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Histopathological liver of one day old and two-week-old chicks exposed to carbofuran during embryonic period

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Abstract

Objective: This study aims to determine the effect of carbofuran exposure during the embryonic period on the histopathological images of the liver of one day old and two-week-old chicks after they hatch.

Method: 60 embryonated chicken eggs from broiler parent stock with an average weight of 62.064 mg were divided into two observation groups: one day old chicks and two-week-old chicks after they hatched. Carbofuran was dissolved in aquabidest and then was injected into the egg yolk at a dose of 0.0106 mg/0.1 ml/egg (P1) and 0.0127 mg/0.1 ml/egg (P2). The control group (P0) was injected with 0.1 ml of aquabidest. All eggs were incubated in an electric incubator until they hatched. The livers of one day and two-week-old chicks were collected for HE staining. The variables observed were the level of liver cell necrosis in the central vein and Kiernan's triangle. Data were analyzed using Kruskal Wallis and it continued with multiple comparison tests.

Results: The results showed that the carbofuran group of one day old chicks treated with at a dose of 0.0106 mg/0.1 ml/egg had a significant effect on necrosis of liver cells in the central vein and in Kiernan's triangle. Moreover,the carbofuran group of two week old chicks treated at a dose of 0.0127 mg/0.1 ml/egg (P2) and 0.0106 mg/0.1 ml/egg (P1) had a significant effect compared to the P0 group ($p<0.05$).

Conclusion Carbofuran insecticide at a dose of 0.0127 mg/0.1 ml/egg was proven to cause the necrosis of liver cells in the central vein and in Kiernan triangle of one day and two-week-old chicks when they were exposed to carbofuran during the embryonic period.

Keywords: Carbofuran; Liver; Embryonic Period; Chick; Pesticide stress

1. Introduction

Increasing agricultural and plantation production such as controlling plant pests, weeds or nuisance plants which can cause a decrease in the quantity and quality of production is currently being actively carried out. Pesticides that enter the agricultural ecosystem, have not only positive impacts, but also negative impacts. The negative impacts come from the toxic nature of pesticides and the lack of knowledge of farmers in using them. Pesticides can also cause environmental pollution which includes contamination of surface water, ground water and air. What is more worrying is the killing of insect predators and organisms not targeted by pesticides and the formation of residues in the environment which have fatal consequences for the survival of creatures which live in polluted areas [1].

Carbamate groups such as carbaryl and carbofuran are widely used in agriculture because their toxic effects are lower than other groups [2]. Carbofuran is very toxic to birds and chickens, and a single granule of carbofuran can kill a small bird [3]. Carbofuran contamination has been found in abundance death of falcons and the residue on the carcass and decreased activity of Choline Esterase (ChE) in the falcons' brain that died eight hours after consuming feed contained carbofuran [4].

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Carbofuran has a working mechanism to inhibit ChE activity in the nervous system of humans, vertebrates and insects [5]. ChE is an enzyme that plays a role in the hydrolysis neurotransmitter of acethylcholine into choline and acetic acid and is involved in the regulatory mechanism of cell proliferation and differentiation [6]. The liver is an organ that is sensitive to the influence of substances with sufficient amount of chemical such as insecticides [7]. Due to its vulnerability as its position in the circulation of body fluids, the liver can easily communicate via the portal vein with substances absorbed from the stomach and intestines. Exposure to lindane in experimental animals such as rats, mice and dogs resulted in damage to the liver, kidneys and nervous system [8]. The liver damage is in line with necrosis (cell death) and fatty liver (accumulation of triglyceride) in the liver cells [9].

If toxic substances are carried in the bloodstream, the liver cells around the central vein are the first to experience necrosis. If the dose of the toxic substance is increased, necrosis will occur evenly in the liver lobules, and it also occurs around Kiernan's triangle [10]. Due to carbofuran residue in egg yolk, it is likely that liver abnormalities will occur in the one day old chicks and these abnormalities will continue in subsequent development (in two weeks old chicks). Therefore, it is necessary to conduct research on the effect of carbofuran exposure at the embryonic period which can result in the death of liver cells around the central vein and Kiernan's triangle of one day old chicks and two week old chick

2. Materials and Method

2.1. Materials

Materials: embryonated chicken eggs produced by Multibreeder Adirama Indonesia Farm Unit 4 Songsong Singosari Village Malang Indonesia, Furadan 3G produced by PT Parama Bina Tani with the active ingredient of 3% carbofuran in granular form and purple in color, aquabidest, anesthetic ether, 70% alcohol as disinfectant, 10% formalin, and paraffin.

