



## Determination of some biomarkers in male rats exposed to oxidative stress and treated with macadamia oil

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<https://doi.org/10.29072/basjs.20260106>

### ARTICLE INFO

Received: 09 September 2025

Accepted: 23 February 2026

Published: 30 April 2026



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### Keywords:

Oxidative stress, hydrogen peroxide, macadamia oil, antioxidants, rats.

### ABSTRACT

Oxidative stress is bad because it destroys cells and damages organs including the heart, liver, and kidneys. Eating additional antioxidants, such as different oils, can help reduce the levels of biomarkers that are associated to oxidative stress. This can help reduce the risk of the diseases progressing. In a study of the protective role of macadamia oil (MO) against oxidative stress induced by H<sub>2</sub>O<sub>2</sub>. Twenty male Wistar rats were randomly assigned to four groups, each consisting of 5 rats. The G 1 (negative control group) was given drinking water, the G2 (positive control group) was given regular feed and drinking water containing only 0.5% H<sub>2</sub>O<sub>2</sub>, the G3 (healthy rats + MO) was given regular feed and drinking water, and daily oral 0.5 ml/kg dose of macadamia oil, while the G 4 (H<sub>2</sub>O<sub>2</sub> +MO) was given regular feed and drinking water containing 0.5% H<sub>2</sub>O<sub>2</sub>, plus a daily oral dose of MO, and measuring some biochemical parameters, such as glucose, uric acid urea and creatinine, AST, ALT, LDH, Troponin I, CK-BM, T. C, T.G, HDL, LDL, CRP, MDA, GSH, weight of animals. Also the rats were euthanized, and the liver, heart, aorta, and kidneys tissues were histopathologically evaluated. The study's results revealed that treatment with H<sub>2</sub>O<sub>2</sub> (G2) caused most biochemical markers to rise significantly, like glucose, urea, creatinine, uric acid, AST, ALT, LDH, Troponin I, CK-BM, and there were big changes in blood lipids, TC, T.G, HDL levels dropped, C-RP levels went up sharply, and GSH levels dropped sharply. MDA levels also went up sharply compared to G1. Even though they began with a lower weight, the positive control group ended up with a greater final weight (217.00 ± 3.89 g) than the negative control group. When treated with MO, on the other hand, (G3 and G4) exhibited a significant recovery, with all indications reverting to normal or near to normal levels. According to the findings, macadamia oil can be used to combat inflammation, antioxidant system facilitation and the preservation of the metabolic equilibrium in cases of oxidative stress.

## 1. Introduction

Oxidative stress is a fundamental biological process associated with the development of many chronic diseases, such as heart disease, diabetes, and neurological disorders. This stress arises from an imbalance between the production of free radicals, known as reactive oxygen species (ROS), and the body's defense capabilities, represented by antioxidant systems [1]. Damage to DNA, RNA, proteins, lipids, the cell plasma membrane, or the inner membrane of mitochondria and the nuclear envelope is associated with impaired circulatory and cardiac function [2]. Hydrogen peroxide ( $H_2O_2$ ) is a common catalyst for studying the effects of oxidative stress in living things because it can make free radicals that hurt cells and tissues, especially in the liver and kidneys. In this study, we need to find out how to protect it from the harmful effects of hydrogen peroxide to avoid causing damage to biological molecules. Macadamia (*Macadamia integrifolia*) is one of the most common nuts all over the world for its health benefits and nutritional value [3,4]. Recently, people were more interested in consuming macadamias due to its impact in reducing inflammation and oxidative stress, as well as lowering the risk of atherosclerosis, hyperlipidemia, and heart disease [5]. Many studies focused on Macadamia oil because of its unique fatty acid composition and bioactivity. Therefore, it is considered as a good source of monounsaturated fatty acids such as oleic acid and palmitoleic acid, both have been found to provide protection against oxidants and lipid peroxidation. Moreover, macadamia oil also comprises of several minor bioactive components such as polyphenols, tocopherols and phytosterol which may enhance its antioxidant potential and cellular protective effects when exposed to oxidative stress [6-10]. Experimental evidence has shown that such oils in the diet may modulate biomarkers of oxidative stress and improve antioxidant defenses in vital organs [11,12]. However, limited information is available regarding the effect of macadamia oil on biochemical biomarkers in male rats in oxidative stress induced by hydrogen peroxide. Therefore, the purpose of this study was to examine the antioxidant and protective properties of macadamia oil in male rats against hydrogen peroxide-induced oxidative stress by evaluation selected biomarkers related to biochemical and oxidative stress.

## 2. Materials and Methods

### 2.1 Sample collection

This type of macadamia was collected by searching the city of Sulaymaniyah within the Kurdistan Region of Iraq on the local markets. The shells were then discarded, and their pulp placed in clean and dry airtight bags that can be stored in a dry and cool area until it was needed for the experiment. Care was taken during the process of collecting the samples to prevent damage or contamination and to ensure accurate results.

## 2.2 Oil extraction

According to Al-Ta'ee (2014), a continuous Soxhlet apparatus was used to extract oil from macadamia pulp. Later, the oil that we got at the end of the extraction process was measured, then poured it into clean, opaque, brown glass bottles with tightly sealed caps. It was then put in the fridge (4°C) until it was needed for chemical or biological analysis [13].

## 2.3 Animal model and housing

Twenty male Wistar strain rats aged two months and weighing between 180 and 220 grams were obtained at the Laboratory Animal House, College of Veterinary Medicine, University of Mosul. They were aerated at a constant temperature of 24 +1 °C and a normal light-dark regime that was 12 hours. It fed the animals special feed and water and also used special drinking bottles. There was a proposal to maintain a good level of hygiene and sterilization of the cage. No treatment was administered for two weeks after which the animals were examined to confirm they were disease-free.

## 2.4 Design of experiment

A total of 20 male Wistar rats were randomly assigned into four categories of 5 rats, and this was done by selecting the rats with similar weights (20 male Wistar rats). The rats were treated after the acclimatization period ended, defined as two weeks, after which the treatment was administered via oral tube feeding once daily for 30 uninterrupted days. The treatments were suggested, taking into consideration the personal needs of the experiment:

**Group 1:** (negative control group): was given only drinking water and regular feed throughout the one-month experimental period.

**Group 2:** (positive control group): was given regular feed and drinking water containing only 0.5% hydrogen peroxide throughout the one-month experimental period.

**Group 3:** (healthy rats + macadamia oil group) was given regular feed with regular drinking water, in addition to a daily oral dose of macadamia oil (0.5 ml /kg of body weight) [14].

**Group 4:** (hydrogen peroxide + macadamia oil group) was given regular feed and drinking water containing 0.5% hydrogen peroxide, in addition to a daily oral dose of macadamia oil (0.5 ml/kg of body weight).

## 2.5 Blood sample collection

After the administration of the ether anesthesia, the eye socket was also used in the harvesting of blood on a special capillary tube, a few seconds after the experiment had been performed using the rats. Test tubes were sterilized and uncoagulated, gel-free, and finely loaded with blood itself. The tubes were centrifuged at 3,000 rpm for 15 minutes to obtain the serum. The serum was subsequently pipetted into small Eppendorf tubes and clean, labelled dry tubes. The serum was stored in a refrigerator at a low temperature (-20°C) until biochemical examinations were performed.

