International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Check for updates

Histopathological overview of mice duodenum (*Mus musculus*) due to carbofuran exposure

Reza Mahendra Yudha Permana, Eka Pramyrtha Hestianah, Widjiati, Widjiati, Maslichah Mafruchati, Budiarto Budiarto and Epy Muhammad Luqman *

Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

International Journal of Life Science Research Archive, 2024, 06(01), 041–046

Publication history: Received on 14 December 2023; revised on 01 February 2024; accepted on 03 February 2024

Article DOI: https://doi.org/10.53771/ijlsra.2024.6.1.0029

Abstract

The aim of this study was to explore small bowel tissue injury through duodenal histopathology of mice (*Mus musculus*) exposed to carbofuran. The measured parameter was duodenal epithelial integrity. The experiment involved thirty female mice, divided into four treatment groups: P0 (control) was not exposed to carbofuran but substituted with 0.9% NaCl; P1 was exposed to 0.0104 mg/kg Body Weight of carbofuran; P2 was exposed to 0.0208 mg/kg Body Weight of carbofuran; P3 was exposed to 0.0407 mg/kg Body Weight of carbofuran. The experimental design was a completely randomized design with four treatments and five replications. Data were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney test if there were significant differences among treatment groups. The results suggested that carbofuran exposure caused desquamation and epithelial erosion, with the effective dose for duodenal histopathology change being 0.208 mg/kg Body Weight.

Keywords: Duodenum; Carbofuran; Mice; Epithelial Integrity; Pesticide Stress

1. Introduction

Pesticides widely used in agriculture to increase fruit, vegetable, and grain yields have been found to contain pesticide residues [1]. Commonly used carbamate pesticides in agriculture include carbofuran, aldicarb, and carbaryl [2]. Carbofuran, frequently used in agriculture, has positive impacts on agricultural development but leaves residues on livestock and crops [4]. Residues of pesticides in food can harm non-target organisms [5]. Carbofuran has a wide spectrum and is highly toxic to mammals [6]. High mammalian toxicity occurs through oral and inhalation exposure [7].

Oral carbofuran exposure can trigger the formation of Reactive Oxygen Species (ROS) [8]. ROS formation leads to oxidative stress, resulting in tissue damage and cell death [9]. Insecticides that enter the body orally undergo a digestive process similar to food. In the digestive tract, the small intestine is responsible for digesting food and water entering the body. Additionally, the intestine can be a primary portal for the entry of unwanted substances into the body, such as drugs and pesticides [10]. The duodenum is the most proximal part of the small intestine that first contacts chyme for absorption and distribution throughout the body, transporting chyme into the liver for detoxification via the portal vein [11].

Oral exposure to insecticides can cause gastrointestinal hypermotility and malabsorption [12]. Malabsorption and gastrointestinal hypermotility lead to poor digestion and disruption of the protective barrier of the gastrointestinal tract [13], resulting in mucosal layer irritation due to epithelial cell shedding [14].

* Corresponding author: Epy Muhammad Luqman, Email: epy-m-l@fkh.unair.ac.id

This research serves as an initial study on the dangers of carbofuran to the gastrointestinal tract. This preliminary study aims to provide information about the dangers of carbofuran and can be continued by future researchers as an effort to minimize pesticide poisoning.

2. Materials and Methods

2.1. Materials

The materials used in this study were Balb/c mice, carbofuran (Furadan® 3 GR - PT Bina Guna Kimia - MDL MFCD00041819), complete chicken feed CP 593 (PT. Charoen Pokphpand Indonesia), PDAM drinking water, chloroform, 70% alcohol, 0.9% physiological NaCl, 10% formalin, and cotton. The research instruments used in this study were mice cages, mice feeders and waterers, disposable syringes (3 ml and 10 ml), a light microscope, a stomach tube, surgical equipment (forceps, scalpel, surgical scissors), gloves, masks, anesthesia jars, test tubes, test tube racks, test tube stoppers, Petri dishes, alcohol spray, analytical scales, label paper, blotting paper, and medicine cups.