2.2. Tools

The tools used in this research: hatching machine, egg candler, scales, disposable syringe of 10 ml and 1 ml, test tube, spatula, scissors, tweezers, scalpel, petridish, electric drill with 1 mm drill bit, chicken coop, heating lamp, microscope and ointment pot as a place for sample collection.

2.3. Preliminary Research

To determine the teratogenic dose in this chicken breed, an approach of LD_{50} in the chicken of 25 mg/kg BW and in the metabolic and pharmacokinetic properties of carbofuran in the chicken mother was conducted so that the results obtained could show the real dose in environmental conditions. The total concentration of carbofuran that was able be found in the mother's body was 91.8% [11], so the potential for carbofuran to form residues in egg yolk was 8.2%. The teratogenic dose given was based on these fractions which do not kill chicken embryos and have the potential to cause teratogenic effect.

Furadan 3G used in the research contained the active ingredient of 3% carbofuran, the potential for carbofuran to form residues in yolk was 8.2%, the weight of the eggs used was 62.064 g and the LD_{50} in chickens was 25 mg/kg BW so that doses were obtained from the calculations to have teratogenic potential as shown in table 1.

Table 1 Doses of carbofuran exposure based on LD_{50} fractions of yolk from embryonated chicken egg

Fractions of LD_{50}	Carbofuran injected into yolk (mg/egg)	Furadan 3G injected into yolk (mg/egg)
½ LD_{50}	0.0636	2.1197
¼ LD_{50}	0.0318	1.0599
1/6 LD_{50}	0.0212	0.7066
1/8 LD_{50}	0.0159	0.5299
1/10 LD_{50}	0.0127	0.4241
1/12 LD_{50}	0.0106	0.3534

The dose fractions used in the primary research were dose fractions that had a survival rate of more than 50% after 10 days of incubation [12].

2.4. Primary Research

This research was a completely randomized design with 3 treatments, each treatment had 10 replications. The control (P0) was injected with 0.1 ml aquabidest, the P1 group was injected with carbofuran at a dose of 0.0106 mg/0.1 ml/egg and the P2 group was injected with carbofuran with a dose of 0.0127 mg/0.1 ml/egg.

Embryonated eggs and incubators were disinfected using 70% alcohol spray, then they were labeled according to group using a pencil. The egg was drilled in the blunt part using a drill with a diameter of 1 mm, then an injection was made into the hole using a 1 ml disposable syringe with a 23 G needle. The injection was carried out into the yolk with a volume of 0.1 ml per egg. Control group was injected with Aquabidest without using carbofuran. The injected eggs were covered with paraffin and taped. The treated eggs next were placed in an incubator with a temperature of 38^0C and humidity of 60-80%. During the incubation process until hatching, the stability of the incubator temperature was observed and the eggs were rotated from day 3 to day 18 three times a day. Liver collection was carried out after eggs hatched at the age of one day and two weeks and histological preparations were made with Hematoxyline Eosin (HE) staining.

2.5. Observed variables

The variables observed were the necrosis level of liver cells in the central vein and Kiernan's triangle of one day old and two week old chicks exposed to carbofuran during the embryonic period. Observations were carried out under a microscope with 100X and 400X magnification in 3 fields of view, then the assessement was made based on the necrosis level of liver cells (table 2). The assessment results of the observation score were taken in the form of the mean of the liver histopathological score when observation on necrosis of liver cells in the central vein and Kiernan's triangle was conducted.

Table 2 Histopathological scores of liver

Score	The necrosis level of liver cells
0	The necrosis of liver cells does not occur
1	The necrosis of liver cells reaches < 25 % of view field
2	The necrosis of liver cells between 25-50 % of view field
3	The necrosis of liver cells between 50-75 % of view field
4	The necrosis of liver cells reaches >75 % of view field

2.6. Data Analyses

Data analyses were carried out using Kruskal Wallis nonparametric analysis. If there were differences between treatments, it was proceeded with a multiple comparison test.

3. Results

3.1. Preliminary Research

After the fraction of the LD_{50} carbofuran dose was degraded until it had the equivalent exposure of Furadan 3G to embryonated chicken eggs, the research obtained fractions of 1/10 and 1/12 LD_{50} which had a survival rate of more than 50% after 10 days of incubation in a hatching machine.