## 2.6 Biochemical assays

Biochemical test glucose, uric acid, urea, and creatinine measurement by manuscript (Biolabo-France), ALT, and AST measurement spectrophotometrically by manuscript (Biolabo-France), while MDA, GSH measurement by ELISA kit manuscript (SunLong-China) based on Sandwich – ELISA kit. The concentration of triglycerides and cholesterol, LDL, HDL in the blood serum was estimated using the analysis kit provided by the French company Biolabo [15].

## 2.7 Heart function test

We used an Ichroma kit from Geodudanji 1-gil, Dongnae-myeon, Chuncheon-si, Gangwon-do, Republic of Korea, to find out how much troponin I was in the serum. We used a kit from Biolabo France [16] to check the levels of CK-MB. We were able to find the activity of lactate dehydrogenase (LDH) by using the [17] method, which turns pyruvate into lactate in the presence of NADH.

## 2.8 Histological preparations

The animals were put to sleep, and their blood was taken. Then, they were killed by cervical dislocation, and microscopic slides were made using the method in [18]. The pins held the animal's limbs in place in a dissection tank. Sharp scissors were used to make an incision in the shape of an upside-down "T" from the beginning of the abdominal cavity to the end of the upper sternum. A scalpel was used to cut out all the internal organs, such as the liver and kidneys. The liver, heart, and aorta were then put in a Petri dish with 0.9% physiological saline (NaCl) to wash them. After that, they were put in a fixation solution (10% formalin) for 48 hours. Next, a step-by-step plan for the samples was made [14].

## 2.9 Statistical analysis

Data was conveyed as a (mean value  $\pm$  SE). An analysis of variance (ANOVA) was conducted using a one-way approach. The GraphPad Prism 5 software was subsequently used to compare the different groups through the Tukey test. Otherwise, the P 0.05 was considered statistically significant.

## 3. Results

### 3.1. Serum glucose levels

The results in the Table 1 indicate that glucose levels were highest ( $P \leq 0.01$ ) in the positive control group, while G3 and G4 showed values close to the negative control group. The first group (negative healthy control) revealed a normal level of glucose ( $3.92 \pm 0.29$  mmol/L), while the second group (positive control), which was exposed to oxidative stress resulting from the administration of 0.5% hydrogen peroxide, showed higher concentrations of glucose ( $8.19 \pm 0.36$  mmol/L). The third group G3 (healthy rats treated with macadamia oil), showed glucose level of ( $4.25 \pm 0.25$  mmol/L), with no significant difference compared to the negative healthy control group. The group with hydrogen peroxide plus macadamia oil showed a substantially higher decline in glucose levels ( $3.67 \pm 0.22$  mmol/L) than the group that simply got hydrogen peroxide. When compared to the healthy groups, there wasn't a massive difference.

**Table 1: Comparison between difference groups in Glucose**

Group	Mean $\pm$ SE of Glucose (mmol/L)
Negative control (-ve)	3.92 $\pm$ 0.29 b
Positive control (+ve)	8.19 $\pm$ 0.36 a
G3	4.25 $\pm$ 0.25 b
G4	3.67 $\pm$ 0.22 b
L.S.D.	**
P-value	0.0001

Means having with the different letters in same column differed significantly.

\*\* ( $P \leq 0.01$ ).

### 3.2. Kidney function parameters

The kidney function parameters results are shown in Table 2, where there was a significant increase ( $P \leq 0.01$ ) in urea ( $12.48 \pm 0.86$  mmol/L), creatinine ( $125.76 \pm 20.32$   $\mu$ mol/L) and uric acid ( $214.62 \pm 13.81$   $\mu$ mol/L) levels in the positive control group G2 compared to the negative control group G1, which had the lowest levels of urea ( $4.25 \pm 0.35$  mmol/L), creatinine ( $48.93 \pm 6.50$   $\mu$ mol/L), and uric acid ( $73.87 \pm 4.39$   $\mu$ mol/L). Group 3 (G3) (Macadamia Oil Only): The kidney function markers were similar to those of the healthy group G1 and did not differ significantly, which means that macadamia oil does not hurt kidney function. Group 4 (G4 - Hydrogen Peroxide + Macadamia Oil): Even though they were exposed to hydrogen peroxide, this group had lower levels of kidney function markers: urea ( $3.73 \pm 0.32$  mmol/L), creatinine ( $52.65 \pm 4.63$   $\mu$ mol/L), and uric acid ( $64.51 \pm 3.85$   $\mu$ mol/L), which are closer to the normal levels in G1. This shows that macadamia oil may repair kidney damage caused by oxidative stress.

**Table 2: Comparison between difference groups in Kidney functions**

Group	Means $\pm$ SE		
	Urea (mmol/L)	Creatinine ( $\mu$ mol / L)	Uric acid ( $\mu$ mol / L)
<b>Negative control (-ve)</b>	4.25 $\pm$ 0.35 b	48.93 $\pm$ 6.50 b	73.87 $\pm$ 4.39 b
<b>Positive control (+ve)</b>	12.48 $\pm$ 0.86 a	125.76 $\pm$ 20.32 a	214.62 $\pm$ 13.81 a
<b>G3</b>	4.49 $\pm$ 0.46 b	47.33 $\pm$ 6.81 b	68.16 $\pm$ 5.48 b
<b>G4</b>	3.73 $\pm$ 0.32 b	52.65 $\pm$ 4.63 b	64.51 $\pm$ 3.85 b
<b>L.S.D.</b>	1.629 **	34.29 **	23.938 **
<b>P-value</b>	0.0001	0.0003	0.0001

Means having with the different letters in same column differed significantly.

\*\* ( $P \leq 0.01$ ).

### 3.3. Liver function

Table 3 show how different treatments affect liver function indicators. The levels of both AST and ALT were much higher ( $P \leq 0.01$ ) in the positive control group G2 that was exposed to hydrogen peroxide  $H_2O_2$ . The average AST levels were  $275.00 \pm 16.81$  IU/L, and the ALT levels were  $112.20 \pm 7.41$  IU/L. In the negative control group G1, the levels were  $180.60 \pm 5.41$  IU/L and  $33.60 \pm 2.58$  IU/L, respectively. in contrast, G3 and G4 groups exhibited significantly reduced levels of AST ( $158.40 \pm 6.41$  IU/L and  $153.80 \pm 8.49$  IU/L, respectively) and ALT

( $38.20 \pm 3.04$  IU/L and  $41.60 \pm 5.24$  IU/L, respectively) compared to the positive control group ( $275.00 \pm 16.81$  IU/L for AST and  $112.20 \pm 7.41$  IU/L for ALT).

