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The Effectiveness of Star Anise (Illicium Verum) Extract Nanoemulsion on Malondialdehyde (MDA) and Blood Glucose Levels (BGL) and Histopathological Features of Alloxan-Induced Rat Liver

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ABSTRACT
Diabetes mellitus is a collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. High MDA levels in the liver are associated with liver damage and non-alcoholic fatty liver disease (NAFLD). This study aims to test and analyze the effectiveness of star anise (Illicium verum) extract nanoemulsion preparations on reducing MDA levels, KGD, and liver histopathology in alloxan-induced male Wistar rats (Rattus norvegicus). The sample of rats used was 24 in 4 groups (control, treatment 1, 2 and 3) where the extract preparation in the treatment was star anise (Illicium verum) extract nanoemulsion with concentrations of 10% and 20%. Administration of star anise extract nanoemulsion, especially at a concentration of 20%, showed a significant decrease in blood glucose levels approaching the value of the negative control group (p = 0.333).

Keywords: *Star anise, Nanoemulsion, Liver, KGD, MDA*

INTRODUCTION
Diabetes mellitus is a collective term for a heterogeneous group of metabolic disorders whose primary finding is chronic hyperglycemia. The cause is impaired insulin secretion or impaired insulin action, or usually both (Petersmann et al., 2019). Diabetes consists of two main types: type 1, which accounts for approximately 5% of cases, and type 2, which accounts for 90%–95%. Although carbohydrate metabolism is most clearly impaired and forms the basis for diagnostic biochemical tests, lipid metabolism is also negatively affected, and abnormalities in protein metabolism, although less pronounced, are also present. For example, fasting free fatty acid and triglyceride levels are elevated, and tissue uptake of amino acids, particularly branched-chain amino acids, in response to insulin is impaired (Genuth et al., 2018).
Diabetes mellitus has numerous microvascular and macrovascular complications, including periodontitis, which is the sixth most common complication of diabetes mellitus (Pavithra et al., 2025). Diabetes promotes the accumulation of reactive oxygen species (ROS) in metabolic pathways, which can lead to oxidative stress and a decrease in antioxidants (Choosong et al., 2021). Oxidative stress can be defined as a disturbance in the balance of antioxidants and prooxidants caused by various factors such as aging, drug action and toxicity, inflammation, and/or addiction. In general, oxidative stress is the excessive formation or/and inadequate removal of highly reactive molecules such as reactive nitrogen species (RNS) and ROS. Oxygen is a highly reactive species that has the ability to become part of potentially harmful and damaging molecules (Ali et al., 2023).
MDA can be produced enzymatically as a byproduct (Cordiano et al., 2023). Malondialdehyde is one of the biomarkers of diabetes mellitus. MDA acts as a marker

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system, so it can be used to diagnose abnormal reactions that develop in diabetic patients and other complications associated with diabetes mellitus. Undesirable free radical reactions occur after lipid toxicity due to red blood cell membrane peroxidation, which leads to the development of microvascular complications such as retinopathy in diabetics (Laddha et al., 2019). High levels of MDA in the liver are associated with liver damage and non-alcoholic fatty liver disease (NAFLD). Interestingly, patients with NAFLD have been shown to have significantly higher levels of MDA and other oxidative markers compared to patients with chronic viral hepatitis (Kumar et al., 2013).

One plant that can be used for phytotherapy is star anise extract. Star anise is a well-known botanical species known for its diverse bioactive compounds. Besides being a culinary spice, this plant has been widely used in traditional medicine (Kačániová et al., 2024). Numerous compounds, such as volatiles, seco-prezizaane-type sesquiterpenes, phenylpropanoids, lignans, flavonoids, and other constituents, have been identified from star anise extract (Yang et al., 2021). This plant is also used in traditional Moroccan medicine as an antidiabetic agent (Amtaghri et al., 2022).

Based on the background explanation, the researcher was interested in seeing the effectiveness of the nanoemulsion preparation of star anise (Illicum verum) extract on MDA and blood glucose levels as well as the histopathological features of the liver tissue in alloxan-induced mice.

