Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

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The effect of carbofuran administration on protoplasmic astrocyte cell necrosis in the cerebrum of mice (*Mus musculus*)

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Magna Scientia Advanced Biology and Pharmacy, 2024, 11(01), 039–043

Publication history: Received on 13 December 2023; revised on 26 January 2024; accepted on 29 January 2024

Article DOI: https://doi.org/10.30574/msabp.2024.11.1.0011

Abstract

The aim of this study was to investigate the exposure of the insecticide carbofuran on the necrosis of protoplasmic astrocyte cells in the cerebrum of mice (*Mus musculus*). Twenty mice were divided into four groups with five replicates each. The control group (P0) received 0.5% physiological NaCl, P1 (carbofuran 0.0833 mg/kg BW), P2 (carbofuran 0.0417 mg/kg BW), P3 (carbofuran 0.0208 mg/kg BW), and carbofuran was administered for ten days. On the 12th day, brain necropsy and HE staining were performed to observe the necrosis of protoplasmic astrocyte cells in the mice cerebrum. The necrosis of protoplasmic astrocyte cells was analyzed using ANOVA followed by Duncan's test. The results showed that carbofuran caused necrosis of protoplasmic astrocyte cells ($p < 0.05$), and the necrosis increased with increasing carbofuran dosage ($p < 0.05$).

Keywords: Carbofuran; mice; Protoplasmic astrocytes; Necrosisl; Pesticide stress

1. Introduction

In an effort to improve the quality and productivity of agricultural products, the use of pesticides to control crop pests is often unavoidable. Excessive and uncontrolled pesticide use often poses a risk of poisoning. Examination of cholinesterase and hemoglobin levels in the blood of horticultural farmers in the Tejosari Village, Ngablak District, Magelang Regency, Indonesia, showed a pesticide poisoning rate of 96.2% [1]. Duck deaths occurred in Indramayu, Indonesia, after being grazed in rice fields, and carbofuran insecticide was found in their livers and rice field water [2]. The excessive and continuous use of pesticides can lead to various consequences, including pesticide residue accumulation in agricultural products, environmental pollution in agriculture, reduced productivity, and human poisoning with adverse health effects [3].

Oral administration of carbofuran has been shown to stimulate reactive oxygen species (ROS) in the brains of rats. Administration of a dose of 1 mg/kg of carbofuran for 28 days orally can increase malondialdehyde (MDA) levels [4]. Subacute intraperitoneal carbofuran administration has been shown to increase oxidative stress in the brain with increasing doses. Increased oxidative stress can decrease the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the brain [5]. Decreased catalase activity in response to carbofuran induction can reduce protection against free radicals. A decrease in the stimulant activity of SOD and catalase leads to the vulnerability of the brain to oxidative stress induced by carbofuran [6].

ROS also causes DNA damage by altering the deoxyribose structure into peroxyl radicals and other carbonyl products. This condition leads to DNA strand breaks that result in cell mutations or death [7]. Changes in oxidative stress and mitochondrial function affect cognitive and motor functions due to carbofuran contamination. Carbofuran has been

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shown to alter the balance of pro-oxidants and antioxidants in the brain and increase oxidative stress [8]. ROS formed in astrocyte cells will damage the astrocyte cell membrane, as the cell membrane is rich in polyunsaturated fatty acids (PUFA) that are easily damaged by free radicals [9]. The damaged cell membrane can lead to the death of protoplasmic astrocyte cells. Therefore, research on the effects of carbofuran insecticide on the necrosis of protoplasmic astrocyte cells in the cerebrum of mice (*Mus musculus*) is needed.

2. Material and methods

This research was conducted in the Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

2.1. Materials

The materials used for this study were Balb/C mice, carbofuran insecticide (Furadan 3GR, MDL MFCD00041819), complete chicken feed CP 593 (PT. Charoen Pokhphand Indonesia), tap water, ether/chloroform, distilled water, 70% alcohol, physiological NaCl, formalin 10%, and cotton. The instruments used in this study included mice cages, syringes, sondes, scalpels, forceps, scissors, mice feeding and drinking containers, pipettes, Erlenmeyer flasks, reaction tubes, gloves, and masks. The experimental animals in this study were female Balb/C mice aged 10 weeks with a body weight ranging from 25-30 grams, obtained from the Pusat Veterinaria Farma (PUSVETMA) in Surabaya, Indonesia. This study used 20 mice selected randomly and divided them into four treatment groups with five replicates each.

