Cover letter/ Declaration required for peer-review

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Inbox

Mark Rana <markrana7@manuscriptscientific.com> Fri, Jun 25, 2021, 10:15 AM

to me

Dear **Dr. Epy Muhammad Luqman,**

We had just finished the pre-quality check of your manuscript entitled "**Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Duodenum in Mice (Mus musculus) at Lactation Period Exposed to Carbofuran**"As per the International Standard protocol, we request you to please fill the attached Cover Letter and submit us as soon as possible by duly signing it. It is a mandatory requirement for proceeding ahead for the Peer Review of your submission by our editors and reviewers.

Once we receive the duly signed cover letter, we will immediately proceed ahead for Peer Review and other publication processes.

We request you to submit it at your earliest convenience so that the publication process will not be delayed.

Awaiting your early response as soon as possible.

Thanks & Regards
Mark Rana.

epy muhammad luqman <epy-m-l@fkh.unair.ac.id> Sun, Jul 4, 2021, 10:08 PM

to Mark

Surabaya Indonesia, July 4, 2021

Dear Mark Rana

I am submitting a revision of a manuscript entitled " "“Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Duodenum in Mice (Mus musculus) at Lactation Period Exposed to Carbofuran”. I had corrected manuscript according the reviewer comments:

1. Table 1 was addressed in the text first and then presented.
2. The figures were placed right after Table 2 and were addressed in the Discussion section.
3. Figures have been enhanced (or be bigger), so it can easily observe the intestinal mucosa and the effects of different treatments.
4. The figures had their captions better specified.

5. I have agreed to change the sentence structure and grammar in the manuscript to make it look effective.

Thank you

--

Dr. Epy Muhammad Luqman
Badan Kerjasama dan Manajemen Pengembangan
Universitas Airlangga

epy muhammad luqman <epy-m-l@fkh.unair.ac.id> Mon, Aug 16, 2021, 8:55 PM

to Mark

Surabaya Indonesia, August 16, 2021

Dear Mark Rana

I am submitting a revision of a manuscript entitled " "“Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Duodenum in Mice (Mus musculus) at Lactation Period Exposed to Carbofuran”. I had added the conclusion to the manuscript.

Mark Rana <markrana7@manuscriptscientific.com> Aug 30, 2021, 10:29 PM

to me

Dear **Dr. Epy Muhammad Luqman,**

Thank you for your email.
Hope you are doing well .Apologies for the delay in response. We have received your revised manuscript. We will update you soon on further processes.

5-JVMR-1-1-Muhammad Luqman_Galley Proof & Fee Details

External

Inbox

Niti Singh <nitisingh7@manuscriptscientific.com> Tue, Sep 21, 2021, 1:33 AM

to me

Dear **Dr. Epy Muhammad Luqman,**
Greetings from Manuscript Scientific Services!
We are hereby attaching your final galley proof of your article to be published in our Journal.
Article Number: 5-JVMR-1-1-Muhammad Luqman
Article Title: Potency of Kebar Grass (*Biophytum petersianum*) Extract to Histopathological of Mice (*Mus musculus*) Duodenum at Lactation Period Exposed to Carbofuran
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Manuscript No: **JARR-1-1-03**

Manuscript Title: **"Potency of Kebar Grass (Biophytum petersianum) Extract to Histopathological of Duodenum in Mice (Mus musculus) at Lactation Period Exposed to Carbofuran"**

Article Type: **Research Article**

Reviewer Assigned: **Prof.**

Date Sent to Reviewer: **01/07/2021**

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Editorial Assistant: **Mark Rana**

Editorial Office: **Manuscript Scientific Services, Governed by, ResGenesis Technology Solutions Pvt. Ltd., Plot No 85, Ramjeet Nagar, Albatiya Road, Jagdish Pura Agra- 282007, Uttar Pradesh, INDIA.**

Email Address: **markrana7@manuscriptscientific.com**

REVIEW QUESTIONS

1. Title

Does the title address the topic specified in the paper? Yes X No □

Do you recommend another Title? Yes X No □

If yes, please specify.

