Number 4

Meditory
(The Journal Of Medical Laboratory)

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HEPATOPROTECTIVE EFFECT OF BLACK CUMIN (Nigella sativa) AGAINST THE LIVER Aspartame-induced Rattus norvegicus BASED ON HISTOPATHOLOGICAL FEATURES

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Article history
Posted, Dec 5th,2024
Reviewed, Nov 29th, 2024
Received, Sept 10th , 2024

Abstract

Background*: In Indonesia, aspartame artificial sweeteners are contained in foods and drinks that are often consumed and increase every year. Excessive consumption of aspartame can cause alterations in the histopathological picture of the liver characterized by degeneration and cell necrosis. Hepatoprotectors are compounds that have antioxidant benefits so that they can protect the liver by reducing oxidation reactions in liver damage. One of the herbs that functions as a hepatoprotector is black cumin which contains thymoquinone.* ***Aims****: This study aims to determine hepatoprotective effect of black cumin (Nigella sativa) against the liver aspartame-induced Rattus norvegicus based on histopathological features.* ***Methods****: This study was experimental with 5 treatment groups including 1 normal group, 1 negative group and 3 aspartame and black cumin treatment groups.* ***The results****. The results of the study were based on the ANOVA test, which was 0.002 so that based on the significance value of <0.05, it can be concluded that there is an average difference between groups.* ***Conclusions****: Based on the Anova test and tukey test it can be concluded that black cumin has a protective effect on the liver histopathology of Rattus norvegicus in the form of normal cells and degenerated cells in fourth group (K4).*

Keywords*: : Aspartame, Black Cumin, Hepatoprotective, Histopathological features*

1. Introduction

Foods and drinks that contain artificial sweeteners in the form of aspartame are widely consumed in various countries including Indonesia. Cahyadi (2008) stated that the use of synthetic sweeteners such as aspartame is widely chosen by the food and beverage industry because the price is relatively cheap and has 200 times the sweetness content compared to sugar (sucrose). These foods and drinks can pose various kinds of health hazards if consumed in excessive amounts. Health problems that arise are related to organs such as the liver, kidneys, stomach, pancreas, esophagus, brain, and others (1).

According to data from the Food and Drug Supervisory Agency (2014), for the food and beverage category, the maximum limit of aspartame consumption is 50 mg / kg body weight. Excessive consumption of aspartame can cause significant changes in the histopathological picture of rat livers characterized by hydrophyic degeneration of hepatocyte cells and cell necrosis (cell death). When aspartame enters the human body, it is broken down into three compounds, namely aspartic acid, phenylalanine, and methanol (2).

The use of aspartame is not allowed for people who have brain damage because the content of phenylalanine can cause severe brain damage (3). Taking a low dose of aspartame can increase methanol levels in the body (2). While taking aspartame with excessive doses can increase the occurrence of hepatotoxicity in the human liver. In the study of Rafwiani *et al* (2018), giving energy drinks containing artificial sweeteners in the form of aspartame at doses of 50 mg / kg body weight and 75 mg / kg body weight orally for 28 days can cause damage to rat liver histopathology with the onset of hydrophyic degeneration of hepatocyte cells and cell necrosis in the liver of male *Rattus norvegicus* (4).

The liver has many functions such as metabolism, storage, the process of removing waste substances in the body, synthesis, and the process of cleansing toxins in the body. The function of detoxification in the liver is to prevent or eliminate harmful substances that enter the body. Potentially toxic compounds will be carried to the liver through the portal vein. The liver will mutate this compound through first pass metabolism (4). Hepatoprotectors are compounds that contain molecules that prevent the oxidation process so that they can reduce liver damage and can protect liver cells from toxic substances that harm the liver (5).

Black cumin or *Nigella sativa* contains many active ingredients, especially thymoquinone, which can help overcome toxicity by chemicals. The antioxidant properties of thymoquinone can prevent the effects of ROS (Reactive Oxygen Species) produced. Increasing catalase activity and playing a role in protecting liver tissue from damage is the use of thymoquinone (6). This study aims to determine hepatoprotective effect of black cumin (*Nigella sativa*) against the liver aspartame-induced *Rattus norvegicus* based on histopathological features. This research has passed the code of ethics test with letter number KEPK/UMP/42/VIII/2023.

