GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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The influence of carbofuran insecticide exposure on the number of ovarian follicles in mice (*Mus musculus*)

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GSC Biological and Pharmaceutical Sciences, 2024, 26(01), 125–129

Publication history: Received on 27 November 2023; revised on 06 January 2024; accepted on 08 January 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.26.1.0004

Abstract

Objective: The aim of this research was to investigate the histopathological damage to the ovaries of mice (*Mus musculus*) caused by exposure to carbofuran.

Method: Twenty female mice aged 10 weeks with a body weight ranging from 25-30 mg were used in this study. The mice were divided into four groups, with each group consisting of five replicates. The control group (P0) received 0.5% physiological NaCl, while the treatment groups (P1, P2, and P3) were exposed to carbofuran at doses of 0.0833 mg/kg BW, 0.0417 mg/kg BW, and 0.0208 mg/kg BW, respectively, for ten days. On the 12th day, ovarian necropsy and HE staining were performed to observe the number of primary, secondary, tertiary, and Graafian follicles. Data analysis was conducted using ANOVA and BNJ tests.

Result: The results of this research showed that oral administration of carbofuran leads to a decrease in the number of secondary, tertiary, and Graafian follicles. High doses, such as 0.0833 mg/kg body weight, resulted in a more pronounced reduction in the number of secondary, tertiary, and Graafian follicles compared to the control group.

Conclusion: A reduction in the number of secondary, tertiary, and Graafian follicles with increasing doses of carbofuran.

Keywords: Carbofuran; Mice; Ovarian; Follicles; Pesticide stress

1. Introduction

Synthetic pesticides have become a problem in Indonesia, particularly due to their excessive use in agriculture, leading to acute and chronic poisoning in both animals and humans [1]. Carbamate insecticides commonly used in agriculture include carbofuran, aldicarb, and carbaryl. Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is a broad-spectrum insecticide used for pest control in agriculture [2]. Carbamates, generally used as insecticides, nematicides, and acaricides, are often found in soil, air, food, and water. Carbofuran is highly toxic to humans and wildlife through oral and inhalation exposure routes [3]. Repeated consumption of carbofuran-contaminated food over an extended period is a concern due to potential teratogenic, neurotoxic, and reproductive effects [4].

Carbofuran was highly toxic to mammals and has been reported to cause embryotoxic and teratogenic effects [5]. Other effects of carbofuran exposure include adrenal and reproductive system damage in Passer domesticus birds. In female animals exposed to carbofuran, it can disrupt the reproductive system [6]. Carbofuran could inhibit the action of acetylcholinesterase (ChE) enzymes, leading to the accumulation of acetylcholine (Ach) and resulting in poisoning symptoms [7]. Accumulated unhydrolyzed acetylcholine causes vasoconstriction in blood vessels, affecting the supply

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of nutrients to cells and tissues [8]. This also impacts the ovaries, reducing nutrient supply, and subsequently inhibiting oogenesis [9].

Other insecticides, such as organophosphates, have been shown to cause ovarian degeneration, leading to increased follicular atresia due to hormonal imbalances. One such imbalance occurs in reproductive hormones LH and FSH [10]. Organophosphate insecticides have been reported to decrease ovarian weight, reduce the number of various ovarian follicles in rats, and increase follicular atresia [11]. They have also been found to reduce the number of primary, secondary, and tertiary follicles in rats [12]. Information on the effects of carbofuran on ovarian reproductive organs is limited. Therefore, research is needed to elucidate the influence of carbofuran on ovarian follicle development.

2. Material and methods

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

The materials used in this study included 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. Instruments used included mouse cages, syringes, sondes, scalpels, forceps, scissors, mouse feeding and drinking containers, pipettes, Erlenmeyer flasks, reaction tubes, gloves, and masks. 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, were used as experimental animals. A total of 20 mice were randomly selected and divided into four treatment groups, each with five replicates.

2.1. Determination of Dosage

The LD_{50} approach (the dose that can kill 50% of the test animals) for carbofuran in rats ranged between 1-2.5 mg/kg [13]. The Furadan used in the study contained 3% active carbofuran. Based on this, lower doses were administered that would not cause mouse deaths but could induce organ damage. The LD50 value obtained was 0.5 mg/kg BW, 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).

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. The mice were exposed to carbofuran for 10 days, and on the 12^{th} day, ovarian necropsy and HE staining were performed to observe the number of primary, secondary, tertiary, and Graafian follicles.

