



## Beneficial effects of kebar grass (*Biophytum petersianum* klotzsch) ethanol extract to increase motor reflex and spatial memory in mice offspring (*Mus musculus*) from lactating mothers exposed to carbofuran

Epy Muhammad Luqman<sup>1,\*</sup>, Eka Pramyrtha Hestianah<sup>1</sup>, Widjiati Widjiati<sup>1</sup>,  
Suryo Kuncorojakti<sup>1</sup>, and Viski Fitri Hendrawan<sup>2</sup>

<sup>1</sup>Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

<sup>2</sup>Department of Reproduction, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia.

### Abstract

**Background and purpose:** This study aimed to determine the potency of kebar grass ethanol extract to overcome an increase in cerebellar neuronal cell necrosis, which has an impact on decreasing motor reflex function and spatial memory of mice from lactating mothers exposed to carbofuran.

**Experimental approach:** Forty lactating mice were divided into four groups, 10 each; including control, T1 (carbofuran 0.0125 mg/day), T2 (vitamin C 5 mg + carbofuran 0.0125 mg/day), T3 (kebar grass extract 3.375 mg + carbofuran 0.0125 mg/day). The mice were orally administered with carbofuran, vitamin C, and kebar grass extract on days 0 to 14 postnatal. On the 15<sup>th</sup> day, brains of the mice were necropsied to measure the levels of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH), H&E staining; motor reflex tests were performed on 10-day-old mice, and the mice aged 30 days were tested on their swimming and spatial memory.

**Findings / Results:** Carbofuran caused an increase in MDA, GSH, neuronal cell necrosis, surface righting reflex, a decrease in SOD, swimming ability, and spatial memory. Kebar grass extract and vitamin C administration decreased MDA, GSH, neuron necrosis, surface righting reflex, and increased SOD, swimming ability, and spatial memory.

**Conclusion and implications:** Exposing to carbofuran in lactating mice caused brain oxidative stress, impaired motor reflexes, and spatial memory in mice offspring. Kebar grass extract and vitamin C administration prevented brain oxidative stress and inhibited disorders in motor reflexes, and spatial memory in mice offspring. Kebar grass extract administration was more effective than vitamin C.

**Keywords:** Carbofuran; Cerebellum; Kebar grass; Lactation; Pesticide stress; Vitamin C.

### INTRODUCTION

Furadan, with the active ingredient carbofuran, is widely used in agriculture and it has been proven that residues are found on soil, surfaces, rainwater, and food can harm organisms that are not the target of insecticides (1). Carbofuran exposure produces motor reflex anomalies in newborns, as well as abnormalities in the development of brain function in children, such as a diminished ability to remember and focus power (2).

Contamination by carbofuran in laboratory animals induced oxidative stress and impairs cognitive, memory, and motor functions (3). The cerebral cortex, cerebellum, and brainstem all suffer from severe oxidative damage as a result of carbofuran induction (4).

#### Access this article online



Website: <http://rps.mui.ac.ir>

DOI: 10.4103/1735-5362.343086

\*Corresponding author: E.M. Luqman  
Tel: +62-315992785, Fax: +62-315993015  
Email: epy-m-l@fkh.unair.ac.id

The brain is vulnerable to oxidative damage due to the relatively low enzymatic and non-enzymatic antioxidant systems and the large amounts of free radicals produced due to the high oxygen demand (5). Oral carbofuran administration has been demonstrated to raise malondialdehyde (MDA), and decrease superoxide dismutase (SOD) in the brains of mice throughout embryonic and lactation periods, indicating that it stimulates reactive oxygen species (ROS) (6,7). The cerebellum develops from the middle of the pregnancy until a few days after the fetus is born (8). The cerebellum is responsible for controlling movement, maintaining balance, regulating position, and coordinating body motions. In the human brain, the cerebellum is thought to play a role in motor function as well as cognitive activities (9). Oxidative damage caused by free radicals also plays a central role in cognitive decline and spatial learning and memory (10). Protective markers of oxidative stress (MDA) and antioxidant (glutathione, GSH) in the brain have been investigated to observe the relationship between spatial memory and oxidative stress (11). The increase in ROS that induces neuronal cell death due to carbofuran exposure is able to reduce motor reflex function and memory ability.

Antioxidant compounds overcome oxidative damage caused by nutritional imbalances, xenobiotics, and strenuous physical activity. Biosystems include two types of antioxidants, non-enzymatic molecules such as GSH and vitamins (C, D, and E) and enzymatic indicators such as SOD, catalase, and GSH. Kebar grass (*Biophytum petersianum* Klotzsch) is a plant that belongs to the Oxalidaceae family that is found to naturally and widely grow in Kebar District, West Papua, Indonesia (12). Kebar grass contains flavonoids, vitamins E and A as antioxidants to neutralize toxic agents, prevent damage caused by toxic agents and maintain cell health. Flavonoids, which serve as primary antioxidants, are free radical acceptors so that they can inhibit free radical chain reactions in lipid oxidation, which can prevent membrane damage (13). Vitamin E can inhibit oxidation reactions by binding to vitamin E radicals and producing free vitamin E which functions again as an antioxidant. Beta carotene works by

reacting with free radicals and causing free radicals to become stable (14).