Potency of Kebar Grass (*Biophytum petersianum*) Extract to Histopathological of Mice (*Mus musculus*) Duodenum at Lactation Period Exposed to Carbofuran

2. Abstract (if any)

Is the abstract specific and representative of the paper? Yes X No □

Are main results and conclusion presented? Yes X No □

3. Introduction (if any)

Is the problem being addressed clearly emphasized? Yes X No □

Are the research question and objectives clearly identified? Yes X No □

4. Theoretical Foundation

Do the arguments build on existing theory? Yes X No □

Has the author(s) cited the relevant literature? Yes X No □

Are propositions/hypotheses important for the area of research? Yes X No □

5. Research Design and Methods

Is the research design adequate? Yes X No □

Is the sample size adequate? Yes X No □

Is there sufficient detail to enable the reader to duplicate the analysis? Yes X No □

6. Results

Is the statistical analysis clearly presented? Yes X No □

Are the results presented in a logical sequence to support the hypotheses? Yes X No □

7. Discussion

Are data and results presented in a succinct manner? Yes X No □

Is the relationship between results with previous research relevant? Yes X No □

Are reasons for differences in results with previous research clearly stated? Yes X No □

Are possible directions for future research stated? Yes □ No X

8. Conclusions

Are conclusions stated briefly in a logical order? Yes X No □

9. General Aspects

Is the paper well structured? Yes X No □

Is the paper well written and readable? Yes X No □

Are references accurate and complete? Yes X No □

Is line of reasoning convincing? Yes X No □

Would you recommend this paper to your colleagues? Yes X No □

GENERAL/SPECIFIC COMMENTS TO THE AUTHOR

For paper improvement please provide in the following constructive comments in the form of a detailed review on the most important aspects to be addressed by the author(s). This part is intended to be communicated to the author(s):

The authors are off to a good start; however, Table 1 must be first addressed in the text and then be presented. The figures should be placed right after Table 2 and be addressed in the Discussion section. Figures must be enhanced (or be bigger), so it can easily observe the intestinal mucosa and the effects of different treatments. In addition, the figures need to have their captions better specified, for example, indicating the meaning of the letters placed in each image. Overall, I would like to congratulate the authors for this article.

EVALUATION RESULTS (choose any one)

Considering the present version of this paper, it is: "**1-5**"

1. **Not developed enough and must be "Rejected"**
2. **Revise and resubmit with "Minor Corrections" X**
3. **Conditional acceptance with proposed revisions must be completed with "Major Revisions"**
4. **Acceptable in its current form/"Accepted without changes"**
5. **"Strongly Recommended"**

RECOMMENDED CORRECTIONS

Page No.	Para No.	Line No.	Existing	Change to
1	2	34	Residue on agricultural crops will impact	Residue on agricultural crops impact
1	2	36	corns, and rice with the value of 12–102 ppb can	corns, and rice (value of 12–102 ppb), which can
2	1	52	Mothers have hyperphagia	Mother has hyperphagia
2	4	82	formalin 10%, and alcohol.	formalin 10%, and alcohol were also used.
2	7	98	Determination on the dose of Kebar grass used was 0.135 mg/g BW/day	Determination on the Kebar grass dose used was 0.135 mg/g BW/day
7	2	228	The group of T3 and T4 showed	Groups T3 and T4 showed
7	3	234	The group of T5 and T6 also showed	Groups T5 and T6 also showed

7	5	250	The group of T3 and T4 showed	Groups T3 and T4 showed
7	5	254	The group of T5 and T6 showed	Groups T5 and T6 showed
8	1	277	The group of T5 and T6 showed	Groups T5 and T6 showed

Research Article

Potency of Kebar Grass (*Biophytum petersianum*) Extract to Histopathological of Mice (*Mus musculus*) Duodenum ~~in Mice (*Mus musculus*)~~ at Lactation Period Exposed to Carbofuran

Muchammad Manhum Mawarid[1], Maslichah Mafruchati[1], Tri Wahyu Suprayogi[1], Widjiati[1], Viski Fitri Hendrawan[2], Epy Muhammad Luqman[1*]