LITERATURE REVIEW

Risk factors for diabetes mellitus include adults with a history of cardiovascular disease, hypertension (≥140/90 mmHg or currently undergoing hypertension therapy), HDL cholesterol levels <35 mg/dL (0.90 mmol/L) and/or triglyceride levels >250 mg/dL (2.82 mmol/L), physical inactivity, and other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans), among others. Furthermore, patients with prediabetes and women diagnosed with gestational diabetes mellitus are at risk of developing diabetes. People living with diabetes are at risk of developing macrovascular complications such as cardiovascular disease and microvascular complications (such as diabetic kidney disease, diabetic retinopathy, and neuropathy). These complications lead to increased mortality, blindness, kidney failure, and decreased quality of life in individuals with diabetes (Cole & Florez, 2020).

The liver plays a crucial role in detoxification related to alcohol, amphetamines, hormones, steroids, and barbiturates. Its primary function is to prevent the excessive accumulation of these substances and mitigate any potential negative effects. While metabolic detoxification is generally beneficial, there are instances where it can negatively impact hepatocytes. One example of the adverse effects of alcohol abuse is the potential harm it can cause to hepatocytes, primarily due to the byproducts of alcohol metabolism, including acetaldehyde and hydrogen. These metabolic byproducts lead to increased adipose tissue accumulation, potentially impairing liver function (Sumadewi, 2023).

METHODS

The type of research used in this study is quantitative experimental, employing a true experiment or laboratory experimental design. Experimental research is conducted by controlling all external variables that could influence the experimental activities. This study used a post-test only control group design.This study used a post-test only control group design to determine and analyze the effectiveness of using star anise (Illicum verum) extract nanoemulsion in improving MDA and blood sugar levels in alloxan-induced male white rats of the Wistar strain.

The sample of this study24 Wistar rats were used in each experimental group. The test animals were randomly divided into 4 groups.. The experimental group was divided into positive and negative control groups, treatments 1 and 2. The test animals were acclimatized

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for 7 days in the laboratory.Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. Research procedures include: acclimatization of test animals, preparation of star anise extract soaking star anise powder using 96% ethanol solvent with a concentration of 2000 ml for 3 days, phytochemical test of star anise extract to see the content of secondary metabolites in the extract, making star anise extract nanoemulsionwith 96% ethanol,Preparation of test animals, administration of treatment. Treatment groups 1 and 2 were given extract doses with concentrations of 10% and 20%, for the positive control nothing was given, and the negative control mice were induced with alloxan and given metformin. All test animals were given treatment for 21 days. MDA and KGD levels were then monitored, followed by histopathological examination of the liver. The data from the study were tabulated and analyzed using SPSS (Statistical Package for Social Science).

RESULTS

Phytochemical testing was carried out to identify the content of secondary metabolite compounds contained in star anise extract, which is suspected to have potential as a therapeutic agent. Based on the results of the phytochemical tests carried out, it can be concluded that star anise extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids.

In this study, the test animals received preconditioning treatment in the form of alloxan induction to induce diabetes mellitus. After being injected with alloxan, the mice were incubated for 3 days and blood glucose measurements were taken to determine whether the mice had diabetes mellitus. The mice were declared diabetic if their blood glucose was higher than 200 mg/dl. The successful induction condition was indicated by blood sugar levels reaching more than or equal to 200 mg/dl. Blood sugar levels were measured on days 1, 14, and 21. The changes that occurred were observed and the following results were obtained:

Table 1 Results of Blood Sugar Level Observations

No	Group	Blood Sugar Level (mg/dL) Mean ± SD Day 1	Day 14	Day 21
1	Negative Control	92.5± 8.24	94.16± 8.47	96± 8.92
2	Positive Control	252.5± 4.92	213.33 ± 5.24	118± 7.82
3	Treatment 1	253.66± 4.08	218.16 ± 6.04	123.16± 6.61
4	Treatment 2	253.3± 4.50	197.5± 4.08	100.8± 10.00

On the 14th day, the average blood sugar levels in the positive control group, treatment group 1, and treatment group 2 decreased but were not yet included in the normal blood sugar category. On the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given 10% concentration of star anise extract nanoemulsion, and treatment group 2 given 20% concentration of star anise extract nanoemulsion experienced a decrease so that blood sugar levels returned to normal, namely below 135 mg / dl. Treatment group 2 experienced the most significant decrease and was closer to the negative control group compared to the other groups. Treatment group 1 given 10% concentration of star anise extract nanoemulsion was the group with the least decrease compared to the other groups but was still included in the normal category.