2.2. Observed Variables

Histological observations of mouse ovaries were conducted using an Olympus® CX-41 microscope with a 100X magnification on five fields of view for each ovary. The number of ovarian follicles in mice was counted and averaged for each observed field of view. The variables observed included the number of primary, secondary, tertiary, and Graafian follicles.

2.3. Data Analysis

Data analysis was performed using Analysis of Variance (ANOVA) with the Statistical Programs for Social Scientific (SPSS) software. If significant differences were found at a significance level of 5%, a Bonferroni test (BNJ) was conducted to determine the honest significant difference.

3. Results

Observations of the number of primary, secondary, tertiary, and Graafian follicles in the ovaries of Balb/C mice (*Mus musculus*) were performed microscopically using H.E. staining. Evaluation was carried out for each mouse ovary, and the number of primary, secondary, tertiary, and Graafian follicles was counted using an Olympus® CX-41 microscope with a 100X magnification. Statistical data analysis for primary, secondary, and Graafian follicles was conducted using ANOVA, while for tertiary follicles, the Kruskal-Wallis test was used due to the non-normal distribution of the data.

Table 1 Number of Primary, Secondary, Tertiary, and Graafian Follicles in the Ovaries of Mice Exposed to Carbofuran for 10 Days

Group	Number of Follicles			
	Primary (Mean±SD)	**Secondary (Mean±SD)**	**Tertiary (Mean±SD)**	**de Graafian (Mean±SD)**
P0	$20.88^{b} \pm 0.96$	$11.48^{c} \pm 0.17$	$0.64^{b} \pm 0.76$	$4.36^{c} \pm 0.35$
P1	$23.28^{b} \pm 0.52$	$8.52^{b} \pm 1.70$	$0.00^{a} \pm 0.00$	$1.60^{b} \pm 0.48$
P2	$21.68^{b} \pm 1.99$	$6.76^{ab} \pm 0.77$	$0.08^{a} \pm 0.17$	$0.84^{a} \pm 0.16$
P3	$16.84^{b} \pm 1.83$	$6.24^{a} \pm 1.34$	$0.28^{b} \pm 0.62$	$2.08^{b} \pm 0.52$

Note: Different superscripts in the same column indicate significant differences (P<0.05). The control group (P0) was given 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).

From this study, it is evident that groups P0 (control), P1 (carbofuran 0.083 mg/kg BW), P2 (carbofuran 0.0417 mg/kg BW), and P3 (carbofuran 0.0208 mg/kg BW) showed no significant difference in the number of primary follicles between them. However, there were significant differences observed for secondary, tertiary, and Graafian follicles. This indicates a decrease in the number of secondary, tertiary, and Graafian follicles with increasing doses of carbofuran.

The results of the Kruskal-Wallis test showed a significant difference in the number of tertiary follicles ($p<0.05$), followed by the Mann-Whitney test. The Mann-Whitney test results comparing the groups showed no significant differences ($p>0.05$) between P0 and P2, P0 and P3, P1 and P2, P1 and P3, and P2 and P3.