2.2. Determination of Dosage

This study used the LD_{50} approach (the dose that can kill 50% of the test animals) for carbofuran between 1-2.5 mg/kg in rats [10]. The Furadan used in the study contained 3% active carbofuran. Based on this dosage, a reduction in dosage was performed that would not cause mice deaths but could cause organ damage. The LD_{50} value obtained was 0.5 mg/kg BW. The doses administered were doses that did not cause death in the test animals but could cause organ damage, resulting in doses of 1/24 LD_{50} (0.0208 mg/kg BW), 1/12 LD_{50} (0.0417 mg/kg BW), and 1/6 LD_{50} (0.0833 mg/kg BW).

2.3. Method

Twenty Balb/C mice were divided into four treatment groups (P0, P1, P2, and P3) with five replicates each. The control group (P0) received physiological NaCl. P1 received a dose of 1/6 LD_{50} carbofuran, P2 received a dose of 1/12 LD_{50} carbofuran, and P3 received a dose of 1/24 LD_{50} carbofuran. Carbofuran was administered orally using a sonde. Carbofuran was given for 10 days, and on the 12th day, brain necropsy and HE staining were performed to observe the necrosis of protoplasmic astrocyte cells in the mice cerebrum.

2.4. Histopathological Examination

Histological observation of mice brain was performed using a microscope at magnifications of 400 and 1000 times, with five different fields of view observed. Each histological preparation was assessed for the degree of histopathological damage to protoplasmic astrocyte cells in the cerebrum. Changes observed included changes in protoplasmic astrocyte cells that experienced necrosis (pyknosis, karyorrhexis, and karyolysis). Protoplasmic astrocyte cells that underwent necrosis had a darker nucleus (pyknosis), a ruptured nucleus of protoplasmic astrocyte cells (karyorrhexis), the nucleus of protoplasmic astrocyte cells was no longer visible (karyolysis), and they appeared wrinkled and homogenous compared to normal astrocyte cells.

2.5. Data Analysis

This study used a Completely Randomized Design (CRD), and the necrosis of protoplasmic astrocyte cells was analyzed using ANOVA followed by Duncan's test with a significance level of 5%.

3. Results and discussion

Histopathological images of the mice cerebrum (*Mus musculus*) showed necrosis in protoplasmic astrocyte cells in all groups exposed to carbofuran (P1, P2, and P3), while the control group (P0) showed no necrosis. Protoplasmic astrocyte cells that underwent necrosis had a darker nucleus (pyknosis), a ruptured nucleus of protoplasmic astrocyte cells (karyorrhexis), the nucleus of protoplasmic astrocyte cells was no longer visible (karyolysis), and they appeared wrinkled and homogenous compared to normal astrocyte cells (Figure 1).

Analysis using ANOVA and Duncan's distance test showed a significant difference between groups ($p<0.05$). All groups exposed to carbofuran showed necrosis of protoplasmic astrocyte cells, with higher doses of carbofuran resulting in greater and significant necrosis among groups (P1, P2, and P3) (Table 1).

Table 1 Protoplasmic astrocyte cell necrosis after exposure to carbofuran for 10 days

Group	Necrosis Score (Mean±SD)
Control (P0)	0
P1 1/6 LD_{50} (0.0833 mg/kg BW) carbofuran	$10.44^{c} \pm 0.17$
P2 1/12 LD_{50} (0.0417 mg/kg BW) carbofuran	$5.28^{b} \pm 0.68$
P3 1/24 LD_{50} (0.0208 mg/kg BW) carbofuran	$3.68^{a} \pm 0.19$

Note: Superscripts with the same letter indicate no significant difference ($p > 0.05$).