**Table 3: Comparison between different groups in Liver functions**

Group	Means $\pm$ SE	
	AST (IU/L)	ALT (IU/L)
Negative control (-ve)	$180.60 \pm 5.41$ b	$33.60 \pm 2.58$ b
Positive control (+ve)	$275.00 \pm 16.81$ a	$112.20 \pm 7.41$ a
G3	$158.40 \pm 6.41$ b	$38.20 \pm 3.04$ b
G4	$153.80 \pm 8.49$ b	$41.60 \pm 5.24$ b
L.S.D.	30.898 **	14.87 **
P-value	0.0001	0.0001

Means having with the different letters in same column differed significantly.

### 3.4. Heart function

Table 4 illustrates the results of heart function parameters like CK-BM (Creatine Kinase-MB), Troponin I, and LDH (Lactate Dehydrogenase). The positive (+ve) group, which only got hydrogen peroxide, had much higher levels of all heart parameters than the negative (healthy) group. For instance, LDH levels were  $641.60 \pm 60.24$  U/L, Troponin I levels were  $63.80 \pm 3.65$  ng/L, and CK-BM levels were  $398.20 \pm 51.10$  ng/L. The negative group, on the other hand, had low levels of these parameters ( $163.60 \pm 9.41$  U/L,  $5.47 \pm 0.38$  ng/L, and  $49.20 \pm 4.59$  ng/L, respectively). The groups of G3 and G4 showed a lot of reduction in the cardiac parameters as registered. G3 recorded levels of LDH ( $150.60 \pm 10.14$  U/L), Troponin I ( $4.16 \pm 0.36$  ng/L), and CK-BM ( $43.80 \pm 3.28$  ng/L), while G4 recorded ( $172.00 \pm 15.01$  U/L), ( $3.69 \pm 0.39$  ng/L) and ( $42.00 \pm 3.30$  ng/L) for LDH, Troponin I and CK-BM, respectively.

**Table 4: Comparison between different groups in Heart functions**

Group	Means $\pm$ SE		
	LDH (U/L)	Troponin I (ng/L)	CK-BM (ng/L)
Negative control (-ve)	$163.60 \pm 9.41$ b	$5.47 \pm 0.38$ b	$49.20 \pm 4.59$ b
Positive control (+ve)	$641.60 \pm 60.24$ a	$63.80 \pm 3.65$ a	$398.20 \pm 51.10$ a
G3	$150.60 \pm 10.14$ b	$4.16 \pm 0.36$ b	$43.80 \pm 3.28$ b
G4	$172.00 \pm 15.01$ b	$3.69 \pm 0.39$ b	$42.00 \pm 3.30$ b
L.S.D.	95.357 **	5.563 **	77.224 **
P-value	0.0001	0.0001	0.0001

Means having with the different letters in the same column differed significantly. \*\* ( $P \leq 0.01$ ).

### 3.5. Lipid profile

Table 5 shows the results of lipid profile parameters. Some of these numbers include triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein. The positive (+ve) group, which only got hydrogen peroxide, had much higher levels of cholesterol levels ( $7.13 \pm 0.38$  mmol/L), triglycerides level ( $2.918 \pm 0.20$  mmol/L), LDL levels ( $5.635 \pm 0.25$  mmol/L), compared to the healthy group G1 ( $3.04 \pm 0.19$  mmol/L,  $0.774 \pm 0.04$  mmol/L,  $1.615 \pm 0.28$  mmol/L, respectively). The two groups that received macadamia oil (G3 and G4) had lower total cholesterol levels ( $2.86 \pm 0.12$  and  $3.25 \pm 0.16$  mmol/L, respectively), triglycerides ( $0.579 \pm 0.04$ , and  $0.561 \pm 0.05$  mmol/L, respectively), LDL ( $0.932 \pm 0.10$ ,  $1.096 \pm 0.07$  mmol/L, respectively). According to HDL, the highest value was recorded in the healthy group (-ve) ( $3.07 \pm 0.24$  mmol/L), while it decreased sharply in the (+ve) group ( $0.604 \pm 0.07$  mmol/L), reflecting the negative effect of oxidative stress on HDL cholesterol. However, groups G3 and G4 showed a significant improvement in HDL levels ( $1.663 \pm 0.10$  and  $1.668 \pm 0.09$  mmol/L), indicating the role of macadamia oil in improving HDL ratios.

**Table 5: Comparison between different groups in Lipid profile**

Group	Means $\pm$ SE			
	T. Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Negative control (-ve)	$3.04 \pm 0.19$ b	$0.774 \pm 0.04$ b	$3.07 \pm 0.24$ a	$1.615 \pm 0.28$ b
Positive control (+ve)	$7.13 \pm 0.38$ a	$2.918 \pm 0.20$ a	$0.604 \pm 0.07$ c	$5.635 \pm 0.25$ a
G3	$2.86 \pm 0.12$ b	$0.579 \pm 0.04$ b	$1.663 \pm 0.10$ b	$0.932 \pm 0.10$ c
G4	$3.25 \pm 0.16$ b	$0.561 \pm 0.05$ b	$1.668 \pm 0.09$ b	$1.096 \pm 0.07$ bc
L.S.D.	0.709 **	0.324 **	0.431 **	0.604 **
P-value	0.0001	0.0001	0.0001	0.0001

Means having the different letters in the same column differed significantly.

\*\* ( $P \leq 0.01$ ).

### 3.6. GSH, MDA, and C-RP levels

Table 6 shows a statistical comparison of the levels of C-reactive protein (CRP), reduced glutathione (GSH), and malondialdehyde (MDA) in different experimental groups.

The C-reactive protein (CRP) level was greatest in the positive (+ve) group, it was an average of  $59.60 \pm 15.75$  mg/L. The negative group (-ve), on the other hand, only got  $6.80 \pm 1.35$  mg/L.

The values of CRP dropped from  $8.40 \pm 1.46$  mg/L to  $8.40 \pm 1.47$  mg/L in both Group G3 (which just had macadamia oil) and Group G4 (which had hydrogen peroxide plus macadamia oil). The positive group had a significant drop in glutathione (GSH) levels ( $1.626 \pm 0.27$   $\mu$ mol/L). On the other hand, groups G3 and G4 had levels that were still rather high ( $7.93 \pm 0.43$  and  $6.92 \pm 0.78$   $\mu$ mol/L, respectively), which are close to those of the control group.

As for malondialdehyde (MDA) levels, the positive (G2) group showed a significant increase ( $5.95 \pm 0.46$   $\mu$ mol/L), compared to the healthy control group (G1) ( $1.271 \pm 0.19$   $\mu$ mol/L), while groups G3 and G4 recorded significantly lower levels ( $1.290 \pm 0.23$  and  $1.099 \pm 0.10$   $\mu$ mol/L, respectively), similar to the negative group, indicating the protective effect of macadamia oil against lipid peroxidation.