This study aimed to find out the potency of kebar grass (*Biophytum petersianum* Klotzsch) ethanol extract to overcome an increase in free radicals by an increase in MDA and GSH, a decrease in SOD, and cerebellar neuronal cell necrosis, which has an impact on decreasing motor reflex function and spatial memory in mice (*Mus musculus*) from lactating mothers exposed to carbofuran. This study was expected to provide a reference on the potency of kebar grass ethanol extract to reduce free radicals by decreasing MDA and GSH levels, increasing SOD, and decreasing the neuronal cell necrosis, motor reflexes in mice: swimming ability and surface righting reflex (swimming direction and head angle position), as well as a memory test of the eight-arm radial maze. In addition, the obtained data can also be used to provide information to the public about the dangers caused by the careless use of carbofuran, especially in lactating mothers.

## MATERIALS AND METHODS

Ten-week lactating mice (*Mus musculus*) weighing 25-35 g obtained from the Veterinary Center Farma Surabaya and the experimental study went through the following stages: synchronization of the oestrus cycle of mice using pregnant mare serum gonadotropin (PMSG; Folligon, Intervet Inc., Boxmeer, Netherlands) and human chorionic gonadotropin (hCG; Chorulon<sup>®</sup>, Intervet Inc., Boxmeer, Netherlands), examination on mice gestation through vaginal plug observation and administration of carbofuran by gavage for 14 days in lactating mothers. On the 15<sup>th</sup> day of lactation, neuronal cell necrosis in the brains of mice offsprings was counted and behavioral tests of mice consisting of swimming ability, surface righting reflex (direction and position of swimming) were performed on 10-day-old mice, and memory tests of the eight-arm radial maze were performed on mice aged 30 days old. The research procedure was conducted according to the permit by testing the code of ethics committee of experimental animals with the number 1.KE.107.06.2019 at the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.

Kebar grass (*Biophytum petersianum* Klotzsch) ethanol extract, carboxymethyl cellulose Na, 70% ethanol, carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol N-methylcarbamate 98%) were obtained from Aldrich Chemistry with Bellstain Registry number 1428746, USA. Materials used in this study were pellet feed for mice, aquadest as a solvent for carbofuran, vitamin C, drinking water, husks as a base for cages, 10% formalin, and alcohol for brain sampling and fixation. The tools used were plastic, cages, and wire mesh for experimental animal cages, drinking containers, sonde needles, test tubes. Microscopic examination was performed by counting neuronal cell necrosis with hematoxylin and eosin staining (H&E, Millicell®-HA, Merck, Germany). Under a microscope (Olympus® CX-41), three slices of each sample were observed and examined.

**Synchronization of the estrous cycle using PMSG and hCG hormones**

For 7 days, 10-week old female mice (*Mus musculus*) weighing 25-35 g were adapted to the habitat. On the 8<sup>th</sup> day, PMSG was given at a dose of 5 IU/head, and hCG was injected at a dose of 5 IU/head on the 10<sup>th</sup> day. Male mice aged 12 weeks were subsequently mated. After that, the mice were maintained in cages and fed *ad libitum*.

**Examination on mouse gestation**

A gestation examination was done on the 11<sup>th</sup> day, and if a vaginal plug was found in the female mice's vulva, that day was regarded as day 0 of gestation. The expecting mothers were then separated into five cages to give birth.

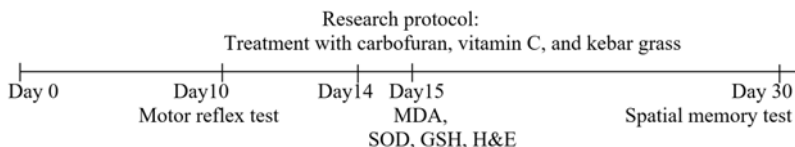
**Preparation of kebar grass ethanol extract**

Dried kebar grass was used in this research. The extract of kebar grass was made at the

Pharmacognosy and Phytochemical Laboratory of the Faculty of Pharmacy, Universitas Airlangga. The dried grass was boiled in distilled water. A total of 350 g of *Simplicia* of kebar grass which has been mashed were macerated in a tube for 3 × 24 h with a 70% ethanol solvent ratio of 1:10, then filtered and the dregs are macerated again 2 times with the same treatment. The macerate was evaporated by a rotary evaporator at a temperature of 30-40 °C to form a thick extract. The extract was put in a bottle and stored in the refrigerator.