2. Research Methods

Therapy Provision

Rattus norvegicus are treated for 7 days of acclimatization to the laboratory environment such as temperature, air, and humidity. Acclimatized rats were divided into 5 groups, group 1 is the group treated with experimental animals that are not given aspartame and are not given black cumin as a normal control, group 2 is the group that is given aspartame 100mg/200 g body weight/day without black cumin as a negative control, group 3 is the treatment group that is given aspartame with a dose of 100mg/200 g body weight/day and black cumin with a dose of 0.1 mL/200 g BB/day, Group 4 is the treatment group given aspartame with a dose of 100mg/200 g body weight/day and black cumin with a dose of 0.2 mL/200 g BB/day, and group 5 is the treatment group given aspartame with a dose of 100mg/200 g body weight/day and black cumin with a dose of 0.3 mL/200 g BB/day. The administration of aspartame and black cumin is carried out for 21 days.

Animal Termination Process

Rattus norvegicus were terminated using ether a day after giving pure aspartame and black cumin for 21 days, termination was done by cotton given ether then put in a container without air circulation then closed tightly, make sure the rat was euthanized and then liver organs were taken to be examined.

Creation of Tissue Preparations

Liver tissue histology processing is carried out in a manual way. The liver tissue is cut with a sharp knife of about 5mm then washed or rinsed with a 0.9% NaCl solution to clean the blood that is still in it. Once clean, insert the liver tissue pieces into a tissue cassette embedding and then fixate it using a 10% NBF solution for 90 minutes to preserve the liver tissue. Furthermore, the process of removing water from the liver tissue so that it can be thinly sliced, namely dehydration, is carried out by inserting the liver tissue in stratified alcohol, which is 80%, 90%, 95% and absolute alcohol (2 times) for 5 minutes each. Then clearing was carried out using xylol I solution for 1 hour and xylol II for 2 hours.

Furthermore, embeding and blocking is a process to replace the clarifying material with liquid paraffin so that the liver tissue is easily cut using microtomes, the paraffin block is allowed to stand at room temperature for 10 hours and then incubated in the freezer. Then cutting, which is the process of cutting tissue on the block using microtomes at a thickness of 3-5 mm and taken with a glass object in a floating bath at a temperature of 55-60°C. Previously, glass objects were given glycerol albumin so that the liver tissue could stick perfectly to the surface of the glass object.

Furthermore, staining hematoxylin and eosin is carried out. The first staining process is deparafinization by inserting the liver tissue in xylol 2 times for 5 minutes, then put in alcohol for 5 minutes. Then the preparation is flooded with aquadest for 10 minutes. After that, soaking

hematoxylin paint with a time of 5 minutes, then the preparation is washed with flowing water. When finished, the tissue is soaked in eosin paint with 2 minutes and rinsed with xylol. Furthermore, network mounting is carried out with enthelan. Finally, the preparation has been covered with deck glass. Observed using a microscope at 400x magnification (7).

3. Results and Discussions

This study aimed to determine the hepatoprotective effect of black cumin with stratified doses, namely 0.1 mL/200 g BB/day, 0.2 mL/200 g BB/day, and 0.3 mL/200 g BB/day on macroscopic and microscopic images of aspartame-induced *Rattus norvegicus* liver at a dose of 100 mg/200 g body weight of rats tested for 21 days. The observations analyzed are macroscopic and microscopic images on the histology of *Rattus norvegicus* livers.

Previously, black cumin was carried out phytochemical tests, namely tests of metabolite compounds contained in the content of black cumin in the form of flavonoids, antraquinones, terpenoids, and saponins. Flavonoids, interquinones, terpenoids and saponins are chemicals contained in black cumin. The chemical content consists of several active components that can overcome the toxicity caused by chemicals. One of the active components of black cumin that is useful as a hepatoprotector is thymoquinone. The presence of thymoquinone can be seen by interquinone test on black cumin. The following are the phytochemical test results contained in table 1.