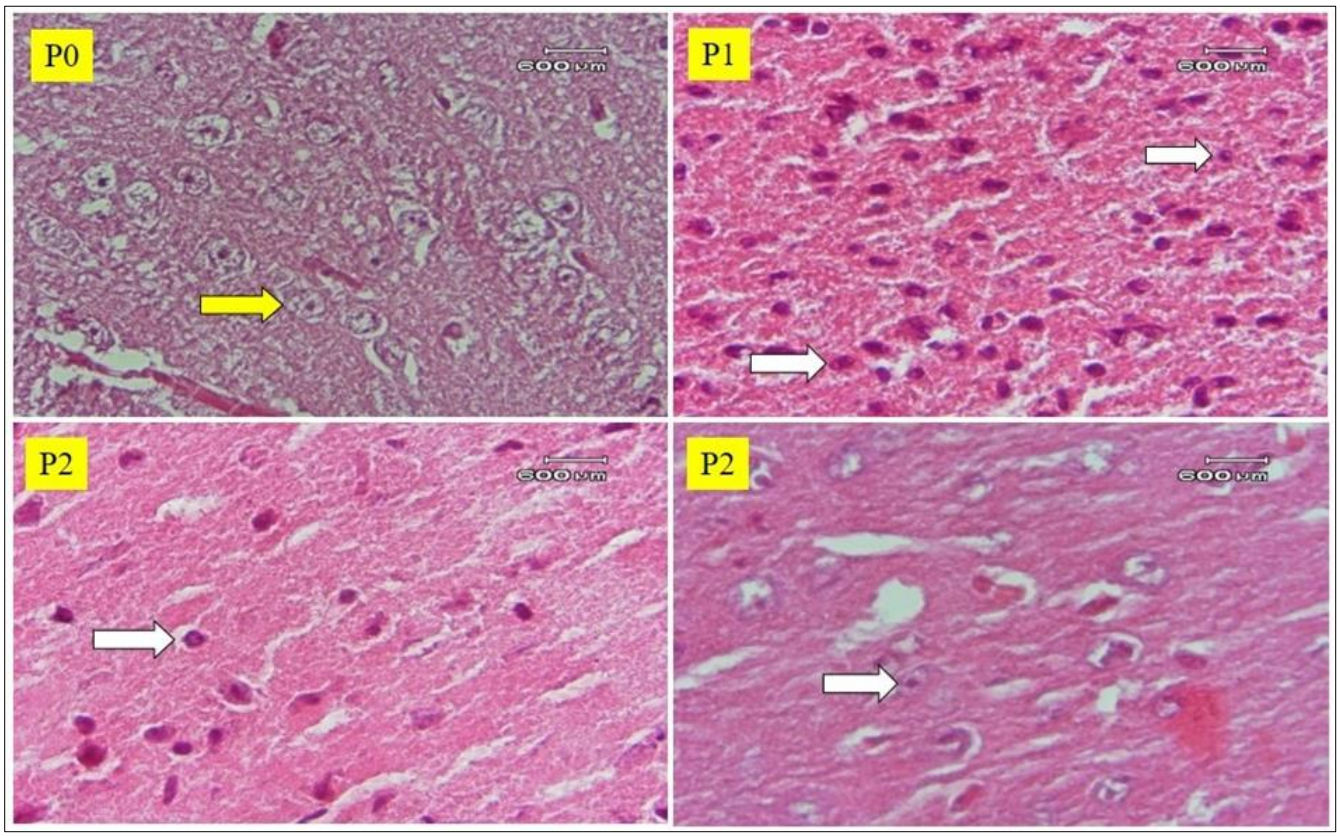

Figure 1 Histopathology of mice brain after exposure to carbofuran for 10 days. H.E staining; 1000x magnification; Olympus CX-41 microscope. Yellow arrows indicate normal cells and white arrows indicate cells experiencing necrosis. The control group (P0) was given 0.5 physiological NaCl, P1 (carbofuran 0.0833mg/kg BW), P2 (carbofuran 0.0417 mg/kg BW), P3 (carbofuran 0.0208 mg/kg BW)