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This research was aimed to find out the effect of the Kebar grass extract on a decrease in villi damage in the duodenum of mice at lactation period due to exposure to carbofuran such as congestion, edema, and neutrophil infiltration. This research used forty-two mice at lactation period, divided into seven treatment groups, each group consisting of six mice. The treatment groups consisted of C (aquadest); T1 carbofuran 0.0125 mg (1/4 LD_{50}); T2 carbofuran 0.00625 mg (1/8 LD_{50}); T3 Kebar grass extract 3,375 mg + carbofuran 1/4 LD_{50}; T4 Kebar grass extract 3.375 mg + carbofuran 1/8 LD_{50}; T5 vitamin C 5 mg + carbofuran 1/4 LD_{50}; and T6 vitamin C 5 mg + carbofuran 1/8 LD_{50}. All groups were treated for 14 days and on day 15 all mice were slaughtered for histopathological observation on their duodenum. The data were analyzed using the Kruskal-Wallis test and the Mann-Whitney test with a significance of ~~P~~p<0.05. The results showed that carbofuran administration could increase congestion, edema, and neutrophil infiltration (~~P~~p<0.05). The administration of Kebar grass extract was proven to reduce congestion, edema, and neutrophil infiltration and a decrease in congestion, edema, and neutrophil infiltration was better when it was administered Kebar grass extract compared to vitamin C (~~P~~p<0.05).

Keywords: Kebar grass extract, carbofuran, lactation period, duodenum.

INTRODUCTION

Carbamate insecticides are commonly used to get rid of pests attacking crops or fruits particularly carbofuran [1]. Residue on agricultural crops ~~will~~ impact on health of humans and livestocks, which is not the main target of using insecticide. The results of a research [2] found carbofuran residue in animal feed such as hay, corns, and rice ~~with the~~ (value of 12–102 ppb), which can cause residue in the meat as much as 110–269 ppb and in the cattle serum as much as 167–721 ppb. Carbofuran exposure can cause free radicals in the form of Reactive Oxygen Species (ROS), which will impact on the death of cells in the body including intestinal epithelial cells [3]. ROS can cause membrane lipid peroxidation so fatty acid chains in cell membranes are broken and then it causes injury in cells [4]. Exposure to toxic subtances can cause inflammation in intestinal lamina propia so it will cause villi damage, congestion, edema, neurophilic infiltration [5]. Malonaldehid (MDA) and SOD are used as indicator to lipid peroxidation and necrosis [6, 7]. The presence of antioxidant compounds and an increase in ROS, which is imbalanced will lead to oxidative stress and cause the damage on DNA, lipid, and protein [8].

Kebar Grass *(Biophytum petersianum)* is a plant containing flavonoids and wildly grows in Papua, Indonesia [9]. Phenolic compounds serve as antioxidants, which have mechanism to reduce and capture free radicals [10]. Vitamine E as lipid antioxidants is able to extinguish free radicals and functions as membrane stabilizer [11]. Vitamin A as antioxsidants works by weakening peroxyl radicals and inhibiting lipid oxidation [12].

Mothers haves hyperphagia at lactation period, which causes unstable condition of the mothers. The change in intestinal mucosa leads to an increase in the number of mucosal ephithelial cells, which causes the change in villi condition [13]. Females that are at lactation period are more susceptible than females that are not at lactation period are due to higher stress level, which triggers susceptibility to toxic subtances [14]. The intestine can also be the main tract or portal in which a substance undesired by the body such as toxic substances, like insecticides and drugs enters the body [15, 16]. Insecticides can cause necrosis, which leads to hipermotility and malabsorption due to a decrease in the number of erythrocyte cells [17]. Entry of toxic substances can cause free radicals to increase endothelial permeability and intestinal mucosa so it leads to infiltration of inflammatory cells in intestinal mucosa [18, 19]. Cell membrane permeability increases so that absorption is impaired and leads to a clinical symptom, that is diarrhea [20].

This research is was carried out to find out the potency of Kebar grass extract, which contains antioxidants to prevent free radicals in order to decrease histopathological damage of the mice's duodenum. Several indicators on the level of duodenum damage were observed: ephitelial damage, congestion, edema, and infiltrasi neutrofil.

MATERIALS AND METHODS

The experimental design in this research used a completely randomized design (CRD) using 42 pregnant mice divided into seven groups with six replications. This research has beenwas approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga (1.KE.107.06.2019).

Materials

The experimental animals used in this research were female mice *(Mus musculus)* weighing 20-30 grams from the Center for Veterinaria Farma Surabaya Indonesia,, Kebar grass *(Biophytum petersianum Klotzsch)*, CMC Na, Ethannol 70%, carbofuran (2,3- Dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate 98%) from Aldrich Chemistry with Bellstain Registry number 1428746 Product of USA, feed pellets for mice, aquadest, vitamin C, drinking water, husks as the base of the cage, ether, formalin 10%, and alcohol were also used.