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Next, MDA, ALT, and AST levels were measured to determine the effectiveness of the star anise extract nanoemulsion. Afterward, the histopathology results of the mice's livers were examined and the data analyzed.

Reporting Research Results

The following are the results of observations on MDA levels in test animals during the treatment process:

Table 2 Results of Observation of MDA Levels After Treatment

No	Group	LevelMDA(nmol/mL) Mean ± SD Day 1	Day 14	Day 21
1	Negative Control	1.78 ± 0.20	1.78 ± 0.20	1.83 ± 0.21
2	Positive Control	11.76 ± 0.59	7.04 ± 0.52	3.04 ± 0.63
3	Treatment 1	11.86 ± 0.44	8.42 ± 0.53	3.23 ± 0.52
4	Treatment 2	11.65 ± 0.51	6.91 ± 0.56	2.42 ± 0.66

On the 14th day, the MDA levels of the test animals were re-measured to observe the changes that occurred. The negative control group that was not induced by alloxan and was only given distilled water showed MDA levels with an average of 1.78 ± 0.20 nmol/mL. The positive control group that was given metformin had an average of 7.04 ± 0.52 nmol/mL. Treatment group 1 that was given a nanoemulsion of star anise extract with a concentration of 10% had an average of 8.42 ± 0.53 nmol/mL. Treatment group 2 with a nanoemulsion of star anise extract with a concentration of 20% had an average of 6.91 ± 0.56 nmol/mL. On the 14th day, the average MDA levels in the positive control group, treatment group 1, and treatment group 2 decreased. On the 21st day, the MDA levels of the mice were re-measured to observe the changes that occurred in all treatment groups. The negative control group obtained an average result of 1.83 ± 0.21 nmol/mL. The positive control group obtained an average result of 3.04 ± 0.63 nmol/mL. Treatment group 1 which was given 10% star anise extract nanoemulsion obtained a result of 3.23 ± 0.52 nmol/mL. Treatment group 2 which was given star anise extract nanoemulsion with a concentration of 20% obtained an average result of 2.42 ± 0.66 nmol/mL.

Observations of changes in ALT levels were carried out periodically, namely on day 1, day 14, and day 21. The following are the results of observations on ALT levels of test animals during the treatment process:

Table 3 Results of ALT Level Observations After Treatment

No	Group	ALT Level (IU/L) Mean ± SD Day 1	Day 14	Day 21
1	Negative Control	23.88 ± 0.84	23.89 ± 0.84	23.92 ± 0.84
2	Positive Control	51.07 ± 0.78	34.04 ± 0.65	26.07 ± 1.40
3	Treatment 1	51.86 ± 0.87	36.12 ± 0.69	26.99 ± 0.67
4	Treatment 2	51.75 ± 0.91	32.30 ± 0.86	23.89 ± 1.05

On the 14th day, the ALT levels of the test animals were measured again to see the changes that occurred. The negative control group that was not induced by alloxan and was only

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given distilled water showed ALT levels with an average of 23.89 ± 0.84 IU/L. The positive control group that was given metformin got an average of 34.04 ± 0.65 IU/L. Treatment group 1 that was given a nanoemulsion of star anise extract with a concentration of 10% got an average of 36.12 ± 0.69 IU/L. Treatment group 2 with a nanoemulsion of star anise extract with a concentration of 20% got an average of 32.30 ± 0.86 IU/L. On the 14th day, the average ALT levels in the positive control group, treatment group 1, and treatment group 2 decreased. On the 21st day, the ALT levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group obtained an average result of 23.92 ± 0.84 IU/L. The positive control group obtained an average result of 26.07 ± 1.40 IU/L. Treatment group 1 which was given 10% star anise extract nanoemulsion obtained a result of 26.99 ± 0.67 IU/L. Treatment group 2 which was given star anise extract nanoemulsion with a concentration of 20% obtained an average result of 23.89 ± 1.05 IU/L.
Observations of changes in AST levels were carried out after alloxan induction, namely on day 1, day 14, and day 21. The following are the results of observations on AST levels of test animals during the treatment process:

Table 4 Results of Observation of AST Levels After Alloxan Induction

No	Group	AST Level (IU/L) Mean ± SD		
		Day 1	Day 14	Day 21
1	Negative Control	56.47 ± 0.80	56.48 ± 0.80	56.50 ± 0.81
2	Positive Control	97.70 ± 2.88	83.01± 1.14	58.64 ± 1.03
3	Treatment 1	97.06 ± 2.57	84.08 ± 0.68	71.28 ± 1.52
4	Treatment 2	96.7 ± 2.85	80.87 ± 0.55	57.30 ± 1.19

On the 14th day, the AST levels of the test animals were measured again to see the changes that occurred. The negative control group that was not induced by alloxan and was only given distilled water showed AST levels with an average of 56.48 ± 0.80 IU/L. The positive control group that was given metformin got an average of 83.01 ± 1.14 IU/L. Treatment group 1 that was given a nanoemulsion of star anise extract with a concentration of 10% got an average of 84.08 ± 0.68 IU/L. Treatment group 2 with a nanoemulsion of star anise extract with a concentration of 20% got an average of 80.87 ± 0.55 IU/L. On the 14th day, the average AST levels in the positive control group, treatment group 1, and treatment group 2 decreased. On the 21st day, the AST levels of the mice were measured again to see the changes that occurred in all treatment groups. The negative control group obtained an average result of 56.50 ± 0.81 IU/L. The positive control group obtained an average result of 58.64 ± 1.03 IU/L. Treatment group 1 which was given a nanoemulsion of star anise extract at a concentration of 10% obtained a result of 71.28 ± 1.52 IU/L. Treatment group 2 which was given a nanoemulsion of star anise extract at a concentration of 20% obtained an average result of 57.30 ± 1.19 IU/L.
Histopathological observations were then performed using a light microscope at 400x magnification. The aim of these observations was to evaluate the morphological structure and possible pathological changes in the liver tissue. The effects of alloxan and star anise extract induction revealed histopathological changes such as fatty degeneration and necrosis.
In each preparation, the average value of liver damage was calculated according to the category. With the following score: 0 = Normal, no pathological changes; 1 = Degeneration or necrosis focused on one location; 2 = Degeneration or necrosis in several locations; 3 = Necrosis throughout the location. The results of the histopathological examination of the liver of the negative control white rat were normal. The appearance of normal cells is indicated by hepatocyte cells that have round nuclei, and are arranged systematically in a plate-like shape towards the central lobule (central vein).

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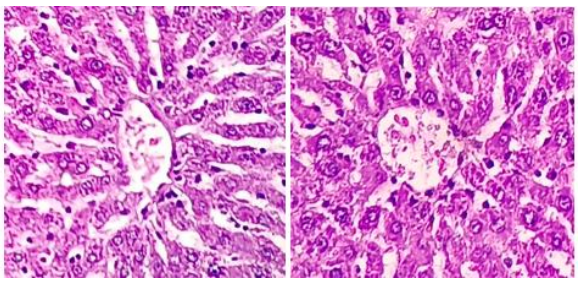

Figure 1 Histopathology of the Liver of the Negative Control Group

The positive control group was given alloxan and metformin induction. This group showed signs of histological liver structure recovery. Microscopically, the hepatocytes began to reorganize around the central vein, indicating the restoration of previously disrupted lobules. Although degeneration was still identified in some hepatocytes, the sinusoid morphology showed improvement toward normal conditions. Furthermore, the central vein structure appeared intact without inflammatory cell infiltration. The presence of mild necrosis indicated that the regenerative process was ongoing, although not yet fully complete.