**Administration of carbofuran and kebar grass**

Mothers who had given birth were divided into four groups: C (control group administered with 0.5 mL of aquadest), T1 (administered with carbofuran ¼ LD<sub>50</sub> 0.0125 mg/day), T2 (administered with vitamin C 5 mg + carbofuran ¼ LD<sub>50</sub> 0.0125 mg/day), and T3 (administered with kebar grass extract 3.375 mg + carbofuran ¼ LD<sub>50</sub> 0.0125 mg/day). The carbofuran dose used was the ¼ LD<sub>50</sub> (0.0125 mg/25g mice/day) (15). The determination of the dosage of kebar grass ethanol extract used in this study refers to research conducted by Labib *et al.* (16). Kebar grass provides an effective antioxidant effect to prevent oxidative stress by 0.135 mg/g BW/day. Carbofuran, vitamin C, and kebar grass were administered orally on days 0 to 14 postnatal (during 14 days of lactation) using a 3 mL syringe. On the 15<sup>th</sup> day, the brains of the mice were necropsied to measure the levels of MDA, SOD, GSH, and H&E staining; motor reflex function tests (neurobehavioral tests): surface righting reflex were conducted on 10-day-old mice; swimming and a spatial memory test of the eight-arm radial maze were conducted on mice aged 30 days (Fig. 1).



**Fig. 1.** Experimental design. Carbofuran, vitamin C, and kebar grass were administered orally on days 0 to 14 postnatal. On the 15<sup>th</sup> day, the brains of the mice were necropsied to measure the levels of malondialdehyde, superoxide dismutase, glutathione, and H&E staining were performed. Motor reflex function tests were conducted on 10-day-old mice, swimming and a spatial memory test were conducted on mice aged 30 days.

### **Measurement of MDA, SOD, and GSH levels using the ELISA method**

The sample was diluted with calibrator diluent RD5-16 (2× dilution). The mixture (75 µL sample plus 75 µL calibrator diluent RD5-16) was then homogenized. The standard assay (standard solution stock, 4000 pg/mL) was prepared with calibrator diluent RD5-16. Fifty µL of assay diluent RD1-54 was put into the well. A 50 µL sample and standard solution were added to the well. The well was covered with an adhesive strip and the solution was gently homogenized in the well. The solution was incubated at room temperature for 2 h. The solution in the well was taken and washed 5 times with 400 µL wash buffer. The well was absorbed using a paper towel. One hundred µL mouse MDA, SOD, and GSH conjugate were added. The well was covered with an adhesive strip and incubated for 2 h at room temperature. The solution in the well was taken and washed 5 times with 400 µL wash buffer. The well was absorbed using a paper towel. One hundred µL of substrate solution was added (by mixing 1:1 color reagent A and reagent B, which were homogenized for 15 min in a dark bottle) to each well. They were incubated at room temperature for 30 min in the dark. A 100 µL stop solution was added to each well and they were mixed gently. The absorbance was read at a wavelength of 450 nm.

### **Staining of mice's brain using H&E**

On the 15<sup>th</sup> day, the mice were sacrificed for taking the brains and making histopathological preparations with H&E staining. Observations of the brains of mice were carried out at five microscopic angles, at the four angles and the center of the preparation, with a magnification of 400× (3).

### **Motor reflex tests**

#### *Surface righting reflex*

Mice aged 10 days were used in this experiment. The mice were placed supine on a flat surface to be evaluated. A stopwatch was used to record the amount of time it took the mice to change positions from supine to prone (17).

#### *Swimming ability*

Mice aged 30 days were used in the experiment. The mice were placed in a water

container filled with warm water (between 27 and 30 °C), and their movements were recorded, head angle position: score 0: dive, score 1: nose above the water surface, score 2: nose and upper head above the water surface, score 3: as in score 2, the eyes are above the water surface, and a fourth of the earlobe is above it, score 4: as in score 3, the entire earlobe is above the water surface; swimming direction: score 1: floating, score 2: swimming in circles, score 3: swimming straight or nearly straight, score 4: sinking (18).

### **Spatial memory test with an eight-arm radial maze memory test**

The tools were made up of eight plastic arms, each measuring 32 cm in length and 5 cm in width, with a diameter of 20 cm in the center. The adaptation phase: the mice aged 30 days were used in the experiment. They were adapted for 7 days and fed pellets and given water every day. The initial phase: the mice were examined in an eight-arm radial maze on the 7<sup>th</sup> day of the adaptation phase. The training phase: before being taught in an eight-arm radial maze, the mice were fasted for 12 h. The mice were then placed in the center of the maze and given 10 min to investigate it. In the test phase, the five-day test phase begins one day after the training period is completed. The test was deemed complete when the mice had entered all arms or 10 min had passed. The number of errors in each session (entering an arm that has already been visited and/or not entering one or more arms in each session were both counted as errors) and the number of accurate choices in entering each arm in each session were the results of observations to be recorded (entering an arm that has not been visited previously in that session). The memory score formula was used to determine each mouse's memory score (19) based on the following equation:

$$\text{Memory score} = \frac{(\text{The correct number of arms}) - (\text{the incorrect number of arms})}{(\text{The correct number of arms}) + (\text{the incorrect number of arms})}$$

One is the maximum memory score that can be achieved. On a scale of -1 to 1, the closer the memory score is to 1, the better the memory of the experimental animals being tested. A score of -1, on the other hand, implies the inverse (all arm entries are incorrect).