Methods

A total of 42 pregnant mice were divided into seven groups: C, T1, T2, T3, T4, T5, and T6. Each group consisted of six replications. The mice were placed in plastic cages covered with wire and using husks as a base. The mice were fed on pellets and drank aquadest water *ad libitum* every day during the research.

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Kebar Grass Extract Preparation

The dried Kebar grass was first boiled in aquadest. 350 grams simplicia of mashed Kebar grass was macerated in a tube for 3x24 hours with 70% ethanol solvent with a ratio of 1:10,. nNext, it was filtered and the pulp was re-macerated twice with the same treatment. The macerated pulp was evaporated using a rotary evaporator at a temperature of 30-40 ^{o}C to form a thick extract. The extract was put in a bottle and stored in the refrigerator.

Dose Determination

Determination on the Kebar grass dose of Kebar grass used was 0.135 mg/g BW/day [21] and the average body weight of the mice used was 25 grams, so the dose used was 0.135 mg x 25 g = 3.375 mg/25g/day. The doses of Carbofuran administered using the LD_{50} fraction for mice

treated for 14 days were ~~¼~~ 1/4 LD_{50} (0.0125mg/25g mouse/day) and 1/8 LD_{50} (0.00625mg/25mg mouse/day) [6, 22].

Treatment

Carbofuran, Kebar grass extract, and vitamin C were administered to the mice from the first day of lactation to the 14th day orally using 1 ml tuberculin. The seven groups were divided into: C (control), T1 ~~was~~ administered carbofuran ~~¼~~1/4 LD_{50} (0.0125 mg/day), T2 ~~was~~ administered carbofuran 1/8 LD_{50} (0.00625 mg/day), T3 ~~was~~ administered carbofuran 1/4 LD_{50} (0.0125 mg/day) + Kebar grass extract 3.375 mg, T4 ~~was~~ administered carbofuran 1/8 LD_{50} (0.00625 mg/day) + Kebar grass extract 3.375 mg, T5 ~~was~~ administered carbofuran 1/4 LD_{50} (0.0125 mg/day) + vitamin C 5 mg, T6 ~~was~~ administered carbofuran 1/8 LD_{50} (0.00625 mg/day) + vitamin C 5 mg. On the 15th day, the mice were slaughtered to take their duodenum and histopalogical preparation ~~was~~ carried out using HE staining.

Observation on Histological Preparation

Microscopic observation on the duodenum was carried out using a 400X magnification microscope by observing five different angles from each slide. The damage observed included epithelial damage, congestion, edema and neutrophil infiltration in duodenal epithelial cells of the mice according to [5] (Table 1).

Table 1: Scoring of intestinal damage

Score	Epithelial damage	Congestion and edema	Neutrophil infiltration
0	Nothing	Nothing	Nothing
1	Damage on the tip of villi	Red blood cells are rarely found in venules	PMN cells rarely exist
2	Damage on half of villi	Red blood cells are found in half of venules	PMN cells exist outside blood vessels (there are approximately 5 cells in each blood vessel)
3	Total damage on villi	Red blood cells are found in more than half of venules	PMN cells upto dilamina propria and epithelium exist

Source: [5]

Data analysis

The scoring results on the changes that occurred in duodenal preparations were then analyzed using the Kruskall Wallis test followed by the Mann Whitney test with the application of SPSS (Statistical Package for the Social Sciences).

RESULTS

Damage to the villi is indicated by the tip of the villi having erosion and rupture (Table 2 and Figure 1). The results of statistical analysis showed that there was a significant difference ($p<0.05$) between group C with groups T1, T2, T3, T5, and T6. There was a significant difference ($p<0.05$) between group T1 with groups T3, T4, T5, and T6. There was a significant difference ($p<0.05$) between group T2 with groups T3, T4 and T6. There was a significant difference ($p<0.05$) between group T4 with groups T5 and T6 (Table 2).