Table 1. Black Cumin Phytochemical Test Results

Content	Information
Flavonoid	The formation of red color
Antarkuinon	The formation of a brownish-red color
Terpenoid	The formation of a brownish-red color
Saponin	Froth does not disappear with HCl addition

Source : Primary data (August, 2023).

The results of macroscopic observations of *Rattus norvegicus* hearts can be seen in table 1 and figure 1. Table 1 is a discriptive data from macroscopic observations of *Rattus norvegicus* liver organs from 5 groups. From the table bellow shows a color change in group K1 with groups K4 and K5. Macroscopic observations also showed that in the K2, K3, K4, and K5 groups there were white spots or spots which were a sign that there was damage to the liver of *Rattus norvegicus*. The presence of white spots on the macroscopic liver is caused by fatty liver that blocks blood flow to the liver and causes the liver to pale in color (8).

Table 2. Results of macroscopic observations of *Rattus norvegicus* liver

Group	Macroscopis	
	Color	Information
Normal Group (K1)	Brownish red	Normal
negative group (K2)	Brownish red	There is a slack
Aspartame and black cumin 0,1 ml (K3)	Brownish red	There is a slack
Aspartame and black cumin 0,2 ml (K4)	Blackish red	There is a slack
Aspartame and black cumin 0,3 ml (K5)	Blackish red	There is a slack

Source : Primary data (September, 2023).

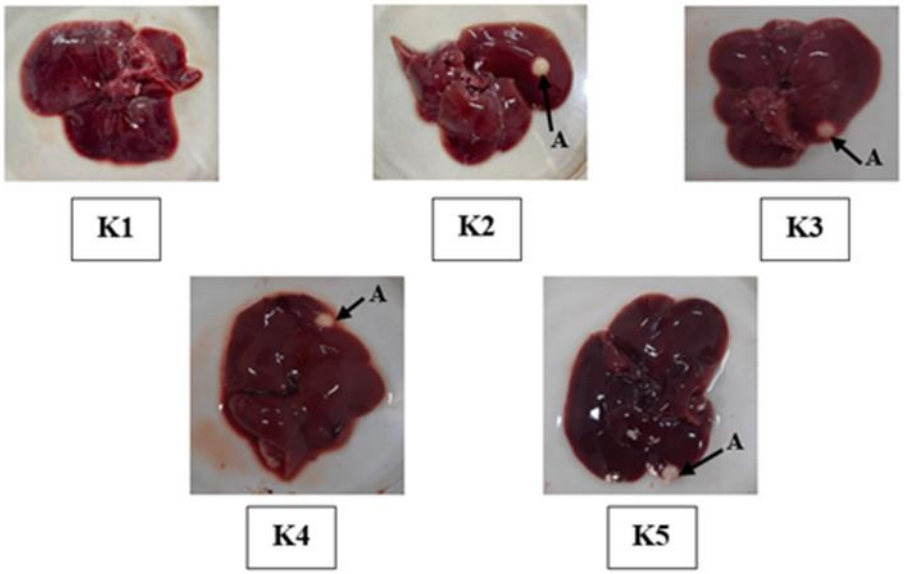

Figure 1. Macroscopic picture of the liver of *Rattus norvegicus*. K1 = Normal group, K2 = Aspartame group, K3 = aspartame and black cumin 0.1 ml, K4 = aspartame and black cumin 0.2 ml, K5 = aspartame and black cumin 0.3 ml (Personal documentation, 2023).

Figure 1 is a documentation of macroscopic observations on the liver of *Rattus norvegicus*. The results of macroscopic observations found color changes in each group. Based on the results of macroscopic observations, it can be seen that all groups have the same color, which is brownish red and there is no color change. However, white spots appeared in the form of fatty in groups K2, K3, K4 and K5 which is a sign that there is damage to the liver of *Rattus norvegicus*.

The presence of white spots on the macroscopic liver is caused by fatty liver that blocks blood flow to the liver and causes the liver to be pale. In macroscopic *Rattus norvegicus* livers, spots or lipid droplets that appear are produced and synthesized as inclusion bodies. When synthesis is inhibited, fat accumulates and fills intracellular and extracellular spaces. Fat accumulation is caused by inhibition of fatty acid oxidation, which among other things is caused by excessive NADH formation.