**Table 6: Comparison between different groups in CRP, GSH, and MDA**

Group	Means $\pm$ SE		
	C- Reactive protein (mg/L)	Glutathione ( $\mu$ mol / L)	MDA ( $\mu$ mol / L)
Negative control (-ve)	$6.80 \pm 1.35$ b	$6.62 \pm 0.72$ a	$1.271 \pm 0.19$ b
Positive control (+ve)	$59.60 \pm 15.75$ a	$1.626 \pm 0.27$ b	$5.95 \pm 0.46$ a
G3	$8.40 \pm 1.46$ b	$7.93 \pm 0.43$ a	$1.290 \pm 0.23$ b
G4	$8.40 \pm 1.47$ b	$6.92 \pm 0.78$ a	$1.099 \pm 0.10$ b
L.S.D.	23.905 **	1.779 **	0.843 **
P-value	0.0006	0.0001	0.0001

Means having the different letters in the same column differed significantly.

\*\* ( $P \leq 0.01$ ).

### 3.7 Effects of Treatments on Animal Weight

The pre- and post-treatment weights of the four rat groups are shown in Table 7. The two weights were significantly different from each other ( $P \leq 0.01$ ). G1 weighed  $218.80 \pm 6.95$  g at the beginning of the trial and  $195.60 \pm 6.04$  g at the conclusion. Since G1's weight dropped significantly throughout the experiment, it's safe to assume that environmental or biological factors were to blame. The positive control group (G2), on the other hand, started with a low weight ( $190.40 \pm 5.67$  g) but ended up with a much higher weight ( $217.00 \pm 3.89$  g). Groups G3 (macadamia oil alone) and G4 (macadamia oil + hydrogen peroxide) began with rather high weights ( $257.00 \pm 4.81$  g and  $251.00 \pm 3.00$  g, respectively) and ended with very comparable weights ( $217.20 \pm 3.59$  g and  $212.80 \pm 3.31$  g, respectively).

**Table 7: Comparison between different groups in Body weight**

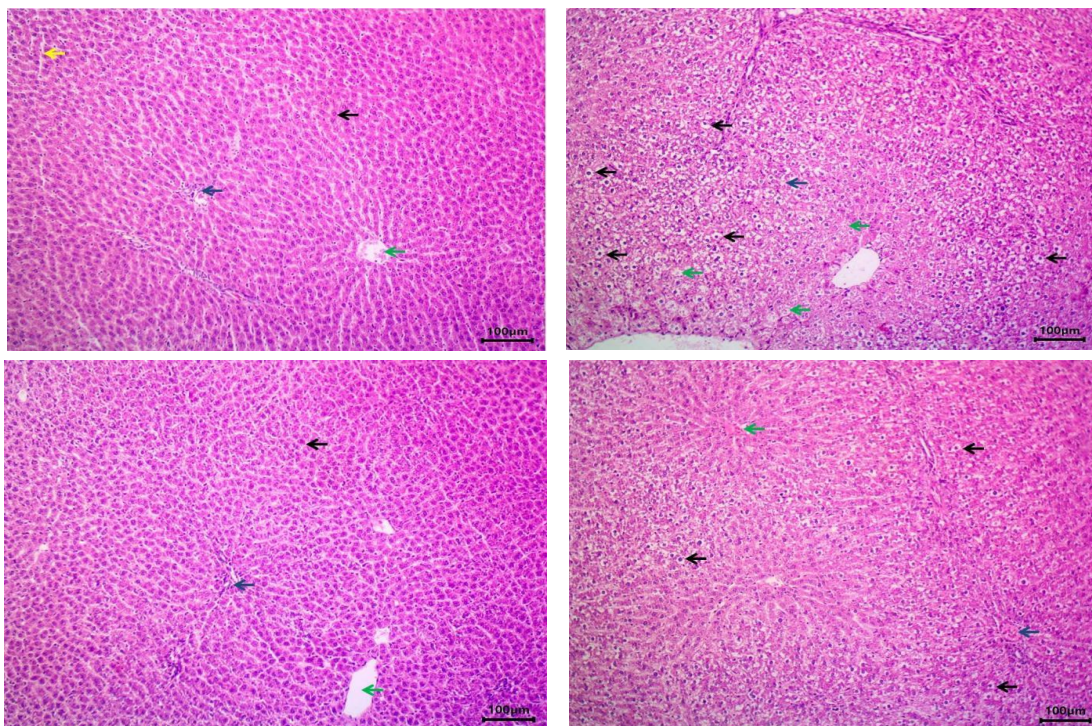
Group	Means $\pm$ SE	
	Initial weight (gm)	Final weight (gm)
Negative control (-ve)	218.80 $\pm$ 6.95 b	195.60 $\pm$ 6.04 b
Positive control (+ve)	190.40 $\pm$ 5.67 c	217.00 $\pm$ 3.89 a
G3	257.00 $\pm$ 4.81 a	217.20 $\pm$ 3.59 a
G4	251.00 $\pm$ 3.00 a	212.80 $\pm$ 3.31 a
L.S.D.	15.92 **	13.037 **
P-value	0.0001	0.0084

Means having the different letters in same column differed significantly.

\*\* ( $P \leq 0.01$ ).

### 3.8 Histological study

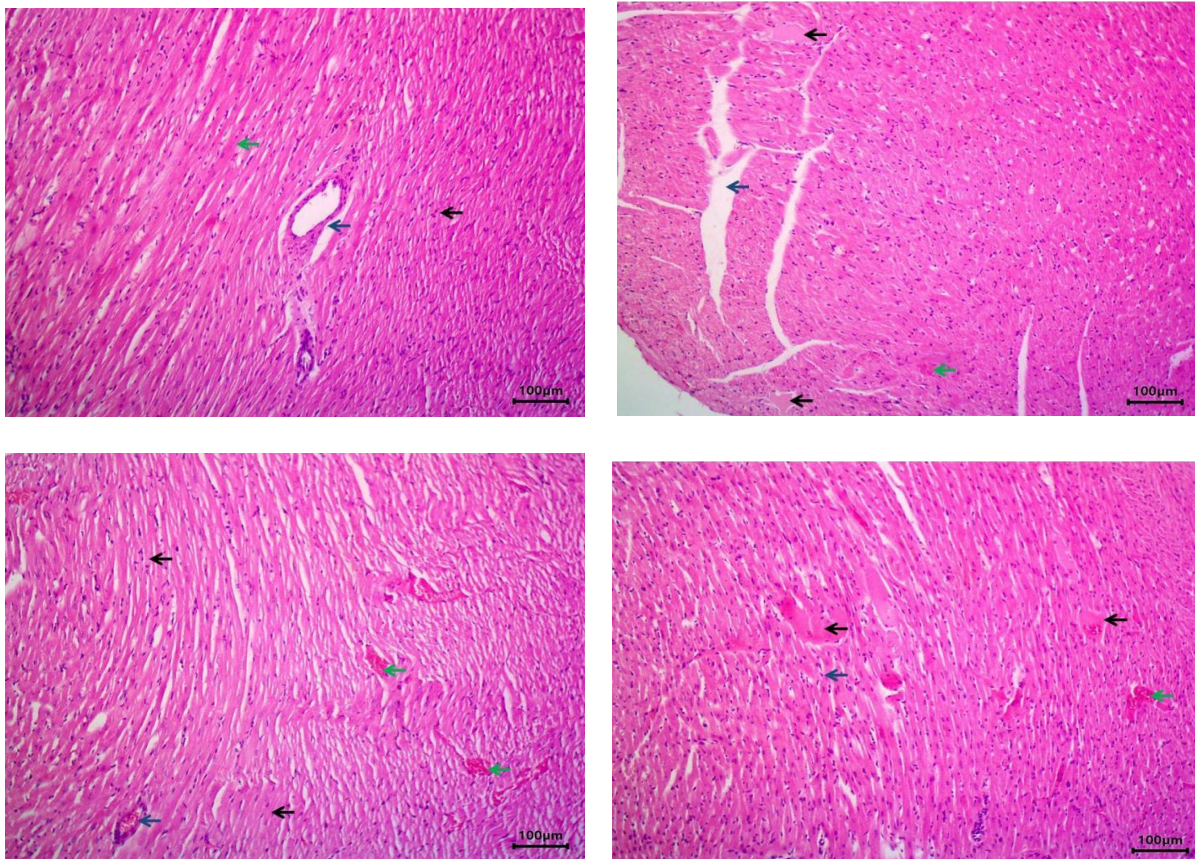
The current research showed how different amounts of hydrogen peroxide and macadamia oil affected the liver, heart, aorta, and kidney tissues. The histopathological characteristics of G1 liver exhibited an intact hepatocyte structure (black arrow), a central vein (green arrow), a portal region (blue arrow), and sinusoids (yellow arrow). G2 had a lot of problems, such as ascites degeneration (black arrows), hepatocyte tumour necrosis (green arrows), and sinusoidal stenosis (blue arrow).