Congestion is known from the accumulation of erythrocytes in the lumen of the blood vessels, whereas edema is characterized by the presence of white spaces in the mucosal lining of the intestine (Table 2 and Figure 3). The results of statistical analysis showed that there was a significant difference ($p<0.05$) between group C with groups T1, T2, T3, T4, T5, and T6. There was a significant difference ($p<0.05$) between groups T1 and T2 with groups T3, T4 and T6. There was a significant difference ($p<0.05$) between groups T3, and T4 with group T5 (Table 2 and ~~f~~Figure 2).

Neutrophil infiltration is characterized by the presence of neutrophil cells around the intestinal mucosal tissue (Table 2 and Figure 3). The results of statistical analysis showed that there was a significant difference ($p<0.05$) between group C with groups T1, T2, T3, T4, T5, and T6. There was a significant difference ($p<0.05$) between group T1 and T2 with groups T3, T4, T5, and T6. There was a significant difference ($p<0.05$) between group T3 and T4 with groups T5 and T6.

Tabel 2: Mean of villi damage, congestion edema, and neutrophil infiltration on the duodenum of mice (*Mus musculus*) at lactation period administered Kebar grass *(Biophytum petersianum* Klotzsch) extract and vitamin C after being exposed to carbofuran.

Treatment	Vili Damage (Mean ± SD)	Congestion Edema (Mean ± SD)	Neutrophil Infiltration (Mean ± SD)
C (control)	0.000^{a}±0.000	0.000^{a}±0.000	0.000^{a}±0.000
T1 (carbofuran 1/4 LD_{50})	2.000^{e}±0.200	2.467^{d}±0.416	2.933^{d}±0.116
T2 (carbofuran 1/8 LD_{50})	1.800^{de}±0.721	2.133^{cd}±0.116	2.800^{d}±0.200
T3 (carbofuran 1/4 LD_{50}+Kebar grass extract 3.375 mg)	0.867^{bc}±0.116	1.067^{b}±0.231	1.400^{b}±0.529
T4 (carbofuran 1/8 LD_{50}+Kebar grass extract 3.375 mg)	0.400^{ab}±0.200	0.933^{b}±0.611	1.333^{b}±0.306
T5 (carbofuran 1/4 LD_{50} + vitamin C)	1.333^{cd}±0.231	1.867^{cd}±0.306	2.267^{c}±0.116
T6 (carbofuran 1/8 LD_{50} + vitamin C)	1.200^{c}±0.734	1.600^{bc}±0.529	2.200^{c}±0.400

Note: Notation a, b, c, d and e (superscript) in the same column shows a significant difference ($p<0.05$).

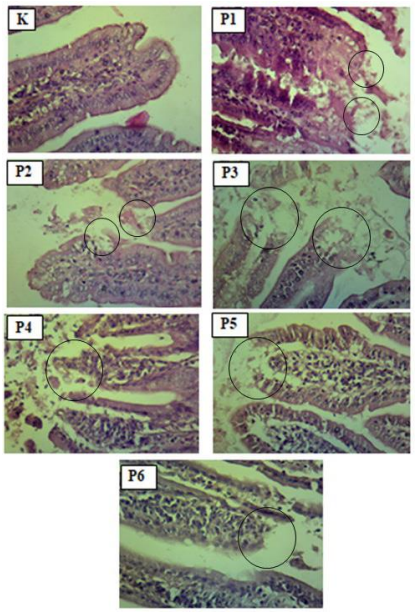

Fig. 1: Villi damage in the duodeum of the mice *(Mus musculus)*. Black circle shows villi damage of duodeum.

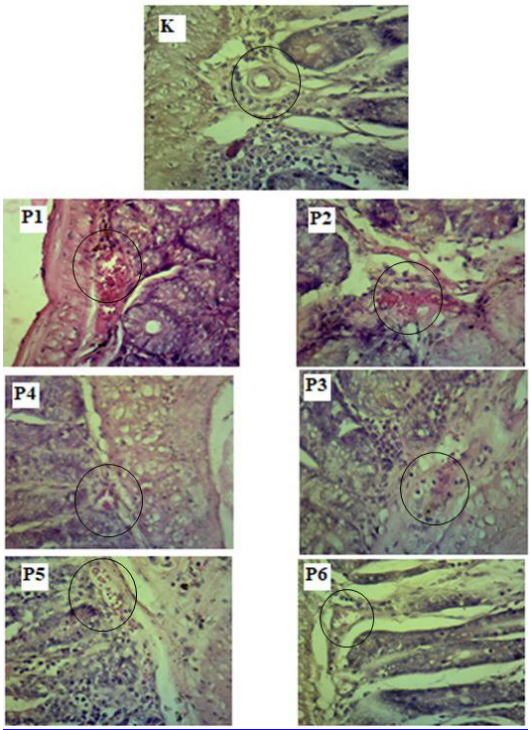

Fig. 2: Congestion and edema in the duodeum of the mice *(Mus musculus)*. Black circle shows congestion and edema of duodeum.