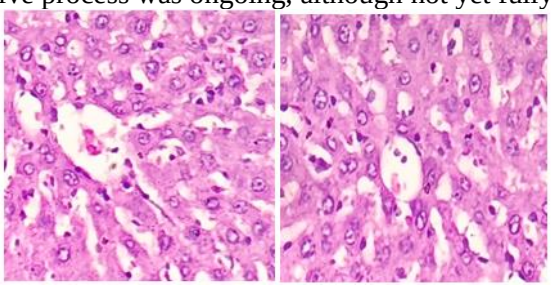

Figure 2 Histopathology of the Liver of the Positive Control Group

Treatment group 1 was given alloxan induction and a 10% concentration of star anise extract nanoemulsion. The rat liver tissue showed quite severe damage, characterized by hepatocyte necrosis in several areas. This necrosis was seen as loss of cell boundaries, shrinking nuclei (pyknosis), and nuclear lysis (karyolysis). The structure of the liver lobules appeared irregular, with disorganized hepatocyte arrangement around the central vein. Hepatic sinusoids were dilated and irregular, and there was mild to moderate infiltration of inflammatory cells around the necrotic areas. These findings indicate significant liver dysfunction due to cellular damage.

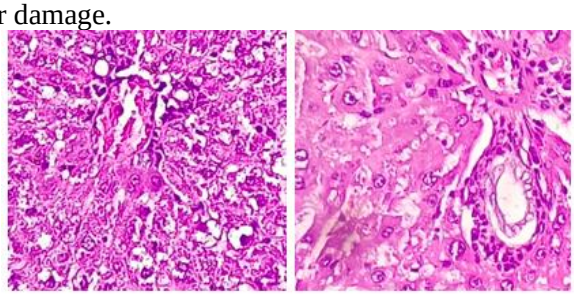

Figure 3 Histopathology of the Liver in Treatment Group 1

Treatment group 2 was induced by alloxan and a 20% concentration of star anise extract nanoemulsion. Treatment group 2 showed signs of improved liver tissue structure, although degeneration was still observed in hepatocyte cells. Hepatocytes began to re-arrange radially from the central vein, indicating restoration of lobular architecture. Little necrosis was found in this group. Sinusoids began to show normal morphology, and the central vein appeared intact without any signs of inflammation.

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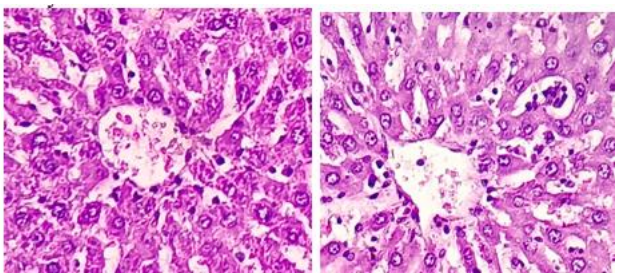

Figure 4 Histopathology of the Liver in Treatment Group 2

Here are the scores:

Table 8 Liver Histopathology Score

Group	Mean Score ± SD	Interpretation
Negative Control	0.16 ± 0.40	No pathological changes
Positive Control	1.66 ± 0.81	Degeneration or necrosis in one area
Treatment 1	2.5 ± 0.54	Degeneration or necrosis in several places
Treatment 2	1.33 ± 0.51	Degeneration or necrosis in one area

In data analysis,The normality test in this study used the Shapiro Wilk test. At the levels of MDA, ALT and AST p value>0.05 Therefore, it can be concluded that the data of the negative control group, positive control, treatment 1, and treatment 2 are normally distributed. In the homogeneity test with the Levene test the MDA significance value is 0.174, the ALT significance value is 0.189, and the AST significance value is 0.415. In the results of One-Way Anova the significance value produced is 0.000 or <0.05. Based on these data it can be concluded that there is a significant difference between the control group and the treatment group.