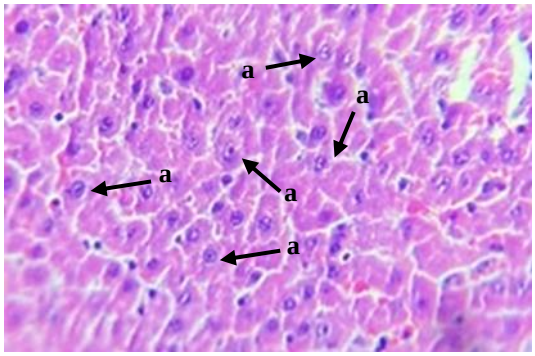

Figure 2. Histopathological features of *Rattus norvegicus* liver in normal group (K1) with magnification of 400x. Remarks : A = Normal Cells (Personal documentation, 2023).

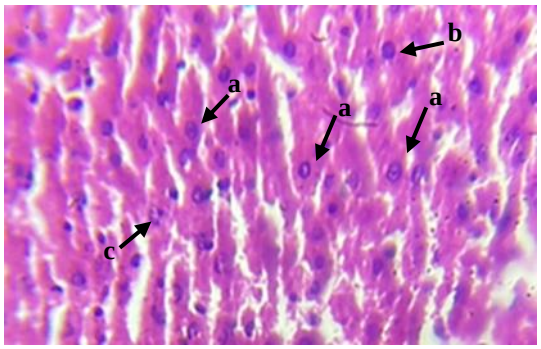

Figure 3. Histopathological features of *Rattus norvegicus* liver in the treatment group (K4) given aspartame and black cumin 0.2 mL with Hematoxylin-Eosin staining and 400x magnification. Remarks : A = Normal Cells, B = Hydropic Degeneration, C = Parenchymatous Degeneration (Personal documentation, 2023).

Based on Figure 2, microscopic observations on K1 show no degeneration and necrosis. While in figure 3, microscopic observations in the K4 treatment group given aspartame and 0.2 mL black cumin showed the presence of relatively many normal cells and a relatively mild level of damage, namely cell swelling (parenchymatous and hydrophyic degeneration).

The results of the study were based on the ANOVA test, which was 0.002 so that based on the significance value of <0.05, it can be concluded that there is an average difference between group. So that continued the Post Hoc Tukey Based on the results obtained, it can be seen that there is a significant difference between the groups, where the K4 group (treatment group 2) is the most effective dose because it has the least difference with the K1 group (normal control) and it can be seen that the administration of black cumin has a hepatoprotective effect on the histopathological picture of the liver of *Rattus norvegicus* after induction of artificial sweeteners in the form of aspartame.

In the scoring assessment, if the preparation is found damage in the form of degeneration, both in the form of parenchymatose and hydropis, the data is still assessed 2. This degeneration damage is mostly found in each treatment except in the normal group or K1 group. Degeneration is a sign of liver damage due to reversible toxins and can still return to normal if

exposure to these toxins is stopped. There are several types of cell damage, degeneration, including parenchymatous and hydropic degeneration.

Morphological changes in dead cells are referred to as cell necrosis. Microscopically, necrosis can be seen from changes that occur in the cell nucleus or cytoplasm. There are 3 kinds of necrosis, namely pycnosis necrosis, cariorexis necrosis, and karyolysis necrosis. Necrosis of picnosis is characterized by reduced cell nucleus size, loss of chromatin and dark color. Caryorexic necrosis is characterized by rupture or destruction of the cell nucleus and scattered chromatin fragments within the cell. Carolysis necrosis is characterized by the loss of the ability of cell nuclei in staining so that they do not look or appear pale (9).