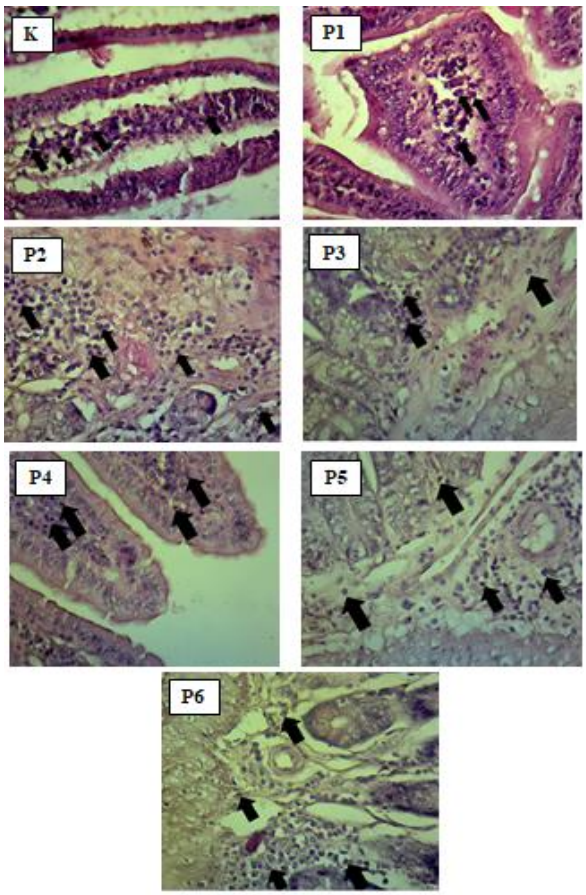

Fig. 3: Neutrophil infiltration in the duodeum of the mice *(Mus musculus)*. Arrow shows neutrophil infiltration of duodenum.

DISCUSSIONS

Carbofuran acts as a systemic poison with extreme toxicity to the stomach [23]. Carbofuran can enter the body through several ways~~,~~ that are~~is~~ absorption through the skin, inhalation and orally [24]. Oral exposure to carbofuran can irritate the digestive tract and can cause oxidative stress [25]. Carbofuran is metabolized by the body by cytochrome P_{450} to 3-hydroxy carbofuran, which can increase its toxic activity [26]. Oxidative stress is an imbalance between free radicals and antioxidants that is triggered by two common conditions, ~~that is~~which are deficiency of antioxidants and excess production of free radicals [27]. Flavonoids in Kebar grass were non-enzymatic antioxidants that also protect cell membranes against oxidation from free radicals. Kebar grass also contains vitamin A and vitamin E. Vitamin A works by reacting with free radicals and causing them to stabilize [28]. Vitamin E functions to protect poly-unsaturated fatty acids and other cell membrane components from free radical oxidation by breaking the lipid peroxide chain. Examination on Clinical chemistry and histopathology is one of the methods used to detect the specific effects of chemical exposure [29, 30].

Villi Damage

The damage is thought to have occurred as a result of exposure to toxic substances [31]. Villi damage was not found at the control group (C). Carbofuran administration at T1 and T2 was able to increase the villi damage in proportion to the increase in the dose administered (Table 2). Inflammatory cell activity occurs when the body is exposed to toxic antigens such as carbofuran, so a non-specific immune response that functions against these antigens at the site of the damage. In addition, the imbalance due to oxidative stress can result in malfunctioned endothelial [32].

~~The~~ gGroups ~~of~~ T3 and T4 showed that villi damage in the mice's duodenum could be reduced (Table 2). The decrease in villi damage was due to vitamin A, vitamin E and flavonoids contained in Kebar grass, which was better at responding the toxic substances of T1 and T2. Flavonoids function as primary antioxidants because they serve as free radical acceptors, so that they can inhibit free radical chain reactions in lipid oxidation, which can prevent membrane damage [33].