DISCUSSION

This study was conducted to test and analyze the effectiveness of a nanoemulsion preparation of star anise (Illicium verum) extract on reducing MDA, KGD, and liver histopathology in male Wistar rats (Rattus norvegicus) induced by alloxan. The background of the study explains that diabetes mellitus is a collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is impaired insulin secretion or impaired insulin effect or usually both (Petersmann et al., 2019).

First, a phytochemical test was conducted to determine the content of the star anise extract nanoemulsion. Based on the results of the phytochemical test, it can be concluded that star anise extract contains secondary metabolites in the form of flavonoids, saponins, tannins, alkaloids, and triterpenoids. Observations were continued on blood sugar levels. Based on the results of blood sugar level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given star anise extract nanoemulsion at a concentration of 10%, and treatment group 2 given star anise extract nanoemulsion at a concentration of 20% experienced a decrease so that blood sugar levels returned to normal, namely below 135 mg / dl. Treatment group 2 experienced the most significant decrease and approached the negative control group compared to the other groups. Treatment group 1 given star anise extract nanoemulsion at a concentration of 10% was the group with the least decrease compared to the other groups but was still included in the normal category.

Based on the results of MDA level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given nanoemulsion of star anise extract at a concentration of 10%, and treatment group 2 given nanoemulsion of star

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anise extract at a concentration of 20% experienced a decrease. Treatment group 2 experienced the most significant decrease and was close to the negative control group compared to the other groups. Treatment group 1 given nanoemulsion of star anise extract at a concentration of 10% was the group with the least decrease compared to the other groups.
Based on the results of ALT level measurements on the 21st day, it can be concluded that the positive control group given metformin, treatment group 1 given nanoemulsion of star anise extract at a concentration of 10%, and treatment group 2 given nanoemulsion of star anise extract at a concentration of 20% experienced a decrease. Treatment group 2 experienced the most significant decrease and was closer to the negative control group compared to the other groups. Treatment group 1 given nanoemulsion of star anise extract at a concentration of 10% was the group with the least decrease compared to the other groups.
Observation of liver condition was observed based on histopathological images of liver tissue. Based on the results of observations in each group, it can be concluded that the negative control group did not experience cell damage so that it got a score of 0.16 ± 0.40. The positive control group given metformin experienced degeneration or necrosis in one place so that it got a score of 1.66 ± 0.81. Treatment group 1 which was given a nanoemulsion of star anise extract with a concentration of 10% experienced degeneration or necrosis in several places so that it got a score of 2.5 ± 0.54. Finally, treatment group 2 which was given a nanoemulsion of star anise extract with a concentration of 20% experienced degeneration or necrosis in one place so that it got a score of 1.33 ± 0.51. These results indicate that administration of star anise extract nanoemulsion at a higher concentration tends to provide a better protective effect on liver tissue than lower concentrations.

CONCLUSION

1. Phytochemical tests showed that star anise extract contains secondary metabolites such as flavonoids, saponins, alkaloids, tannins, and triterpenoids. These compounds are known to play important roles in antioxidant and anti-inflammatory activity, as well as protecting cells against diabetes-related damage.
2. Administration of star anise extract nanoemulsion, especially at a concentration of 20%, showed a significant decrease in blood glucose levels approaching the value of the negative control group ($p = 0.333$).
3. The significant reduction in MDA levels in treatment group 2 indicates that the star anise extract nanoemulsion is capable of reducing oxidative stress. Active compounds such as flavonoids and triterpenoids in the extract are thought to act as antioxidants, scavenging free radicals, thereby reducing lipid peroxidation products such as MDA.
4. High ALT and AST levels are indicators of hepatocellular damage due to oxidative stress. A significant decrease in the levels of these two enzymes in treatment group 2 indicates that administration of 20% star anise extract can help restore liver function impaired by alloxan induction, even to nearly normal levels.
5. Based on liver tissue analysis, treatment group 2, with a 20% concentration, showed minimal tissue damage (necrosis in only one area), compared to treatment group 1, which showed more extensive damage. This supports the claim that high doses of star anise nanoemulsion have a protective effect on hepatocytes.

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