**Figure 1.** Show effect hydrogen peroxide and macadamia oil on tissue liver rats

Most of the histological characteristics of G3 demonstrated that the hepatocyte structure (black arrow), central vein (green arrow), portal region (blue arrow), and sinusoids (yellow arrow) were all intact. G4 had moderate degeneration of hepatocytes (black arrows) and congestion of the central vein (green arrow) and portal vein (blue arrow).

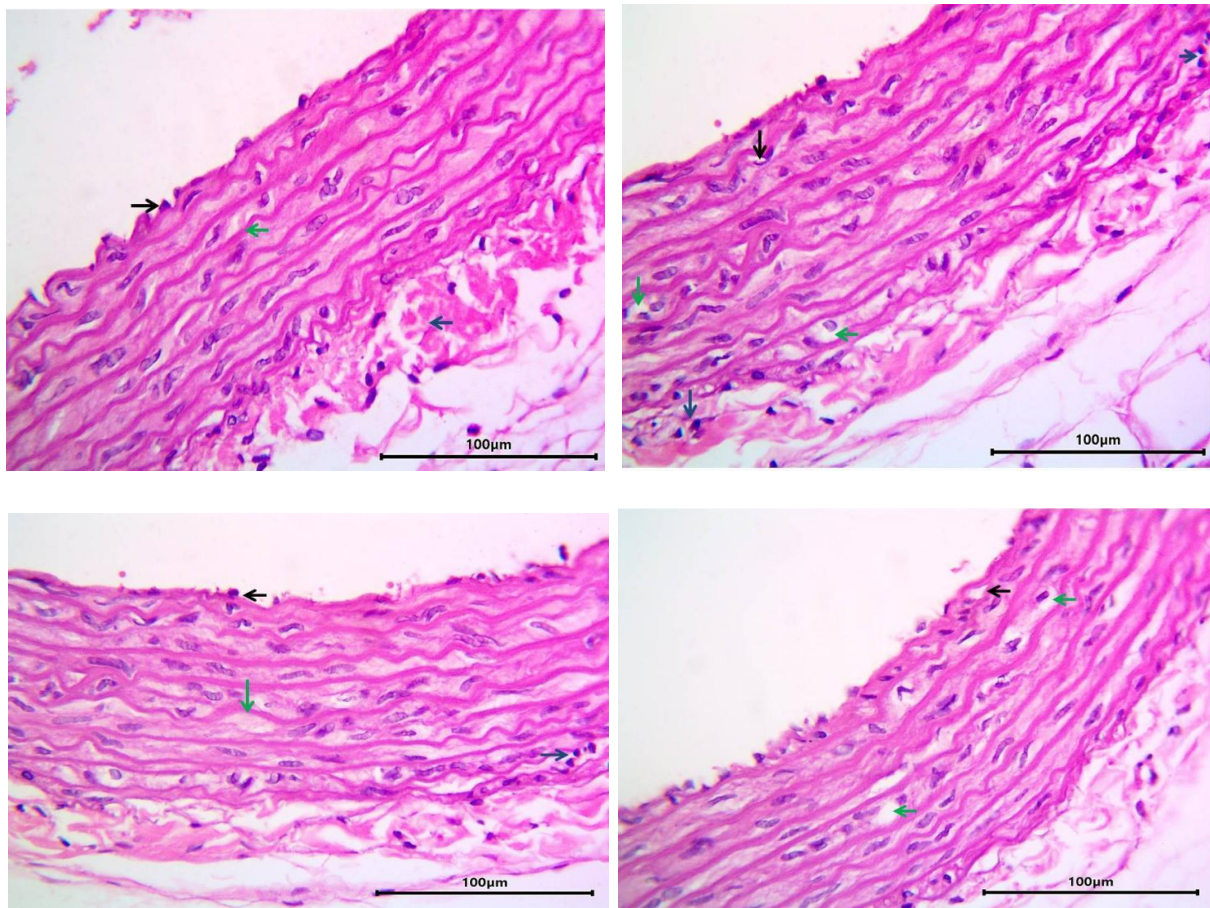
The histopathological characteristics of the **rat heart** in G1 demonstrated that the cardiomyocytes (black arrow), myocardial fibres (green arrow), and blood arteries (blue arrow) all had normal tissue structures. G2 had cardiomyocyte death (black arrow), hyaline degeneration (green arrow), and swelling (blue arrow). G3 exhibited a normal tissue structure of cardiomyocytes and fibres (black arrow), bleeding (green arrow), and blood vessels that were too full (blue arrow). G4 indicated that the cardiomyocytes and fibres were breaking down (black arrow) and there was a little amount of bleeding (green arrow).



