Digestive organs of mice A: Aquades 0.5 ml (P0), B: Yogurt 0.5 (P1), C: LH 25 mg/kg BW + Y (P6), D: lh 50 mg/kg BW + Y (P7), E: LH 75 mg/kg BW + Y (P8).

Based on the results of the study conducted for 28 days, it was shown that there was no difference in behavior in mice (*Mus musculus*) paractuan, mice (*Mus musculus*) negative control (P0), and mice (*Mus Musculus*) positive (P1) and there were no mice (*Mus musculus*) who experienced diarrhea. In addition, there is also no swelling in the stomach and intestines. The anatomical conditions of the stomach and intestines in mice (*Mus musculus*) given a combination of Black Pepper extract (*Piper nigrum* L) and yogurt (P3) were the same as the conditions of the stomach and intestines in mice (*Mus musculus*) given yogurt (P1), but in P4 and P5 the anatomy of the stomach and intestines was closer to the anatomy of the stomach and intestines at P0 (figure 2).

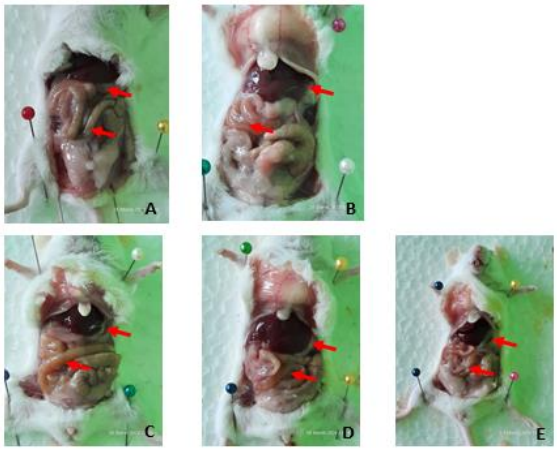

Figure 2. Digestive organs of mice A: Aquades 0.5 ml (P0), B: Yogurt 0.5 (P1), C: LH 25 mg/kg BW + Y (P6), D: lh 50 mg/kg BW + Y (P7), E: LH 75 mg/kg BW + Y (P8).

Bacteria asam lactate (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) ferments processed milk into yogurt which is a food technology product. The content of protein, calcium, phosphorus, and other nutrients is also found in yogurt. Yogurt is generally good for health (Banerjee,2017), especially to neutralize the spicy taste in the stomach and prevent the growth of pathogenic bacteria in the digestive tract (Kurniati,2018;Dempsey, 2022; Nematollahi, 2020). In addition, yogurt also has more protein content than milk. This is due to the expansion of proteins from the combination of microbes and protein content from organisms. Yogurt contains probiotics, especially good microbes that help overcome gastrointestinal infections, so it can help the digestive system to function more smoothly (Puringing, 2022) . In addition, probiotics can also reduce intestinal barrier dysfunction caused by cytokines. Pretreatment of intestinal epithelium with S. thermophilus and L. acidophilus or with the commensal bacterium Bacteroides thetaiotaomicron has also been shown to prevent decreased resistance due to transepithelial electrical activity induced by TNF-α and IFN-γ (Aleman, 2023). Yogurt also has a low antioxidant content, but the antioxidants in yogurt will increase significantly when combined with natural ingredients that contain high antioxidants, such as Black Pepper (Piper nigrum L) (Shori, 2021). Yogurt also contains lactic acid bacteria that are able to kill microbes and other bacteria (Hendarto,2021; Okfrianti,1018). Lactic acid will not stimulate excess stomach acid production and is able to reduce stomach irritation (Andriani,2010).

4. CONCLUSION

In this study, it can be concluded that: The combination of Black Pepper extract (*Piper nigrum* L) and yogurt does not cause acute toxicity to the stomach and small intestine in mice (*Mus musculus*), so that there is no death until the 14th day. The combination of Black Pepper extract (*Piper nigrum* L) and yogurt did not cause subacute toxicity to the stomach and small intestine in mice (*Mus musculus*), so there was no death until the 28th day. In this study, the researcher suggested that the researcher further conduct research on the identification of the balance of intestinal microbiota given Black Pepper (*Piper nigrum* L) extract and Yogurt.

ACKNOWLEDGEMENTS

The Directorate of Research, Technology and Community Service of the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia has provided funding in the 2024 fiscal year, so that research, scientific publications, and the creation of research posters are carried out as expected. LPPM Annuqayah University who has facilitated our research, so that it can be completed as expected. All parties involved for their participation in completing this research.