2.2. Methods

2.2.1. Dose Determination

This study used an LD50 dose of carbofuran of 0.5 mg/kg BW in mice [15]. The doses used in the study were 0.0417 mg/kg BW, 0.0208 mg/kg BW, and 0.0104 mg/kg BW [16]. The Furadan used in this study contained 3% carbofuran.

2.2.2. Animal Preparation

The mice (*Mus musculus*) were acclimated for seven days to reduce stress and adapt to the environment. The mice were placed in plastic cages and provided with ad libitum food and water. Subsequently, they were randomly selected and divided into four treatment groups (P0, P1, P2, P3), each with five replicates.

2.2.3. Treatment

Female mice (*Mus musculus*) that had been acclimated and randomly selected using simple random sampling were divided into four treatment groups and exposed to carbofuran. The control group (P0) was not exposed to carbofuran and was substituted with 0.9% physiological NaCl, while group P1 was exposed to a dose of 0.0104 mg/kg BW of carbofuran, group P2 was exposed to a dose of 0.0208 mg/kg BW of carbofuran, and group P3 was exposed to a dose of 0.0417 mg/kg BW of carbofuran. Oral carbofuran exposure was administered for 10 days [16].

2.2.4. Histological Preparation

After 10 days of carbofuran administration, euthanasia was performed on the 12th day by placing the mice in an anesthesia jar containing chloroform. Subsequently, a surgical procedure was performed to remove the duodenum. The removed duodenum was placed in a medicine cup containing 10% formalin, and histological preparations were made using Hematoxylin and Eosin (HE) staining.

2.2.5. Histological Preparations

Microscopic observation of mice duodenal histological preparations was performed using a light microscope at 400x magnification, with observations made in five different fields of view for each mice duodenal histological preparation. The level of histopathological damage to duodenal epithelium was assessed using a scoring system.

2.2.6. Observed Variables

Histological preparations of the duodenum from each mice were made by taking sections from the beginning, middle, and end of the duodenum. The observed variable was the histopathological appearance of the duodenum, which included epithelial integrity, with a score assigned to each field of view in each histological preparation [17]. Each field of view was observed for changes five times and averaged. The average was then calculated for each group based on the number of samples.

Table 1 Histopathological assessment of duodenal epithelium using Barthel Manja's mucosal integrity score [17]

Score	Mucosal Epithelial Integrity
0	No pathological changes
1	Desquamation of epithelium
2	Surface epithelial erosion (gap 1-10 epithelial cells/lesion)
3	Epithelial ulceration (gap > 10 epithelial cells/lesion)

2.2.7. Data Analysis

A Completely Randomized Design (CRD) was used in this experiment. Data analysis was performed using statistical tests, with the Kruskal-Wallis test conducted using the Statistical Programs for Social Scientific (SPSS) software. If significant differences were found among treatment groups, the Mann-Whitney test was used for comparisons.

3. Results and Discussion

Based on the research, various treatments were applied, including the control group (P0), which was not exposed to carbofuran but was substituted with 0.9% NaCl, and three carbofuran-exposed groups: P1 (0.0104 mg/kg BW), P2 (0.0208 mg/kg BW), and P3 (0.0417 mg/kg BW). The NaCl and carbofuran exposure were given orally for 10 days to study the histopathological appearance of the duodenum in female mice (*Mus musculus*).

Based on the calculation with the assessment of duodenal histopathological appearance using the Barthel Manja's mucosal integrity scoring method, all groups were tested using the Kruskal-Wallis test in SPSS. If the results showed significant differences ($p < 0.05$), the Mann-Whitney test was performed to compare between treatment groups.

Table 2 Duodenal Epithelial Integrity Assessment Due to Carbofuran Administration for 10 Days.

Group	**Mean ± SD**
P0: control	$1.26^{a} \pm 0.176$
P1: carbofuran administration 0.0104 mg/kg BW	$1.30^{a} \pm 0.196$
P2: carbofuran administration 0.0208 mg/kg BW	$2.10^{b} \pm 0.203$
P3: carbofuran administration 0.0417 mg/kg BW	$2.23^{b} \pm 0.436$

Note: The same superscript in different rows in the same column indicates no significant difference ($p < 0.05$).