Carbofuran works by inhibiting the activity of acetylcholinesterase enzymes in the nervous system of insect pests. The accumulation of acetylcholine neurotransmitters at nerve synapses disrupts nerve communication and leads to paralysis and death in target insects [11]. However, when carbofuran exposes non-target organisms such as humans or other organisms, it can cause acute poisoning. Oral administration of carbofuran can stimulate ROS. ROS activity is required in the phagocytosis process in the immune system. Excessive ROS can be harmful to the body and can trigger the formation of highly reactive hydroxyl radicals (OH°). Hydroxyl radicals are one of the most dangerous free radicals for the body and can damage DNA, proteins, and unsaturated fatty acids that are essential components of cell membranes [12].

The attack of hydroxyl radicals on cell membranes can result in lipid peroxidation, leading to the breakage of fatty acid chains into various toxic compounds for cells, such as malondialdehyde (MDA) and various hydrocarbons that can cause

severe and harmful damage to cell membranes [13]. Protoplasmic astrocyte cell necrosis is caused by significant physical or chemical damage to the cells. Necrosis is characterized by changes in cell nucleus morphology, such as pyknosis, karyorrhexis, and karyolysis. Pyknosis is the process of condensation and solidification of the cell nucleus, making it appear denser and darker with H.E staining, and the cell nucleus appears more compact and dark. Karyorrhexis is characterized by a shattered nucleus that forms scattered chromatin fragments, while karyolysis is characterized by a disappearing cell nucleus [14].

Astrocytes are a type of neuroglial cell that functions as support for the central nervous system, especially in maintaining ion and nutrient homeostasis and providing structural support for nerve cells [15]. In this study, all groups exposed to carbofuran showed necrosis of protoplasmic astrocyte cells, with higher doses of carbofuran resulting in greater and significant necrosis among groups (Table 1). Protoplasmic astrocyte cells that underwent necrosis had a darker nucleus (pyknosis), a ruptured nucleus of protoplasmic astrocyte cells (karyorrhexis), the nucleus of protoplasmic astrocyte cells was no longer visible (karyolysis), and they appeared wrinkled and homogenous compared to normal astrocyte cells (Figure 1).

Higher doses of carbofuran can cause an increase in the death of protoplasmic astrocyte cells because higher carbofuran doses result in greater damage. The increase in protoplasmic astrocyte cell necrosis with increasing carbofuran dosage is a common principle in toxicology known as "dose-response" [16]. Higher doses of carbofuran can increase the death of protoplasmic astrocyte cells because higher doses can cause significant physical or chemical damage to the cells. Higher doses can result in more severe damage, including to protoplasmic astrocyte cells [17].

High doses of carbofuran can also disrupt the metabolism of cells, including protoplasmic astrocytes. This can disrupt the normal function of these cells and cause cell death [19]. High doses of carbofuran can damage cellular structures, including cell membranes and organelles. This can inhibit the normal function of cells and lead to necrosis or cell death [20]. The impact of carbofuran (a toxic substance) on cells depends on the type of toxic substance, its dosage, duration of exposure, and the type of cells involved [18]. Each toxic substance can have different mechanisms of action and different effects on body cells [21]. Therefore, an increase in the dosage of a toxic substance can increase the risk of cell damage and cell death. It is also important to note that high doses of toxic substances are often more dangerous and potentially fatal. Exposure to toxic substances should be avoided as much as possible, and the use of toxic chemicals should be done in accordance with applicable safety guidelines and regulations.

4. Conclusion

Carbofuran doses of 1/6 LD_{50} (0.0833 mg/kg BW), 1/12 LD_{50} (0.0417 mg/kg BW), and 1/24 LD_{50} (0.0208 mg/kg BW) can cause necrosis of protoplasmic astrocyte cells in the mice cerebrum (*Mus musculus*). Increased necrosis of protoplasmic astrocyte cells along with increasing carbofuran doses.

Compliance with ethical standards

Acknowledgments

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).

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