### Data analysis

The average number of neuronal cell necrosis was characterized by pyknosis and karyorexic neuron cells. To evaluate the levels of MDA, SOD, GSH, and the number of neuronal cell necrosis used the ANOVA test which was followed by Duncan's test. The Kruskal-Wallis test was used to assess data on motor reflex ability and memory in mice in the eight-arm radial maze, and if the results were significantly different, the Mann-Whitney was used as a post hoc test. To make statistical calculations easier, the Statistical Product and Service Solution (SPSS) version 20.0 was used.

## RESULTS

In this study, it was shown that the MDA, GSH, neuronal cell necrosis, and surface righting reflex movements were significantly increased in the carbofuran group (T1) compared to the control group. Carbofuran was able to reduce SOD levels, swimming movements according to head angle position, swimming movements according to swimming directions, and spatial memory abilities through the eight-arm radial maze test compared to the control group (Table 1).

In the group administered with kebar grass extract and carbofuran (T3), it was indicated that the levels of MDA, GSH, neuronal cell necrosis, and surface righting reflex movements were significantly reduced compared to the carbofuran group (T1). The kebar grass extract in group T3 was also able to increase swimming movement according to

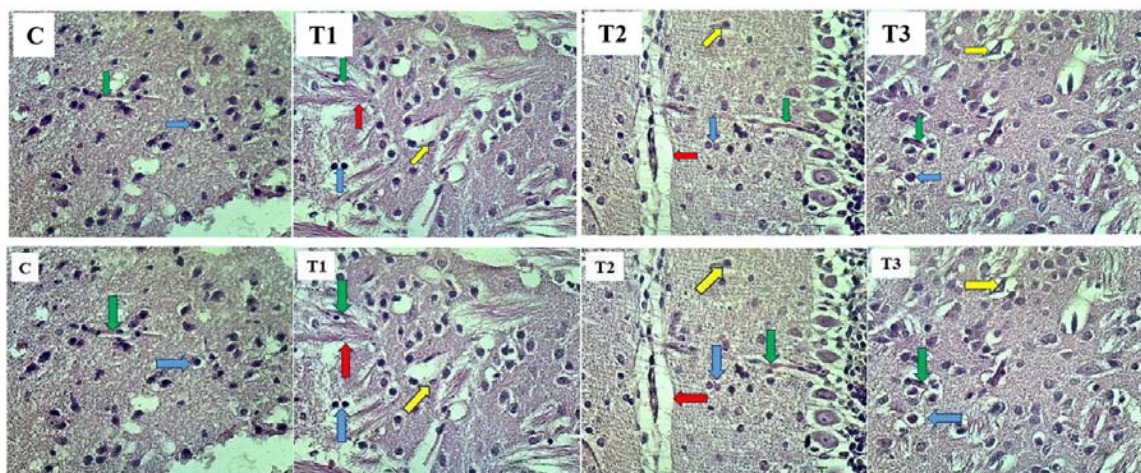
head angle position, swimming movement according to swimming direction, and spatial memory ability through the eight-arm radial maze test compared to the carbofuran group (T1) (Table 1). Except for swimming movements according to swimming directions, decreased levels of MDA, GSH, neuronal cell necrosis, surface righting reflex movements, and decreased swimming movements according to head angle position, and spatial memory ability through the eight-arm radial maze test was observed in the T3 group compared to the control group (Table 1).

The group administered with vitamin C (T2) could not significantly reduce the levels of MDA, GSH, and increase memory ability through the eight-arm radial maze test compared to the carbofuran group (T1). However, vitamin C (T2) was able to significantly reduce neuronal cell necrosis and surface righting reflex. Vitamin C was also able to increase swimming movements according to the head angle and swimming movements according to the direction of swimming. Similarly, the administration of kebar grass ethanol extract and vitamin C (T2) also did not increase SOD levels compared to the carbofuran group. However, The ability of vitamin C to reduce the levels of MDA, GSH, neuronal cell necrosis, and surface righting reflex was lower than the administration of kebar grass ethanol extract (T3). Vitamin C was also unable to increase swimming movements according to swimming direction and the eight-arm radial maze test compared to kebar grass ethanol extract (Table 1).

**Table 1.** Levels of MDA, SOD, GSH, number of neuronal cell necrosis, surface righting reflex, swimming direction and head angle position, eight-arm radial maze test mice from mothers exposed to carbofuran, vitamin C, and kebar grass. All data represent mean  $\pm$  SD.