**Figure 2.** Show effect hydrogen peroxide and macadamia oil on tissue heart rats

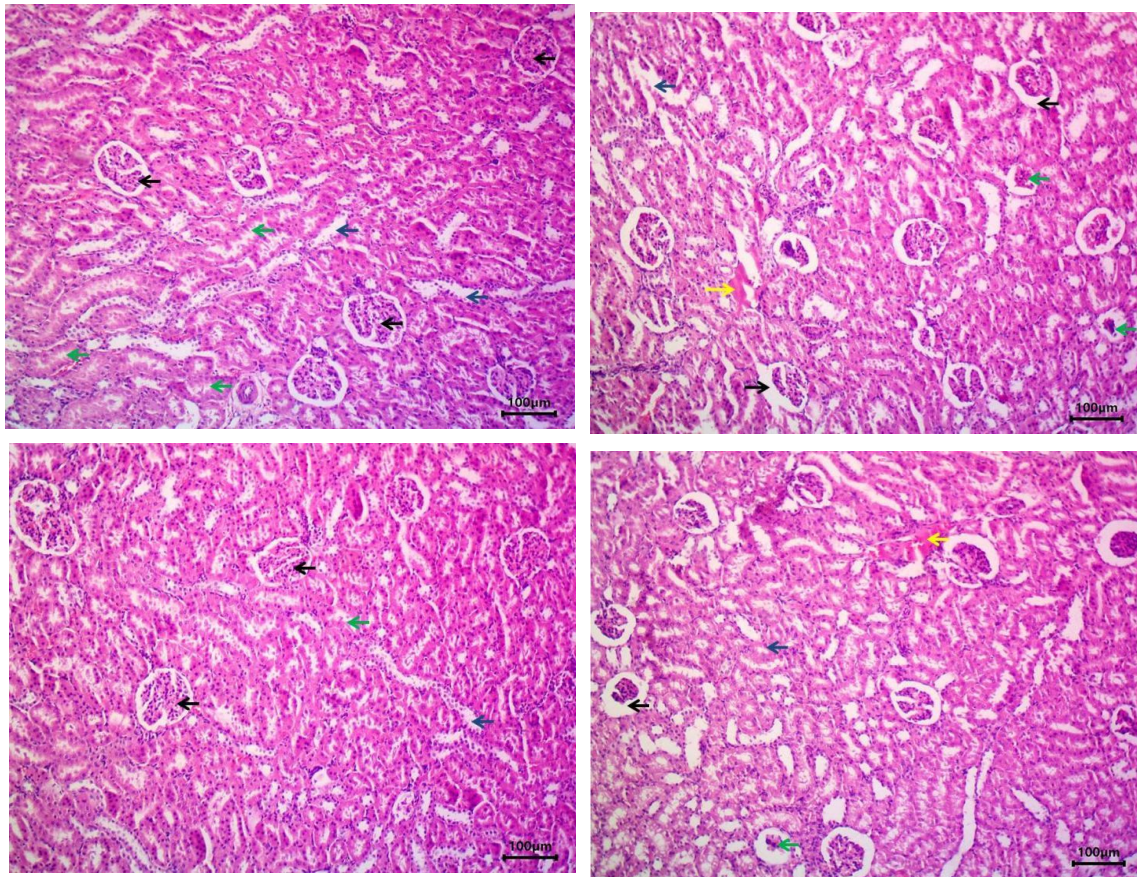
Histopathological features of the **rat aorta** in G1 showed intact histological architectures of the tunica intima with endothelium (black arrow), tunica media with smooth muscle fibers (green arrow) and adventitia (blue arrow). G2 shows foam cells in the tunica intima (black

arrow), and in the tunica media (green arrow) and infiltration of inflammatory cells (blue arrow). G3 showed intact histological architectures of the tunica intima with endothelium (black arrow), tunica media with smooth muscle fibers (green arrow) and and infiltration of inflammatory cells (blue arrow). G4 foam cells in the tunica intima (black arrow), and in the tunica media (green arrow).



**Figure 3.** Show effect hydrogen peroxide and macadamia oil on tissue aorta rats

Histopathological features of the **rat kidney** in G1 showed intact histological architectures of the glomeruli (black arrows), proximal renal tubules (green arrow), and distal renal tubules (blue arrow). G2 shows expansion of Bowman's space of the glomeruli (black arrow), atrophy of glomeruli (green arrow), necrosis of the epithelial cells lining renal tubules (blue arrow) and hyaline deposition (yellow arrow). G3 shows intact histological architectures of the glomeruli (black arrows), proximal renal tubules (green arrow), and distal renal tubules (blue arrow). G4 shows expansion of Bowman's space of the glomeruli (black arrow), atrophy of glomeruli (green arrow), intact renal tubules (blue arrow) and hyaline deposition (yellow arrow).



**Figure 4.** Macadamia oil and effect of hydrogen peroxide on tissue of kidney rats

## 4. Discussion

### 4.1 Effects of treatments on glucose levels

After one month of testing, hydrogen peroxide ( $H_2O_2$ ) and macadamia oil were shown to affect the blood glucose levels of rats, as shown in Table 1. The findings demonstrated that the study groups differed significantly ( $P = 0.0001$ ), which reflects the effect of the various therapies on the physiological balance of blood sugar. The first group (negative healthy control) revealed a normal level of glucose ( $3.92 \pm 0.29$  mmol/L), while the second group (positive control), which was exposed to oxidative stress resulting from the administration of 0.5% hydrogen peroxide, showed higher concentrations of glucose ( $8.19 \pm 0.36$  mmol/L), which is considered as a significant increment in glucose level comparing to the other groups. This shows that oxidative stress may obstruct glucose metabolism due to its direct harmful impact on pancreatic performance and/or induce swelling, causing insulin deficiency [18]. The third group G3 (healthy rats treated with macadamia oil), showed glucose level of ( $4.25 \pm 0.25$  mmol/L), with no significant difference compared to the negative healthy control group. This indicates that the healthy rats who had macadamia oil didn't have any side effects or changes in

their blood sugar levels. The group with hydrogen peroxide plus macadamia oil showed a substantially higher decline in glucose levels ( $3.67 \pm 0.22$  mmol/L) than the group that simply got hydrogen peroxide. When compared to the healthy groups, there wasn't a massive difference. The drop shows that macadamia oil may get rid of metabolic abnormality induced by oxidative stress. The high amounts of monounsaturated fatty acids in macadamia oil, especially oleic acid and palmitoleic acid, may induce reducing blood sugar. Researchers have shown that these substances assist cells in consuming insulin more rapidly and efficiently and attracting glucose from nearby tissues [15-18]. Macadamia oil offers antioxidants because it includes natural phenolic compounds and vitamin E (tocopherol). These assist in minimising oxidative stress that may arise when glucose levels cannot be controlled appropriately, such as when someone has insulin resistance or pancreatic beta cell malfunction [19].

This chemical is also a lipokine, palmitoleic acid, which means it helps keep the body's metabolism in check. It also lowers inflammation and makes insulin work better in the liver and muscles. Macadamia oil may lower blood sugar levels, especially when oxidative stress is generated by oxidising agents such as hydrogen peroxide [20]. This is because it has both anti-inflammatory and insulin-sensitizing effects. Research by Gillingham *et al.* in 2011 found that giving people a diet rich in macadamia oil led to very good blood sugar management and low levels of inflammation in both animals and humans, establishing its viability as an effective nutrient in the prevention of metabolic disorders [21].

#### 4.2 Effects of treatments on Kidney function

Table 2 demonstrates how H<sub>2</sub>O<sub>2</sub> and macadamia oil affect kidney function markers, which are the amounts of urea, creatinine, and uric acid in the blood serum of rats. The findings indicated that there were very significant differences between the experimental groups ( $P < 0.01$ ), which means that the kind of therapy certainly affected how well the kidneys worked. Compared to the negative control group G1, which had the lowest levels of urea ( $4.25 \pm 0.35$  mmol/L), creatinine ( $48.93 \pm 6.50$   $\mu$ mol/L), and uric acid ( $73.87 \pm 4.39$   $\mu$ mol/L), the positive control group G2 had significant and sharp increases in all indicators. This shows that hydrogen peroxide caused significant kidney damage. The elevated level of phosphorus shows that the kidneys are not working properly to get rid of nitrogenous waste.