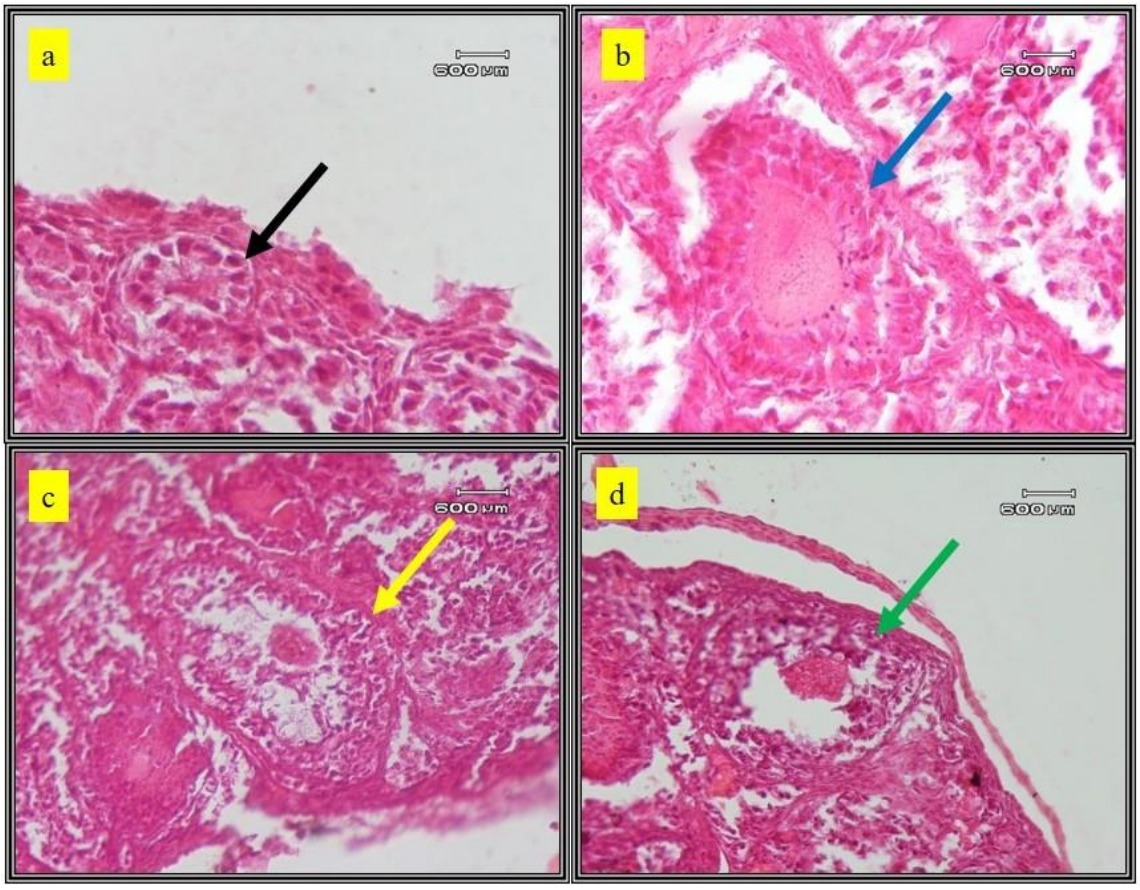

Figure 1 Histopathology of Mouse Ovaries After 10 Days of Carbofuran Exposure, Primary Follicles (a), Secondary Follicles (b), Tertiary Follicles (c), Graafian Follicles (d) in the Ovary of Group P0. (H.E. Staining; 400x Magnification; Olympus CX-41 Microscope)

4. Discussion

The results of this study demonstrate that oral administration of carbofuran in mice leads to a decrease in the number of secondary, tertiary, and Graafian follicles in the ovaries of Balb/C mice (*Mus musculus*). High doses, such as 0.0833 mg/kg body weight, resulted in a more pronounced reduction in the number of secondary, tertiary, and Graafian follicles compared to the control group. Prolonged exposure to high doses of carbofuran can affect ovarian function directly, impacting the hypothalamus-pituitary-ovary system [14]. This disruption interferes with the response generated by the ovaries through hormonal feedback and stops precisely at the release of FSH and LH, which regulate the ovaries to produce essential steroid hormones for follicle growth [15].

This disruption leads to a decrease in the number of secondary, tertiary, and Graafian follicles compared to the control group. Specifically, a higher reduction in the number of Graafian follicles occurred, as the growth of Graafian follicles is directly influenced by the anterior pituitary's functioning [16]. The impaired functioning of the anterior pituitary due to carbofuran intoxication can inhibit the release of FSH, which is essential for follicle growth, resulting in a decreased number of Graafian follicles.

The reduction in the number of follicles, especially Graafian follicles, in carbofuran exposure is caused by the mechanism of carbofuran that can inhibit the action of acetylcholinesterase (AChE) [17]. This leads to disturbances in the pituitary and gonadotropin, affecting ovarian function directly through the effects of AChE on the pituitary, disrupting the balance of steroid hormones and inhibiting follicle growth. Similar results were obtained by Baligar and Kaliwal, showing growth disturbances in the ovaries, follicles, and estrus cycle due to impaired AChE enzyme activity in the pituitary of rats [18]. The reduction in the number of follicles can occur due to the imbalance of essential steroid hormones for normal ovarian function [19]. This hormonal imbalance can disrupt gonadotropin secretion, affecting the central nervous system, as observed in rats exposed to carbofuran [14, 20].

5. Conclusion

The administration of carbofuran insecticide can lead to a decrease in the number of secondary, tertiary, and Graafian follicles in the ovaries of mice (*Mus musculus*). This decrease is correlated with an increase in the dose of carbofuran exposure.

Compliance with ethical standards

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: 2013/110-KE).

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