Variables	Control group	Group T1	Group T2	Group T3
MDA (nmol/mg)	0.14 $\pm$ 0.038 <sup>c</sup>	1.64 $\pm$ 0.08 <sup>a</sup>	1.49 $\pm$ 0.13 <sup>a</sup>	0.88 $\pm$ 0.12 <sup>b</sup>
SOD (ng/mL)	0.75 $\pm$ 0.02 <sup>a</sup>	0.60 $\pm$ 0.01 <sup>b</sup>	0.60 $\pm$ 0.01 <sup>b</sup>	0.65 $\pm$ 0.04 <sup>b</sup>
GSH (ng/mL)	4.90 $\pm$ 1.56 <sup>c</sup>	19.10 $\pm$ 2.44 <sup>a</sup>	18.69 $\pm$ 2.38 <sup>a</sup>	10.12 $\pm$ 2.66 <sup>b</sup>
Neuronal cell necrosis	29.28 $\pm$ 2.74 <sup>a</sup>	46.56 $\pm$ 7.21 <sup>d</sup>	43.15 $\pm$ 1.19 <sup>c</sup>	35.44 $\pm$ 6.11 <sup>b</sup>
Surface righting reflex	0.68 $\pm$ 0.06 <sup>a</sup>	1.96 $\pm$ 0.10 <sup>d</sup>	1.40 $\pm$ 0.10 <sup>c</sup>	0.86 $\pm$ 0.21 <sup>b</sup>
Head angle position	3.00 $\pm$ 0.01 <sup>a</sup>	2.14 $\pm$ 0.37 <sup>c</sup>	2.30 $\pm$ 0.16 <sup>b</sup>	2.46 $\pm$ 0.26 <sup>b</sup>
Swimming direction	3.00 $\pm$ 0.01 <sup>a</sup>	2.04 $\pm$ 0.27 <sup>b</sup>	2.76 $\pm$ 0.24 <sup>a</sup>	2.89 $\pm$ 0.65 <sup>a</sup>
Eight-arm radial maze	1.00 $\pm$ 0.07 <sup>a</sup>	0.85 $\pm$ 0.11 <sup>c</sup>	0.87 $\pm$ 0.15 <sup>c</sup>	0.93 $\pm$ 0.15 <sup>b</sup>

<sup>a, b, c, d</sup>*P* < 0.05, Mean with different letters within a row are significantly different. T1, Carbofuran group; T2, vitamin C; T3, kebar grass extract; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione.



**Fig. 2.** Cerebellum images of each group (C, T1, T2, and T3). Red arrow, astrocytes; yellow arrow, oligodendrocytes; green arrow, microglia; and blue arrow, neuron; magnification, 400 $\times$ . C, Control group; T1, group exposed to carbofuran 1/4 LD<sub>50</sub> 0.0125 mg/day; T2, group exposed to vitamin C 5 mg + carbofuran 1/4 LD<sub>50</sub> 0.0125 mg/day; T3, group exposed to kebar grass extract 3.375 mg + carbofuran 1/4 LD<sub>50</sub> 0.0125 mg/ day.

The mean neuronal cell necrosis number was characterized by increased pyknosis and karyorexic neurons in the T1 group. Administration of kebar grass ethanol extract (T3) and vitamin C (T2) could reduce the number of neuronal cell necrosis even though it has not reached the number of neuron cells in the control group (C). However, the administration of kebar grass ethanol extract (T3) was still better than vitamin C (T2), as is shown in the number of pyknotic and karyorexic neuron cells in Fig. 2.

## DISCUSSION

Carbofuran that is a broad-spectrum insecticide, is lipophilic and can enter mothers' breast milk. Carbofuran exposure to lactating mothers is proven to cause increased MDA levels and brain necrosis in mice that were breastfed for 9 days (15). Increased levels of MDA and non-enzymatic antioxidant GSH and decreased SOD are the beginning of oxidative stress in animal body systems exposed to toxic substances. The results of this study showed that a subacute dose of carbofuran for 14 days of lactation also significantly increased MDA and GSH levels. It showed that carbofuran was able to cause oxidative stress in the brains of mice that were suckling their mothers for 14 days. In this study, there was a significant decrease in SOD due to exposure to carbofuran,

which indicated that oxidative stress had arisen in the brains of mice. A decrease in SOD indicates a failure of the antioxidant system and can cause brain toxicity in mice. Similar observations have been made in the brains of fetal mice whose pregnant mothers were exposed to carbofuran (7). Increased GSH is a mechanism of antioxidant defense to protect the body from oxidative stress by ROS. Similar results were found in the hearts of mice exposed to carbofuran for 28 days (20). Oxidative stress in the brains of mice induced by carbofuran in this study increased MDA and GSH levels and decreased SOD.

The levels of MDA and GSH decreased significantly when kebar grass ethanol extract was administered to the mice, and there was no decrease in MDA and GSH found following the administration of vitamin C. The results of this study were in accordance with the previous studies showing that vitamin C and curcumin could reduce heart and brain GSH but not significantly different (20,21). Meanwhile, the SOD levels did not increase either with the administration of kebar grass ethanol extract or vitamin C. The decrease in MDA and GSH levels due to the administration of kebar grass ethanol extract is due to flavonoids, vitamins A and E contained in kebar grass ethanol extract and is useful in preventing cell damage due to oxidative stress (21).