Table 3 Doses of Furadan 3G dan survival rate after 10 days of incubation

Fractions of LD_{50}	Carbofuran injected into yolk (mg/egg)	Furadan 3G injected into yolk (mg/egg)	Survival rate after 10 days of incubation (%)
½	0.0636	2.1197	0
¼	0.0318	1.0599	0
1/6	0.0212	0.7066	0
1/8	0.0159	0.5299	30
1/10	0.0127	0.4241	100
1/12	0.0106	0.3534	100

Thus, the dose of furadan 3G used as the dose in the primary research was 1/10 and 1/12 LD_{50} of 0.4241 mg/egg and 0.3534 mg/egg respectively which was equivalent to a dose of carbofuran of 0.0127 mg/0.1 ml/egg and 0.0106 mg /0.1 ml/egg.

3.2. Primary Research

After the necrosis of liver cells in the central vein from one day and two week old chicks was observed, the results were obtained as shown in table 4.

Table 4 Results of observation on necrosis level from liver cells obtained from one day and two week old chicks based on central vein observation

Group	P0	P1	P2
	Mean ± SD		
One day old chicks	11.75 ± 6.06^{b}	12.1 ± 8.26^{b}	22.25 ± 6.12^{a}
Two-week-old chicks	8.85 ± 4.59^{c}	14.4 ± 8.11^{b}	23.25 ± 4.43^{a}

Notes: The same superscript letters indicate that they are not significantly different (p>0.05), P0= injected with 0.1 ml of sterile aquabidest solution, P1= injected with carbofuran at a dose of 1/12 LD_{50} (0.0106 mg/egg), P2= injected with carbofuran at a dose of 1 /10 LD_{50} (0.0127 mg/egg).

The results of the Kruskal Wallis analysis indicated that the calculated H value = 7.92 was greater than the H table value (0.05) = 5.99, so the null hypothesis (H0) was rejected, which means there were significant differences between the groups. To determine the differences between groups, a multiple comparison analysis (Z test) was carried out. The calculated Z value was 1.77 and after the test on each group was carried out, it indicated that the difference in the mean of P2 with P0 and P2 with P1 was greater than the calculated Z value of 1.77. , while the mean of P0 and P1 was less than the calculated Z value of 1.77. Thus, from the results of this analysis, it is known that P2 was significantly different from P0 and P1, while P0 was not significantly different from P1. The results of the Kruskal Wallis analysis showed that the calculated H value = 15.197 was greater than the H table value (0.05) = 5.99, so the null hypothesis (H0) was rejected, which means there were significant differences between the groups. To determine differences between groups, multiple comparison analysis (Z test) was carried out. The calculated Z value was obtained at 1.74 and after test was carried out at each group, the difference in the mean of P2 and P1, P2 and P0 groups was obtained with the mean of P1 and P0 was greater than the calculated Z value of 1.74. Thus, from the results of this analysis it indicated that there were significant differences between groups.

Table 5 Results of observation on necrosis level from liver cells obtained from one day and two-week-old chicks based on observation in Kiernan triangle

Group	P0	P1	P2
	Mean ± SD		
One day old chicks	12.25 ± 5.75^{b}	11.85 ± 8.56^{b}	21 ± 4.47^{a}
Two-week-old chicks	8.7 ± 4.64^{c}	14.7 ± 7.70^{b}	22.65 ± 4.56^{a}

Notes: The same superscript letters indicate that they are not significantly different (p>0.05), P0= injected with 0.1 ml of sterile aquabidest solution, P1= injected with carbofuran at a dose of 1/12 LD_{50} (0.0106 mg/egg), P2= injected with carbofuran at a dose of 1 /10 LD_{50} (0.0127 mg/egg).