A high level of creatinine may indicate that the glomerular filtration rate (GFR) is dropping. Inflamed or injured kidneys don't get rid of purines as efficiently, which may lead to raise uric acid levels [22]. When hydrogen peroxide damages cells, it produces additional reactive oxygen

species (ROS), which causes oxidative stress in cells and nitrogenous waste to build up in the blood. These alterations in renal function markers are the outcome [23-25]. Li *et al.*, showed that reactive oxygen species (ROS) may damage renal tissue and boost blood nitrogenous waste levels, which is what we found [26]. One study showed that hydrogen peroxide changes the glomerular filtration rate (GFR) and the way the glomeruli work. The chemical destroys the glomerular barrier layer and the endothelial cells. Hydrogen peroxide also has an immediate influence on tubular epithelial cells, making it difficult for them to take in salt and water. This causes creatinine and urea to seep into the blood, which elevates their levels in the serum [27].

H<sub>2</sub>O<sub>2</sub> has been attributed to the activation of the inflammatory signal transduction pathways, including the NF- $\kappa$ B, after which the production of proinflammatory cytokines like TNF- $\alpha$  and IL-6 is incited. These interactions result in inflammation of tissues in the kidney, high levels of glomerular permeability, and, as a result, higher uric acid levels, an indicator of inflammatory damage to kidney tissue [27]. Group 3 (G3) (Macadamia Oil Only): The kidney function markers were similar to those of the healthy group G1 and did not differ significantly, which means that macadamia oil does not hurt kidney function. It can also keep you safe. Group 4 (G4 - Hydrogen Peroxide + Macadamia Oil): Even though they were exposed to hydrogen peroxide, this group had lower levels of kidney function markers: urea ( $3.73 \pm 0.32$  mmol/L), creatinine ( $52.65 \pm 4.63$   $\mu$ mol/L), and uric acid ( $64.51 \pm 3.85$   $\mu$ mol/L), which are closer to the normal levels in G1. This shows that macadamia oil may repair kidney damage caused by oxidative stress. On the other hand, macadamia oil has clear protective effects. This is probably because it is high in monounsaturated fatty acids and phenolic compounds, which act as antioxidants and keep renal cell membranes from breaking down and keep the glomerular filtration process working properly [28]. A study by Li *et al.* in 2020 found that eating macadamia oil lowered signs of inflammation and oxidative stress in kidney tissue in animal models [29]. This supports this effect. The positive effects of macadamia oil are likely to apply as well. It has been shown that the oil can improve blood flow in the kidneys and stop the spread of inflammation, which is a process that slows down the rate at which waste products build up in the body.

### 4.3 Effects of treatments on Liver function

By measuring the levels of the enzymes AST (aspartate aminotransferase) and ALT (alanine aminotransferase) in rat serum, the results in Table 3 show how different treatments affect liver function indicators. The levels of both AST and ALT were much higher ( $P \leq 0.01$ ) in the positive control group G2 that was exposed to hydrogen peroxide H<sub>2</sub>O<sub>2</sub>. The average AST

levels were  $275.00 \pm 16.81$  IU/L, and the ALT levels were  $112.20 \pm 7.41$  IU/L. In the negative control group G1, the levels were  $180.60 \pm 5.41$  IU/L and  $33.60 \pm 2.58$  IU/L, respectively.

The hydrogen peroxide ( $H_2O_2$ ) group had a big rise in liver function parameters like AST and ALT. This is because  $H_2O_2$  is toxic to hepatocytes and breaks down the integrity of the hepatocyte plasma membrane. ALT and AST are specialised enzymes that are found in high concentrations in the cytoplasm and mitochondria of hepatocytes [30]. They are also useful because they are direct signs of the liver tissues' health. When hepatocytes are damaged, these enzymes enter the bloodstream, leading to elevated serum concentrations [31,32], in contrast, G3 and G4 groups exhibited significantly reduced levels of AST ( $158.40 \pm 6.41$  IU/L and  $153.80 \pm 8.49$  IU/L, respectively) and ALT ( $38.20 \pm 3.04$  IU/L and  $41.60 \pm 5.24$  IU/L, respectively) compared to the positive control group ( $275.00 \pm 16.81$  IU/L for AST and  $112.20 \pm 7.41$  IU/L for ALT). These observations indicated that macadamia oil has hepatoprotective and regenerative activity to minimize liver damages.

#### 4.4 Effects of treatments on Heart function

Table 4 shows how different treatments change heart function markers like CK-MB (Creatine Kinase-MB), Troponin I, and LDH (Lactate Dehydrogenase). All of these markers are okay to use to find damage to the heart and myocardial cells. The positive (+ve) group, which only got hydrogen peroxide, had much higher levels of all heart parameters than the negative (healthy) group. For instance, LDH levels were  $641.60 \pm 60.24$  U/L, Troponin I levels were  $63.80 \pm 3.65$  ng/L, and CK-MB levels were  $398.20 \pm 51.10$  ng/L. The negative group, on the other hand, had low levels of these parameters ( $163.60 \pm 9.41$  U/L,  $5.47 \pm 0.38$  ng/L, and  $49.20 \pm 4.59$  ng/L, respectively). The quickly rising number shows that a lot of heart cells are being damaged by the dangerous hydrogen peroxide. When it breaks down the cell membranes, it lets the enzymes into the blood. When hydrogen peroxide breaks down cell membranes, it makes hydroxyl radicals ( $\bullet OH$ ), which are very reactive and hurt the lipids in heart cell membranes. This breaks down the membranes, which lets enzymes from the heart, such as Troponin I, CK-MB, and LDH, get into the blood [33]. If this level goes up, it means that the cells in your heart are dying or getting worse. Hydrogen peroxide might kill the mitochondria in cells that make energy. The heart's normal function worsens when there are more signals for cell death (apoptosis) and less ATP production [34]. Fibrosis changes the structure of the heart muscle in the affected area. This could be one reason why the CK-MB enzyme activity is higher in the positive (G2) group. Ischaemic heart disease happens when  $H_2O_2$  is injected. These happen when the levels of cardiac proteins and an enzyme called CK-MB in the blood rise. If contractile proteins, cardiac

fibres, and the sarcoplasmic reticulum are damaged, these proteins may leak into the blood. As the membranes of the damaged heart cells become less permeable, the levels of these proteins rise [35]. The groups of G3 and G4 showed a lot of reduction in the cardiac parameters as registered. G3 recorded levels of LDH ( $150.60 \pm 10.14$  U/L), Troponin I ( $4.16 \pm 0.36$  ng/L), and CK-BM ( $43.80 \pm 3.28$  ng/L), while G4 recorded ( $172.00 \pm 15.01$  U/L), ( $3.69 \pm 0.39$  ng/L) and ( $42.00 \pm 3.30$  ng/L) for LDH, Troponin I and CK-BM, respectively. Based on the findings of our present study, it can be said that concentrations of these enzymes decreased markedly in the subject treated with macadamia oil, which can be attributed to the level of effectiveness of preserving the integrity of membranes of the heart cells. Macadamia's anti-inflammatory and antioxidant properties help prevent cardiovascular diseases, such as myocardial infarction, and reduce the risk of heart attacks and deaths from them by protecting heart cell membranes from oxidative damage caused by  $H_2O_2$ . This will lessen the enzymes in blood because they will not be released. According to Tu *et al.*, [36], MO also contributes to heart health as it reduces the blood pressure, cholesterol level, and the incidence of blood clots. The finding revealed that the macadamia oil has a protective effect on the rats, as seen in the result, as it helped reduce the concentration of the enzymes that indicated damage to the heart, though the oxidising agent was used in the form of  $H_2O_2$ .