Based on the Kruskal-Wallis test results, significant differences were found ($p < 0.05$). Subsequently, further analysis was conducted to compare between treatment groups using the Mann-Whitney test. When comparing the same treatment duration, the control group for 10 days showed no significant difference with the group receiving 0.0104 mg/kg BW of carbofuran. However, when compared with the group receiving 0.0208 mg/kg BW and 0.0417 mg/kg BW of carbofuran, significant differences were observed. Comparing groups with the same treatment duration, specifically the 0.0104 mg/kg BW carbofuran treatment with the 0.0208 mg/kg BW and 0.0417 mg/kg BW carbofuran treatments, significant differences were observed. In contrast, there was no significant difference when comparing the group receiving 0.0208 mg/kg BW of carbofuran with the group receiving 0.0417 mg/kg BW of carbofuran. This suggests that the dose affects the histopathological appearance of the duodenum in female mice (*Mus musculus*).

One way to determine the effect of a substance on the gastrointestinal tract is to examine histopathological changes. Based on the results of this research, it is known that the active ingredient in Furadan® 3GR, carbofuran, affects the histopathological appearance of the duodenum. Carbofuran has high toxicity to mammals when orally exposed and inhibits cholinesterase enzymes, inhibiting the hydrolysis of acetylcholine into choline and acetic acid, causing organophosphorus poisoning symptoms. Excessive accumulation of acetylcholine leads to increased gut motility, resulting in clinical manifestations of diarrhea [18].

Oral carbofuran exposure can lead to increased Reactive Oxygen Species (ROS). Increased ROS can occur because carbofuran binds to mitochondria, disrupting mitochondrial function. This disruption leads to ATP depletion and

increased production of reactive oxygen species. Carbofuran can damage the unsaturated fatty acid structure in cell membranes, causing cell walls to become brittle, ultimately resulting in cell death.