The content of vitamin A and vitamin E in kebar grass ethanol extract also functions to react with free radicals and stabilize free radicals. Vitamin A and carotenoids interact with free radicals and prevent cell lipid peroxidation. Vitamin E is a major lipophilic antioxidant that inactivates peroxy radicals through the direct transfer of hydrogen atoms and protects lipid tissues from damage due to free radicals (12). Vitamin C is also a potential antioxidant that has been proven to reduce the free radical load by neutralizing reactive chemical species through oxygen binding, hydroperoxide reduction, and free radical stabilization into neutral and non-toxic materials (14).

The decrease in MDA and GSH levels due to the administration of kebar grass ethanol extract is better than vitamin C because antioxidants from fruits and vegetables are the best sources compared to conventional antioxidant supplements. Higher doses of antioxidant supplements do not replace the need for a healthy diet. Antioxidant supplements of vitamins C and E, as daily antioxidants, can be consumed from fruits and vegetables (22).

In this study, the number of neuronal cell necrosis increased to 59.01 %. When exposed to carbofuran during the peak of neurogenesis, the number of neuronal cells necrosis increased by 662.64% when compared to the embryonic phase (days 14-17 of gestation) (14). Meanwhile, carbofuran exposure at the peak of the lactation period (on the days 1-4 of lactation) increased to 287.87% (15). It was discovered that the response to carbofuran-induced neuronal cell necrosis was significantly dependent on the critical phase of organ development. The administration of kebar grass ethanol extract and vitamin C reduced necrosis by 17.48 and 11.64% compared to the carbofuran group (Table 1).

This showed that the flavonoid contained in kebar grass ethanol extract was more potent in anticipating excess oxidative stress that caused neuronal cell necrosis than vitamin C administration. It is in line with the study by Heo and Lee that indicated that quercetin, one of the major flavonoids in some fruits and vegetables, has much stronger antioxidative activities than vitamin C. Quercetin decreased

oxidative stress-induced neuronal cell membrane damage more than vitamin C (23). The greater antioxidant potency of most flavonoids than vitamin C indicates that one of the strong flavonoids is quercetin.

Oral carbofuran administration has increased MDA and decreased SOD, which has an impact on increasing neuronal cell death throughout the embryonic and lactation periods (6,7). Oxidative damage caused by free radicals also plays a central role in cognitive decline and spatial learning and memory (10). The oxidative stress (MDA) and antioxidant defense (GSH) markers in brain and blood samples were investigated to observe the relationship between spatial memory and oxidative stress. (11). Contamination by carbofuran in laboratory animals induces oxidative stress and impairs cognitive and spatial memory, and motor functions (3). The increase in free radicals that induce neuronal cell death due to carbofuran exposure is able to reduce motor reflex function and memory ability.

In this study, administration of carbofuran caused a significant increase in surface righting reflex and a significant decrease in swimming ability: swimming direction and head angle position as well as spatial memory ability (eight-arm radial maze). Perinatal exposure to carbofuran caused oxidative stress and oxidative stress that may contribute to the decrease in cerebellar structure and the impairment of motor coordination in hypergravity-exposed rat neonates (24). The swimming endurance test is a pharmacological screening method for determining the effect of toxic substances on movement coordination, including testing for both decreased and increased central nerve control. Table 1 shows that there is a decrease in swimming ability according to swimming direction and head angle position. This indicates that carbofuran can shorten the onset of fatigue or reduce swimming endurance. Maternal high levels of the redox-active amino acid homocysteine hyperhomocysteinemia found an increased level of MDA, and decreased SOD in the brain tissues of rats caused impairment of reflex ontogeny, locomotion, muscle strength, and motor coordination (25). Oxidative stress in the brain could cause a decrease in the ability to

swim in mice. The behavioral responses related to depression were evaluated using the modified forced swimming test. The decrease in active swimming carbofuran-treated mice was indicative of despair-like behavior as suggested by the decreased swimming in the T1 group. A depressive-like behavior has been reported in previous studies in which animals received the chlorpyrifos in offspring rats exposed during pregnancy (26).

Administration of kebar grass ethanol extract and vitamin C was able to reduce surface righting reflex time and also increase swimming ability according to head angle position and swimming direction. However, the administration of kebar grass ethanol extract was still better than vitamin C in reducing surface righting reflex time, as well as increasing swimming ability according to swimming direction and head angle position (Table 1). The antioxidant enhances swimming endurance by elevation of the antioxidant capacity of the skeletal muscles, which has thereby highlighted the potential of this natural product as an antioxidant in the treatment of fatigue (27). The study confirmed numerous motoric manifestations of cisplatin-induced neurotoxicity in rats and supplementation with N-acetylcysteine successfully prevented a decreased motor performance (28).