~~The~~ gGroups ~~of~~ T5 and T6 also showed a reduction in villi damage due to exposure to carbofuran (Table 2). The reduction in villi damage was better at responding to the toxic substances of T1 and T2. Vitamin C has a strong ability to reduce and acts as an antioxidant in reacting with hydroxyl [34]. Vitamin C is easily oxidized by atmospheric oxygen or by the enzyme ascrobate oxidase. However, vitamin C is a very powerful antioxidant and can prevent the oxidation process in food and in the body system [35].

Congestion and Edema

In the control group (C) congestion and edema were not found. T1 and T2 could increase congestion and edema in proportion to the increase in dose administered (Table 2). Congestion is a pathological reaction as a manifestation of inflammation due to injury. Congestion changes occur due to vascular hydrostatic pressure and osmotic pressure. Osmotic pressure is the pressure caused by proteins in plasma [36]. Edema is an indication of hydrostatic pressure or an error in imbalanced blood osmotic pressure, increased capillary permeability, lymph, and obstruction. This condition can be caused by toxic chemicals, viruses, bacteria and parasitic diseases [37].

~~The~~ gGroups ~~of~~ T3 and T4 showed that congestion and edema could be reduced (Table 2). The reduction in congestion and edema was due to vitamin A, vitamin E and flavonoids contained in Kebar grass which was better in responding to the toxic substances of T1 and T2. Antioxidant compounds work by inhibiting the oxidation rate of other molecules or neutralizing free radicals [38]. ~~The~~ gGroups ~~of~~ T5 and T6 showed that vitamin C was able to reduce congestion and edema due to exposure to carbofuran (Table 2). The reduction in congestion and edema was better at responding to the toxic substances of carbofuran at T2 to T1. Vitamin C can act as a co-antioxidant by regenerating a-tocopherol radicals [39]. Another characteristic that makes vitamin C an ideal antioxidant is the low reactivity of ascorbyl radicals formed when ascorbic acid reacts with ROS. Ascorbyl radical is not a strong oxidizing agent and reducing agent, so it reacts slowly with oxygen. This cycle produces a strong defense system against free radicals or other oxidant compounds [40].

Neutrophil Infiltration

Neutrophils are one type of white blood cell markers of inflammation or infection. Neutrophils will work when there is a signal from cytokine hormones that indicate the location of inflammation in the body, resulting in an increase in the number of neutrophils in the peripheral blood [41]. In the control group (C) there was no increase in neutrophil infiltration. T1 and T2 could increase neutrophil infiltration in proportion to the increase in

dose administered (Table 2). T3 and T4 groups showed that the amount of neutrophil infiltration in the duodenum of mice could be reduced (Table 2). The decrease in neutrophil infiltration was due to vitamin A, vitamin E and flavonoids contained in Kebar grass, which was better in responding to the toxic substance of T1 and T2. Antioxidants in flavonoids can reduce inhibition of nitric oxide, prevents leucocyte from adhering to blood vessel walls and ~~having interactions~~interacting with other enzyme systems such as COX-1 and COX-2 [42]. These stimuli can be cytokines, bacterial lipopolysaccharides, inflammation or other pathological conditions [43]. Cyclooxygenase-1 (COX-1) plays a role in normal physiological functions such as mucus secretion to protect the digestive mucosa [44]. ~~The g~~Groups ~~of~~ T5 and T6 showed the potency of vitamin C in reducing the amount of nutrophil infiltration due to exposure to carbofuran (Table 2). The reduction in the number of nutrophilic infiltrations was better at responding to the toxic substances of T2 to T1.

The results of this study indicate that administration of Kebar grass extract as an antioxidant is better at reducing the level of villi damage, congestion, edema, and neutrophil infiltration in duodenum of the mice at lactation period compared to vitamin C.

ACKNOWLEDGEMENT~~:~~

The authors express sincere thanks to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding the research, and the Dean of Faculty of Veterinary Medicine Universitas Airlangga for providing all necessary facilities and funds in conducting this research work.

CONFLICT OF INTEREST~~:~~

The authors declare no conflict of interest.

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