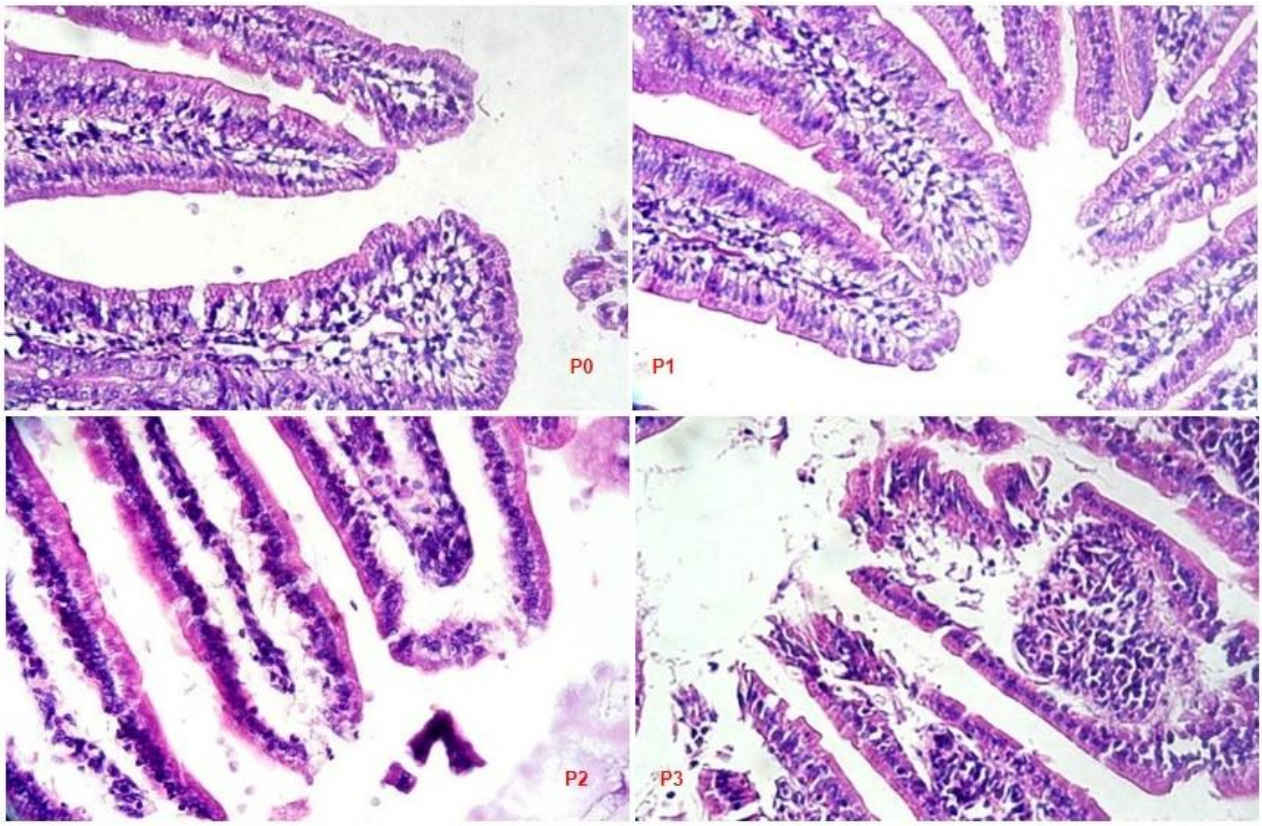

Figure 1 Histopathological appearance of mice duodenum exposed to carbofuran (400x magnification). P0: control shows no pathological changes; P1: Treatment 1 shows epithelial desquamation; P2: Treatment 2 shows surface epithelial erosion; P3: Treatment 3 shows epithelial ulceration. P0: control without carbofuran exposure for 10 days, P1: carbofuran administration 0.0104 mg/kg BW for 10 days, P2: carbofuran administration 0.0208 mg/kg BW for 10 days, P3: carbofuran administration 0.0417 mg/kg BW for 10 days.

Based on the research conducted, it is evident that carbofuran can cause changes in duodenal epithelial integrity. The analysis results indicate significant differences between the control group and the carbofuran-treated group. The control group, when compared to the carbofuran treatment at 0.0208 mg/kg BW and 0.0417 mg/kg BW, showed no significant difference. The histopathological changes observed in the duodenum include epithelial desquamation, surface epithelial erosion, and epithelial ulceration, which are believed to be caused by carbofuran [19].

Carbofuran exposure can lead to the shedding of surface epithelial cells and a reduction in mucus secretion, which is a protective barrier against acid attacks. This is related to the stimulation of the autonomic nervous system, the parasympathetic system, which leads to increased gastric acid secretion [20]. High acidity can suppress mucus production, reducing mucosal resistance and making it easier for surface epithelial cells to become damaged [21].

4. Conclusion

The administration of carbofuran in mice at doses of 0.0208 mg/kg BW and 0.0417 mg/kg BW can lead to histopathological changes characterized by epithelial desquamation and erosion of the epithelial surface. The exposure dosage of carbofuran influences the histopathological changes in the duodenum of mice, and the dose of 0.208 mg/kg BW is an effective dose that can induce histopathological changes in the duodenum, including epithelial desquamation and erosion of the epithelial surface.

Compliance with ethical standards

Acknowledgements

The authors express sincere thanks to the Director of Postgraduate Studies at Universitas Airlangga and the Dean of the Faculty of Veterinary Medicine for providing all necessary facilities and funds for conducting research work.

Disclosure of Conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to the animal handling to ensure no discomfort or pain was caused to the animals during sampling(certificate registration number: 2012/112-KE).

References

[1] Khatun P, Islam A, Sachi S, Islam MZ, Islam P. Pesticides in vegetable production in Bangladesh: A systemic review of contamination levels and associated health risks in the last decade. Toxicology Reports. 2023; 11: 199-211. doi: 10.1016/j.toxrep.2023.09.003

[2] Mdeni NL, Adeniji AO, Okoh AI, Okoh OO. Analytical Evaluation of Carbamate and Organophosphate Pesticides in Human and Environmental Matrices: A Review. Molecules. 2022; 27(3): 618-625. doi: 10.3390/molecules27030618

[3] Tuhumury GNC, Leatemia JA, Rumthe RY, Hasinu JV. Pesticide Residues from Fresh Vegetable Products in Ambon City. Journal of Plant Cultivation. 2012; 12(1): 1-8. doi:10.30598/a.v1i2.284