Carbofuran exposure during lactation (perinatal) significantly decreased learning and memory patterns, as demonstrated by increased latency time to reach food and time spent on the food arm. The number of mice who made mistakes entering the arm of the eight-arm radial maze was used to assess memory function in this study (Table 1). Both the Kruskal-Wallis and Mann-Whitney tests revealed a significant decline, showing that carbofuran exposure may impair memory function in mice. This is in line with a prior study that discovered carbofuran can cause neurobehavioral, neurochemical, and neurophysiological issues. Carbofuran exposure leads to increased expression of caspase 3, and the number of degenerative neurons in the hippocampus, leading to enormous deficits in learning and memory (2). The mechanism of action of carbofuran is compatible with the prevention of memory loss,

so the use of antioxidants as neuroprotective and preventing neuronal death by free radicals is highly recommended (29). Kebar grass ethanol extract administration could improve the memory ability of mice which was not found in the administration of vitamin C. This is in accordance with the research of Barichello *et al.* which states that the administration of antioxidants can prevent hippocampal oxidative stress as a cause of memory impairment (30). Antioxidants in kebar grass ethanol extract were better than vitamin C because antioxidants from fruits and vegetables are the best sources compared to conventional antioxidant supplements, even though high doses of the supplements are administered and it can be dangerous for those who consume them (26).

## CONCLUSION

Exposure of lactating mothers to carbofuran could cause brain oxidative stress, impaired motor reflexes, and spatial memory in mice offspring. Kebar grass ethanol extract and vitamin C administrations could prevent brain oxidative stress and inhibit disorders in motor reflexes and spatial memory in mice offspring. Kebar grass ethanol extract administration was more effective than vitamin C.

### *Acknowledgments*

The authors expressed sincere thanks to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia and Universitas Airlangga for funding research (Grant No. 1408/UN3/2019) and the Faculty of Veterinary Medicine of Universitas Airlangga that has been facilitated the implementation of this research.

### *Conflict of interests statement*

The authors declared no conflict of interest in this study.

### *Authors' contributions*

E.M. Luqman contributed to conceptualization, methodology, and software analysis; E.P. Hestianah contributed to data curation, writing-original draft preparation; W. Widjiati contributed to visualization,



investigation; S. Kuncorojakti supervised the study; V.F. Hendrawan wrote, reviewed, and edited the manuscript. The final version of the article was approved by all authors.

## REFERENCES

- Ramesh M, Narmadha S, Poopal RK. Toxicity of furadan (carbofuran 3% g) in *Cyprinus carpio*: haematological, biochemical and enzymological alterations and recovery response. Beni-Seuf Univ J Appl. 2015;4(4):314-326. DOI: 10.1016/j.bjbas.2015.11.008
- Mishra D, Tiwari SK, Agarwal S, Sharma VP, Chaturvedi RK. Prenatal carbofuran exposure inhibits hippocampal neurogenesis and causes learning and memory deficits in offspring. Toxicol Sci. 2012;127(1):84-100. DOI: 10.1093/toxsci/kfs004.
- Luqman EM, Widjiati, Hestianah EP, Hendarti GA, Yustinasari LR, Hendrawan VF. The carbofuran exposure during lactation period in reducing the motoric reflexes and memory in infant mice (*Mus musculus*). Int J Pharm Res. 2021;13(1):4831-4837. DOI: 10.31838/ijpr/2020.12.04.327
- Kamboj A, Kiran R, Sandhir R. Carbofuran-induced neurochemical and neurobehavioral alterations in rats: attenuation by N-acetylcysteine. Exp Brain Res. 2006;170(4):567-575. DOI: 10.1007/s00221-005-0241-5.
- Devi SA, Chandrasekar BKS, Manjula KR, Ishii. Grape seed proanthocyanidin lowers brain oxidative stress in adult and middle-aged rats. Exp Gerontol. 2011;46:958-964. DOI: 10.1016/j.exger.2011.08.006.
- Zheng GX, Lin JT, Zheng WH, Cao J, Zhao ZJ. Energy intake, oxidative stress and antioxidants in mice during lactation. Dongwuxue Yanjiu. 2015;36(2):95-102. DOI: 10.13918/j.issn.2095-8137.2015.2.95.
- Luqman EM, Sudiana IK, Darmanto W, Achmad AB, Widjiati. Mouse (*Mus musculus*) embryonic cerebral cortex cell death caused by carbofuran insecticide exposure. J Vet Res. 2019;63(3):413-421. DOI: 10.2478/jvetres-2019-0040.
- Syed F, John PJ, Soni I, Inderpal Soni. Neurodevelopmental consequences of gestational and lactational exposure to pyrethroids in rats. Environ Toxicol. 2016;31(12):1761-1770. DOI: 10.1002/tox.22178.
- Koziol LF, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, et al. Consensus paper: the cerebellum's role in movement and cognition. Cerebellum. 2014;13(1):151-177. DOI: 10.1007/s12311-013-0511-x.
- Ataie A, Sabetkasaei M, Haghparast A, Hajizadeh Moghaddam A, Ataie R, Nasiraei Moghaddam S. Curcumin exerts neuroprotective effects against homocysteine intracerebroventricular injection-induced cognitive impairment and oxidative stress in rat brain. J Med Food. 2010;13(4):821-826. DOI: 10.1089/jmf.2009.1278.
- Belviranlı M, Okudan N, Atalık KEN, Öz M. Curcumin improves spatial memory and decreases oxidative damage in aged female rats. Biogerontology. 2013;14(2):187-196. DOI: 10.1007/s10522-013-9422-y.
- Lisangan MM, Syarief R, Rahayu WP, Dharmaputra OS. Effect of kebar grass (*Biophytum petersianum* Klotzsch) leaf extract on the growth and morphological structure of aflatoxigenic *Aspergillus flavus*. Food Res. 2020;4(1):234-243. DOI: 10.26656/fr.2017.4(1).129.
- Sharma P, Jha AB, Dubey RS, Pessaraki M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. 2012;2012:1-26. DOI: org/10.1155/2012/217037.
- Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Al Mahmud J, et al. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. Antioxidants (Basel). 2020;9(8):681-732. DOI: 10.3390/antiox9080681.
- Luqman EM, Widjiati W, Yustinasari LR. Brain cells death on infant mice (*Mus musculus*) caused by carbofuran exposure during lactation period. Kafkas Univ Vet Fak Derg. 2018;24(6):845-852. DOI: 10.9775/kvfd.2018.20045.
- Labib MF, Widjiati, Hernawati T, Luqman EM, Kurnijasanti R, Suprayogi TW. Kebar grass (*Biophytum petersianum* K.) the effect in maintaining mice (*Mus musculus*) sperm quality exposed to dioxin. Int J Res Pharm. 2020;11(3):4977-4981. DOI: 10.26452/ijrps.v11i3.2817.
- Feather-Schussler DN, Ferguson TS. A battery of motor tests in a neonatal mouse model of cerebral palsy. J Vis Exp. 2016;(117):e53569,1-12. DOI: 10.3791/53569.
- Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The mouse forced swim test. J Vis Exp. 2012;(59):3638,1-5. DOI: 10.3791/3638.
- Richter SH, Zeuch B, Lankisch K, Gass P, Durstewitz D, Vollmayr B. Where have I been? Where should I go? Spatial working memory on a radial arm maze in a rat model of depression. PLoS One. 2013;8(4):e62458,1-10. DOI: 10.1371/journal.pone.0062458.
- 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;1-7. DOI: 10.1155/2016/7637931.
- Jaiswal SK, Siddiqi NJ, Sharma B. Carbofuran induced oxidative stress in rat heart: ameliorative effect of vitamin C. ISRN Oxid Med. 2013; 2013:1-10. DOI: 10.1155/2013/824102.