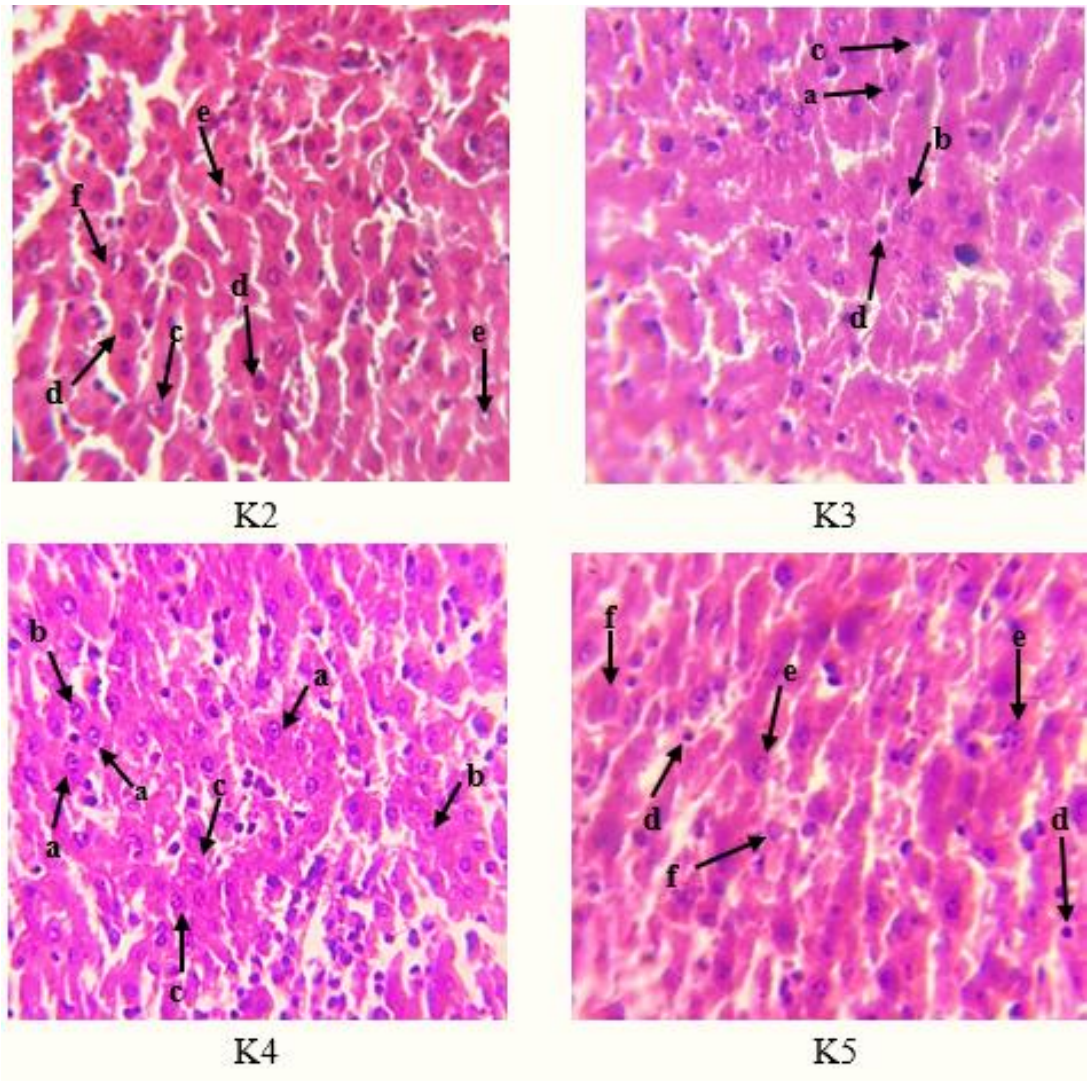

Figure 4. Histopathological picture of the liver of *Rattus norvegicus* in the negative group and treatment with Hematoxylin-Eosin staining and 400x magnification. Description: a = Normal Cells, b = Hydroponic Degeneration, c = Parenchimatous Degeneration, d = Picnotic Necrosis, e = Cariocchylytic Necrosis and f = Cryogenic Necrolysis (Personal documentation, 2023).