[4] Lv X, Chang Q, Li H, Liang S, Zhe Z, Shen S, Pang G. Risk assessment of carbofuran residues in fruits and vegetables at the Chinese market: A 7-year survey. Ecotoxicology and Environmental Safety. 2022; 239: 113667. doi: 10.1016/j.ecoenv.2022.113667

[5] Nofyan E, Lamin S, Patriot IT, Kanedi M. Physiological Effects of Carbofuran on Earthworm Pheretima javanica Gates. Advances in Life Sciences. 2017; 7(2): 21-25. doi: 10.5923/j.als.20170702.02

[6] Alfiansyah H, Ardikoesoema N, Samuel J. Potential for environmental degradation due to the existence of carbofuran in Indonesia. Journal of Forestry and Environmental Business. 2023; 1(1): 66-87.

[7] Gammon DW, Liu Z, Becker JM. Carbofuran occupational dermal toxicity, exposure and risk assessment. Pest Manag Sci. 2012; 68(3): 362–370. doi: 10.1002/ps.2270

[8] Jaiswal SK, Sharma A, Gupta VK, Singh RK, Sharma B. Curcumin Mediated Attenuation of Carbofuran Induced Oxidative Stress in Rat Brain. Biochem Res Int. 2016; 2016: 7637931. doi: 10.1155/2016/7637931

[9] Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F, Peng ZY. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxid Med Cell Longev. 2019; 2019: 5080843. doi: 10.1155/2019/5080843

[10] Lin JH, Chiba M, Baillie TA. Is the Role of the Small Intestine in First-Pass Metabolism Overemphasized?. The American Society for Pharmacology and Experimental Therapeutics. 1999; 51 (2): 135-154.

[11] Thie J. Touch for Health, Practical Instructions for Natural Health with Acupressure Touch. Grasindo. Jakarta. 2007. 100.

[12] Vismaya, Rajini PS. Oral exposure to the organophosphorus insecticide, Monocrotophos induces intestinal dysfunction in rats. Food Chem Toxicol. 2014:71:236-43. doi: 10.1016/j.fct.2014.05.030.

[13] Owens SR, Greenson JK. The pathology of malabsorption: current concepts. Histopathology. 2007; 50: 64–82. doi: 10.1111/j.1365-2559.2006.02547.x

[14] Kusuma AH. Effect of Oral Administration of Gradual Doses of Propoxur on the Histological Picture of the Duodenum of Balb/c Mice. Medical School. Diponegoro University. 2006

[15] Gammon DW, Liu Z, Becker JM. Carbofuran occupational dermal toxicity, exposure and risk assessment. Pest Manag Sci. 2012; 68(3): 362–370. doi: 10.1002/ps.2270

[16] Luqman EM. Effect of Exposure to the Insecticide Carbofuran on ROS Activity, Expression of p53 and Caspase 3 and Cell Death of Embryonic Cerebral Cortex Neurons in Mice (*Mus musculus*). [Doctoral Dissertation]. Medical School. Airlangga University. 2013.

[17] Underwood JCE. General and Systematic Pathology. 6th ed. Churchill Livingstone: Elsevier; 2013.

[18] Corwin EJ. Pathophysiology Handbook. EGC. Jakarta. 2009; 3: 227-265

[19] Mishra S, Zhang W, Lin Z, Pang S, Huang Y, Bhatt P, Chen S. Carbofuran toxicity and its microbial degradation in contaminated environments. Chemosphere . 2020:259:127419. doi: 10.1016/j.chemosphere.2020.127419

[20] Browning KN, Travagli RA. Central Nervous System Control of Gastrointestinal Motility and Secretion and Modulation of Gastrointestinal Functions. Compr Physiol. 2014; 4(4): 1339–1368. doi: 10.1002/cphy.c130055

[21] Liao YSJ, Kuan SP, Guevara MV, Collins EN, Atanasova KR, Dadural JS, Vogt K, Schurmann V, Bravo L, Eken E, Sponchiado M, Reznikov LR. Acid exposure disrupts mucus secretion and impairs mucociliary transport in neonatal piglet airways. Am J Physiol Lung Cell Mol Physiol. 2020; 318(5): L873–L887. doi: 10.1152/ajplung.00025.2020