22. Aminudin A, Andarwulan N, Palupi NS, Arifiantini I. Characteristics and antioxidant activity of kebar grass (*Biophytum petersianum*) extract. *Biosaintifika*. 2020;12(2):178-185.  
DOI: 10.15294/biosaintifika.v12i2.23820.
23. Heo HJ, Lee CY. Protective effects of quercetin and vitamin C against oxidative stress-induced neurodegeneration. *J Agric Food Chem*. 2004;52(25):7514-7517.  
DOI: 10.1021/jf049243r.
24. Sajdel-Sulkowska EM, Nguon K, Sulkowski ZL, Lipinski B. Potential role of oxidative stress in mediating the effect of altered gravity on the developing rat cerebellum. *Adv Space Res*. 2007;40(9):1414-1420.  
DOI: 10.1016/j.asr.2007.08.004.
25. Yakovleva OV, Ziganshina AR, Dmitrieva SA, Arslanova AN, Yakovlev A, Minibayeva FV, *et al*. Hydrogen sulfide ameliorates developmental impairments of rat offspring with prenatal hyperhomocysteinemia. *Oxid Med Cell Longev*. 2018;2018:1-13.  
DOI: 10.1155/2018/2746873.
26. Silva JG, Boareto AC, Schreiber AK, Redivo DDB, Gambeta E, Vergara F, *et al*. Chlorpyrifos induces anxiety-like behavior in offspring rats exposed during pregnancy. *Neurosci Lett*. 2017;641:94-100.  
DOI: 10.1016/j.neulet.2017.01.053.
27. Wu R, Sun Y, Zhou T, Zhu Z, Zhuang J, Tang X, *et al*. Arctigenin enhances swimming endurance of sedentary rats partially by regulation of antioxidant pathways. *Acta Pharmacol Sin*. 2014; 35(10):1274-1284.  
DOI: 10.1038/aps.2014.70.
28. Vukovic R, Kumburovic I, Jovicic N, Velickovic S, Arnaut A, Selakovic D. Antioxidant supplementation with N-acetylcysteine as a protection against cisplatin-induced motor impairment in rats. *Serb J Exp Clin Res*. 2020;5(2):100-105.  
DOI: 10.2478/sjecr-2019-0076.
29. Lalkovičová M, Danielisová V. Neuroprotection and antioxidants. *Neural Regen Res*. 2016;11(6):865-874.  
DOI: 10.4103/1673-5374.184447.
30. Barichello T, Machado RA, Constantino L, Valvassori SS, Réus GZ, Martins MR, *et al*. Antioxidant treatment prevented late memory impairment in an animal model of sepsis. *Crit Care Med*. 2007;35(9):2186-2190.  
DOI: 10.1097/01.ccm.0000281452.60683.96.