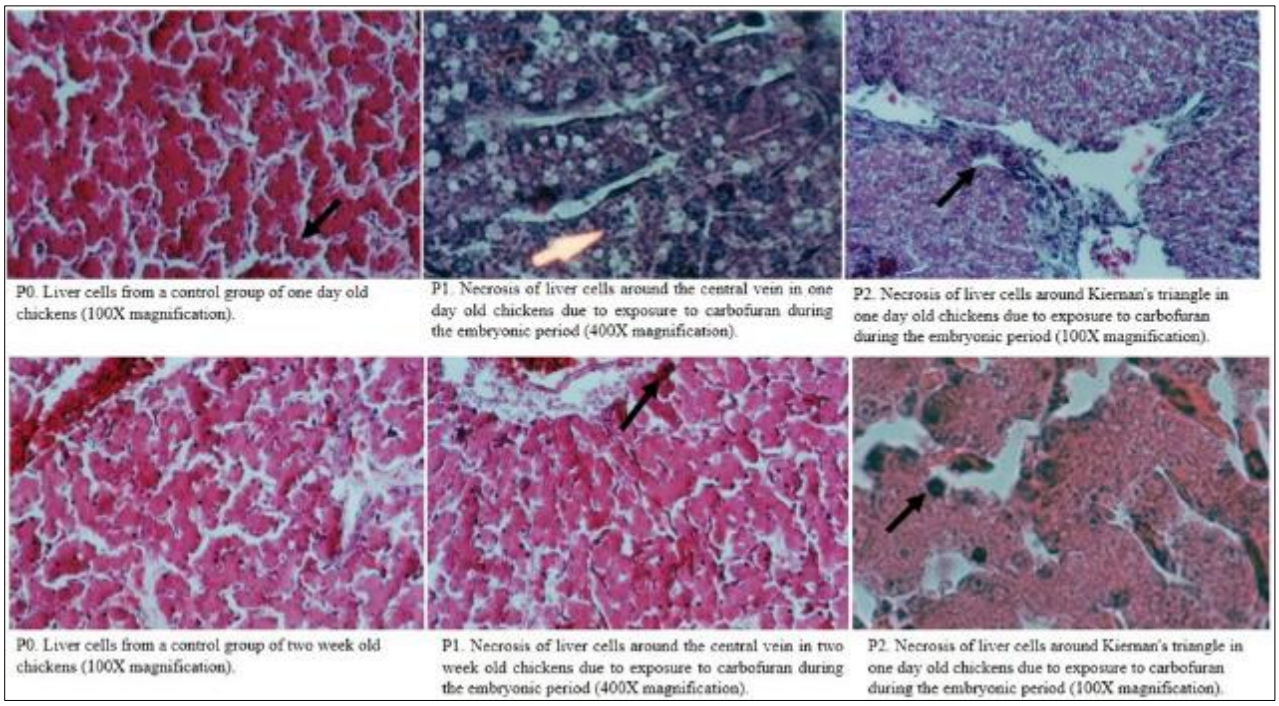

Figure 1 Necrosis of liver cells in central vein and in Kiernan triangle of one day old and two-week-old chicks exposed to carbofuran during embryonal period (magnification 100X)

After observation on the necrosis level of the liver cells in the Kiernan triangle from one day old chicks was carried out, the results were obtained as shown in table 5. The results of the Kruskal Wallis analysis indicated the calculated H value = 4.33 was greater than the H table value (0.05) = 5.99, so the null hypothesis was (H0) was rejected, which means there were significant differences between the groups. To determine differences between groups, multiple comparison analysis (Z test) was carried out. The calculated Z value was 1.69 and after each group was tested, it indicated that there was a difference in the mean of P2 with P0 and that of P2 with P1 which was greater than the Z calculated value of 1.69, while the mean of P0 and P1 was less than the Z calculated value of 1.69. Thus, from the results of this analysis, it is known that the mean of P2 was significantly different from that of P0 and P1, while the mean of P0 was not significantly different from that of P1. After observation on the necrosis level of the liver cells in the Kiernan triangle of two week old chicks was carried out, the results were obtained as seen in table 5. The results of the Kruskal Wallis analysis indicated that the calculated H value = 12.32 was greater than the H table value (0.05) = 5.99, so the null hypothesis was (H0) was rejected, which means there were significant differences between the groups. To determine differences between groups, multiple comparison analysis (Z test) was carried out. The calculated Z value was obtained at 1.74 and after each group was tested, it indicated that the difference in the mean between groups P2 and P1, P2 and P0 and P1 and P0 was greater than the calculated Z value of 1.74. Thus, from the results of this analysis, it indicated that there were significant differences between groups.

4. Discussion

Carbofuran insecticide is a chemical substance used to kill insects that damage plants. Carbofuran is a toxic substance that can have a negative impact on living organisms, including chickens and chicks. When chickens consume feed or food contaminated with carbofuran, this substance can enter the chicken's body and accumulate in body tissues, including in the chicken's egg yolk. This is likely to occur because carbofuran can be persistent in the environment and can be absorbed by plants eaten by chickens [13]. Carbofuran can cause damage to vital organs, including the liver, kidneys and central nervous system. A chicken mother acutely exposed to high doses of carbofuran can experience severe poisoning and even death. If the chicken mother repeatedly eats feed contaminated with carbofuran, this toxin can accumulate in its body tissues, including reproductive organs such as the ovaries. This can result in eggs produced by the chicken containing carbofuran residues, which can then harm newly hatched chicks. Carbofuran can affect embryo development in eggs. This can result in growth abnormalities, defects, or even death in the chicks which grow in the egg [14].