REFERENCES

Ghaidaa Jihadi Mohammed, Aseel Mohammad Omran HMH. (2016). Antibacterial and Phytochemical Analysis of Piper nigrum using Gas Chromatography – Mass Spectrum and Fourier-Transform Infrared Spectroscopy. Int J Pharmacogn Phytochem Res [Internet]. 8(6):977–966. *Available from: https://www.researchgate.net/publication/304945495_Antibacterial_and_phytochemical_analysis_of_piper_nigrum_using_gas_chromatography_-_mass_spectrum_and_fourier-transform_infrared_spectroscopy*

B. Hammouti , M. Dahmani 1, A. Yahyi1, A. Ettouhami1, M. Messali, A. Asehraou, A. Bouyanzer 1 IW and RT. (2019). Black Pepper, the "King of Spices": *Chemical composition to applications. Arab J Chem Environ Res.6(1):12–56.*

Putu Ermi Hikmawanti N, Aulia C, Putri Viransa Department of Pharmacy V, Pharmacy and Science F, Muhammadiyah Hamka U. (2016). Piperine Content in Piperine Black and White Pepper Fruits (Piper nigrum L.) Extracted with Ethanol Concentration Variations Using KLT-Densitometry Method The Content of Piperine Black and White Pepper Fruits (*Piper nigrum* L.) *Extracted with V. Media Farm. 13(2):173–85.*

McNamara FN, Randall A, Gunthorpe MJ. (2005). Effects of piperine, the pungent component of black pepper, at the human vanilloid receptor (TRPV1). *Br J Pharmaco; 144(6):781–90.*

Srinivasan K. (2007). Black pepper and its pungent principle-piperine: A review of diverse physiological effects. *Crit Rev Food Sci Nutr. 47(8):735–48.*

Shori AB, Aljohani GS, Al-zahrani AJ, Al-sulbi OS, Baba AS. (2022). Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic Lactobacillus spp. *Lwt [Internet].153(September 2021):112482. Available from: https://doi.org/10.1016/j.lwt.2021.112482*

Ujjwainee Banerjee, Rakibul Malida, Roshni Panda, Tanima Halder GR. (2017). Variety of Yogurt and ITS Health Aspects a Brief Riview. Int J Innov Pract Appl Res [Internet]. 7(7):56–66. *Available from: https://www.researchgate.net/publication/321028178_variety_of_yogurt_and_its_health_aspects_-_a_brief_review*

Kurniati A, Anugrah A D, Fauz iah A N, Saputri R, Zulfah S. (2018). Community Service: Making Yoghurt with Simple Technology in the East Jakarta Community. *Proabdimas. 1(x):1–2.*

Dempsey E, Corr SC. (2022). Lactobacillus spp. for Gastrointestinal Health: Current and Future Perspectives. *Front Immunol. 13(March 2020):1–15.*

B NMM a, C AMM, B MA a, Nematollahi A. (2020) Probiotic viability in yoghurt: *A review of influential factors. Probiotic Viability Yoghurt Int Dairy J.*

Purwantiningsih TI, Bria MAB, Kia KW. (2022). Levels Protein and Fat of Yoghurt Made of Different Types and Number of Cultures. *J Trop Anim Sci Technol. 4(1):66–73.*

Aleman RS, Moncada M, Aryana KJ. (2023). Leaky Gut and the Ingredients That Help Treat It: A Review. *Molecules. 28(2):1–34.*

Hendarto, David Richard, Arita Putri Handayani, Elisa Esterelita YAH. (2021). Biochemical Mechanism and Optimization of Lactobacillus bulgaricus and Streptococcus thermophilus in Quality Yoghurt Processing. *J Basic Science [Internet]; 8(1):13–9. Available from: https://www.researchgate.net/publication/359175382_Mekanisme_Biokimiawi_dan_Optimalisasi_Lactobacillus_bul*

garicus_dan_Streptococcus_thermophilus_dalam_Pengolahan_Yoghurt_yang_Berkualitas

Okfrianti Y, Bengkulu P, Agustine, Levinna JJ. (2018). Total Identification of Lactic Acid Bacteria (BAL) in Yoghurt with Sucrose and Skim Milk Variations. *J World Nutrition. 1(2).*

Andriani, M., & Lia UK. (2010). The *Characteristic Study in Physic, Chemical, and Organoleptic of Yoghurt With Sweet Potato Extract (*Ipomoea batatas L). *Biomediaca; 3:23–32.*