The picture of necrosis that occurred in the K2 negative control group was different from all test groups because this group was only given aspartame which can be damaging, while K3, K4, and K5 with the treatment of aspartame and black cumin with stratified doses of each group showed differences between degeneration and necrosis. This situation shows that the dose of cumin used is varied enough to produce varying effects as well. The K3 black cumin treatment group has begun to show improvements in liver histopathological features when compared to the K5 group. When compared to the K4 group given black cumin at a dose of 0.2 mL/200 g BB rats, the decrease was smaller with an improved degree of necrosis.

The histopathological picture in the negative control group showed the result that there was a small amount of degeneration and a considerable amount of necrosis in the field of view of each preparation. It showed that aspartame at a dose of 100 mg / 200 g body weight / day for 21 days caused damage to the liver cells of *Rattus norvegicus*. The effects of consuming aspartame for the body, especially the liver, have been mentioned in several studies such as in the study of Haliem *et al* (2018) male *Rattus norvegicus* induced aspartame at a dose of 250 mg / kg bb / day for 2 months showed histopathology of liver tissue with irregular nuclear hepatocytes, cytoplasm containing many vacuoles and few mitochondria, and cell necrosis.

Histopathological features in groups K3, K4, and K5 have been related to the administration of black cumin with stratified doses. In the damage scoring of each treatment, it was found that the K4 group given black cumin at a dose of 0.2 mL / 200 g BB rats gave the most effective results where normal cells were found and reduced damage was found. The second treatment group (K4) used a dose of 0.2 mL / 200 g body weight of rats which dose is the standard daily consumption of black cumin which has been able to provide hepatoprotective effects on the liver of *Rattus norvegicus* induced by artificial sweetener aspartame. The first (K3) and third (K5) treatment groups showed damage results that were not much different from the negative control group. That's because consumption of black cumin that is less than the normal dose and exceeds the normal dose can cause damage to the liver of *Rattus norvegicus*.

The mechanism of liver cell damage by aspartame is that when aspartame is consumed, it will be hydrolyzed in the intestine into two amino acids (Aspartic Acid and Phenylalanine) along with a number of free methanol. Phenylalanine and aspartic acid are amino acids produced by the body naturally. Methanol is known as a substance that can damage liver cells, where

methanol oxidizes to formaldehyde and then into a format. Formaldehyde can cause oxidative stress and also cell damage to various organs, including the liver. Methanol poisoning is associated with mitochondrial damage and increased microsomal proliferation, which results in an excess of oxygen free radicals. Consumption of aspartame, will cause tissue damage because the concentration of its metabolites increases in the blood as a result, several enzymes in the cell go out to the blood circulation due to an increase in the permeability of the cell membrane (2).

In accordance with the research of Risky *et al* (2018), ethanol-induced *Rattus norvegicus* in treatment group 5 given black cumin extracted with a concentration of 50% and 50% ethanol solution 0.01 mL/grBB for 14 days showed that black cumin that had been extracted had a hepatoprotective effect on the liver of *Rattus norvegicus* damaged by ethanol in the presence of mild fat degeneration in microscopic observations of *Rattus norvegicus* livers (6).

Black cumin contains many active ingredients, especially thymoquinone, which can overcome toxicity by chemicals. The antioxidant properties of thymoquinone can reduce oxidative stress and strengthen the antioxidant effects in the body. Thymoquinone, which is a black cumin content, inhibits the hepatic activity of CYP1A1/A2 isozymes which are involved in the biotransformation of xenobiotics that become genotoxic. The antioxidant properties of thymoquinone are also responsible for liver damage caused by parasites. The antioxidant properties of thymoquinone can reduce the impact of ROS (Reactive Oxygen Species) produced. Thymoquinone increases catalase activity and acts as a protector against liver tissue from trauma (10).

4. Conclusions

Based on the results of this study, it can be concluded that the administration of black cumin at a dose of 0.1 mL/200 g body weight /day; 0.2 mL/200 g body weight /day; and 0.3 mL/200 g body weight /day after induced aspartame at a dose of 100 mg/200 g BB/day for 21 days exerted a protective effect against macroscopic and microscopic *Rattus norvegicus* livers. The most effective dose in preventing liver cell damage of *Rattus norvegicus* is the fourth group (K4) which is the second treatment group with aspartame 100 mg / 200 g body weight / day and black cumin 0.2 mL / 200 g body weight / day.

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