In this study, there was an increase in necrosis of liver cells in the central vein and Kiernan's triangle of one day and two week old chicks due to exposure to carbofuran at a dose of 0.0127 mg/0.1 ml/egg during the embryonic period ($p<0.05$). Meanwhile, carbofuran at a low dose of 0.0106 mg/0.1 ml/egg did not have a significant difference compared to the control. The insecticide carbofuran, as previously mentioned, is very toxic. When carbofuran is exposed in high doses, this toxin can have a more damaging impact on living organisms, including the liver of chicks when they hatch. High doses of carbofuran can damage chicks' livers more than low doses or short-term exposure. This is because high doses of carbofuran has a higher toxin concentration in the chicks' bodies. This will increase its ability to damage organs in the body, including the liver. Carbofuran works by disrupting the nervous system and poisoning body cells, which can cause more severe damage when the toxin concentration is higher. In high doses or prolonged exposure to carbofuran, the toxin can accumulate in body organs such as the liver. This accumulation can worsen organ damage and affect liver function. Accumulation of this toxin may not occur at low doses or short exposures. Carbofuran can disrupt the metabolic system in the body. In high doses, carbofuran can cause more serious disorders in metabolism, including metabolism that occurs in the liver. This can result in more significant damage and impaired liver function. The dose and duration of exposure are critical in determining the extent of damage that may occur [15]. High doses or sustained exposure to carbofuran will increase the risk of serious organ damage in chicks [16].

Carbofuran is a highly toxic insecticide, and exposure during the embryonic period can cause various negative impacts on the development and health of the embryo, including on blood vessels such as the central vein and Kiernan's triangle. The mechanism of cell death (necrosis) that occurs in blood vessels is because carbofuran can directly poison blood vessel cells [17]. This can result in structural damage and cell function, which in turn can cause necrosis. Direct exposure of carbofuran to blood vessel cells can damage cell membranes, cell organelles and important molecules in cells. Carbofuran can interfere with blood flow through the blood vessels in several ways. For example, it causes vasoconstriction (narrowing of blood vessels) or changes in blood circulation, which can inhibit the supply of oxygen and nutrients to blood vessel cells. As a result, these cells can die due to lack of oxygen and nutrient supply [18].

Carbofuran is an insecticide that works by disrupting the insect's nervous system. Although the working mechanism of carbofuran on embryos is not the same as that on insects, exposure during the embryonic period can affect the developing nervous system in the embryo. This can cause changes in vascular regulation and can trigger necrosis in certain blood vessels. Exposure to carbofuran can trigger an inflammatory reaction in the body. This inflammation can damage blood vessels and the cells around them. This could be a contributing factor to necrosis [19]. The impact of carbofuran exposure on the embryonic period can vary greatly depending on the dose, duration, and the embryo development point when it is exposed. Exposure to carbofuran during the embryonic period can disrupt the normal development of the embryo and affect various organ systems. Therefore, it is important to prevent carbofuran exposure and other toxic substances during embryonic development to ensure healthy and normal development of the embryo and organs [20].

It is important to note that the use of the carbofuran insecticide and exposure to it in the livestock environment remains controlled and is in accordance with applicable safety and regulatory guidelines to avoid such risks. Using the carbofuran insecticide safely and in accordance with the instructions for proper use can reduce the risk of chicken feed and egg yolk being contaminated with carbofuran. In addition, close monitoring and supervision of insecticide use in the livestock environment can help reduce the potential exposure of chicks to these insecticides.

5. Conclusion

The carbofuran group at a dose of 0.0106 mg/0.1 ml/egg had a significant effect on necrosis of liver cells in the central vein and in Kiernan's triangle of one day old chicks. Moreover, observation carried out on two week old chicks : carbofuran group treated with carbofuran at a dose of 0.0127 mg/0.1 ml/egg and that at a dose of 0.0106 mg/0.1 ml/egg indicated that both doses had a significant effect compared to the P0 control group ($p<0.05$). The carbofuran insecticide at a dose of 0.0127 mg/0.1 ml/egg was proven to cause the necrosis of liver cell in the central vein and Kiernan's triangle of one day and two week old chicks which were exposed to carbofuran during the embryonic period.

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: 2001/11-KE).

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