#### 4.5 Effects of treatments on lipid profile

Table 5 illustrates the rats' blood lipid levels (lipid profiles) after they had various treatments. Some of these numbers include triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein. The results indicated that the groups were quite different from one another ( $P \leq 0.01$ ). Cholesterol levels were considerably greater in the positive control group (G2) that was exposed to hydrogen peroxide ( $7.13 \pm 0.38$  mmol/L) compared to the healthy group (G1) ( $3.04 \pm 0.19$  mmol/L). Because of this, oxidative stress probably increased lipogenesis or threw off the body's metabolic balance. When HepG2 and BRL-3A live liver cells were put in a lab with  $H_2O_2$ , transcription factors like SREBP-1a and SREBP-2 were turned on. Higher levels of triglycerides and cholesterol within cells were caused by an increase in the production of enzymes that make fatty acids and cholesterol [37].

Too much  $H_2O_2$  puts oxidative stress on the liver tissues.  $H_2O_2$  stops the role of both security and defense enzymes like GPX and CAT, which leads to the formation of foetal phonetics and substances like lipid hydroperoxides. This may diminish hepatic efficiency and affect cholesterol metabolism pathway, which may turn it into dangerous forms like oxidised LDL [38]. The two groups that received macadamia oil (G3 and G4) had lower total cholesterol

levels ( $2.86 \pm 0.12$  and  $3.25 \pm 0.16$  mmol/L, respectively). This shows that macadamia oil can protect against high cholesterol levels because it is high in monounsaturated fatty acids and antioxidants. Research by Rus et al. (2023) backs this up by showing that fatty acids may help in controlling total cholesterol [39].

The (+ve) group had a big rise in triglycerides ( $2.918 \pm 0.20$  mmol/L), whereas the other groups saw a distinct drop: (-ve) recorded  $0.774 \pm 0.04$ , G3:  $0.579 \pm 0.04$ , and G4:  $0.561 \pm 0.05$  mmol/L. This means that hydrogen peroxide disrupts lipid metabolism by raising triglycerides. As the H<sub>2</sub>O<sub>2</sub> speeds up, it throws off the organelles in charge of oxidising fatty acids. When the mitochondria get out of balance, it slows down the process of oxidising fats, which raises triglycerides in cells. H<sub>2</sub>O<sub>2</sub> also stops lipolytic enzymes like hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL) from working, which slows down the breakdown of triglycerides and helps them build up. These actions together show how hydrogen peroxide may cause problems with lipid metabolism, especially by boosting blood triglyceride levels, as shown by recent research like [40] and [6]. According to HDL, the highest value was recorded in the healthy group (-ve) ( $3.07 \pm 0.24$  mmol/L), while it decreased sharply in the (+ve) group ( $0.604 \pm 0.07$  mmol/L), reflecting the negative effect of oxidative stress on HDL cholesterol. However, groups G3 and G4 showed a significant improvement in HDL levels ( $1.663 \pm 0.10$  and  $1.668 \pm 0.09$  mmol/L), indicating the role of macadamia oil in improving HDL ratios. These results support the study [41], which confirmed that consuming oils rich in omega-9 enhances HDL. The (+ve) group recorded the highest LDL levels ( $5.635 \pm 0.25$  mmol/L), which is a major indicator of the risk of heart disease and atherosclerosis, while the (-ve) group recorded a decrease:  $1.615 \pm 0.28$ , G3:  $0.932 \pm 0.10$ , G4:  $1.096 \pm 0.07$  mmol/L. This indicates that macadamia oil has an LDL-lowering effect due to its effect in reducing inflammation and oxidative stress, as indicated by Shuai *et al.*, [6].

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) raises cholesterol levels by causing oxidative stress, which causes LDL (bad cholesterol) to oxidise and build up in the blood, which leads to atherosclerosis [42]. H<sub>2</sub>O<sub>2</sub> also lowers HDL (good cholesterol) levels by stopping the gene expression of the protein ApoA-I, which is needed for HDL creation, and by slowing down the activity of the enzyme LCAT, which is needed for HDL maturation. This makes it harder for HDL to do its job of removing cholesterol from the blood [43]. On the other hand, more and more evidence shows that eating vegetable oils high in monounsaturated fatty acids (MUFAs), like MO, can greatly lower lipid buildup by lowering levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate

aminotransferase (AST), while raising levels of high-density lipoprotein cholesterol (HDL-C) [44].

#### 4.6 Effects of treatments on GSH, MDA, and C-RP function

Table 6 shows a statistical comparison of the levels of C-reactive protein (CRP), reduced glutathione (GSH), and malondialdehyde (MDA) in different experimental groups. This shows how hydrogen peroxide and macadamia oil affect indicators of oxidative stress and inflammation. The C-reactive protein (CRP) level was greatest in the positive (+ve) group, which was given pure hydrogen peroxide. It was an average of  $59.60 \pm 15.75$  mg/L. The negative group (-ve), on the other hand, only got  $6.80 \pm 1.35$  mg/L. The values of CRP dropped from  $8.40 \pm 1.46$  mg/L to  $8.40 \pm 1.47$  mg/L in both Group G3 (which just had macadamia oil) and Group G4 (which had hydrogen peroxide plus macadamia oil). The positive group had a significant drop in glutathione (GSH) levels ( $1.626 \pm 0.27$   $\mu$ mol/L), which shows that the antioxidant defence system was weakened by the oxidative stress generated by H<sub>2</sub>O<sub>2</sub>. On the other hand, groups G3 and G4 had levels that were still rather high ( $7.93 \pm 0.43$  and  $6.92 \pm 0.78$   $\mu$ mol/L, respectively), which are close to those of the control group. This shows that macadamia oil is good for the antioxidant system and helps cells fight oxidative stress.

As for malondialdehyde (MDA) levels, the positive (G2) group showed a significant increase ( $5.95 \pm 0.46$   $\mu$ mol/L), compared to the healthy control group (G1) ( $1.271 \pm 0.19$   $\mu$ mol/L), while groups G3 and G4 recorded significantly lower levels ( $1.290 \pm 0.23$  and  $1.099 \pm 0.10$   $\mu$ mol/L, respectively), similar to the negative group, indicating the protective effect of macadamia oil against lipid peroxidation. The liver makes C-reactive protein (CRP) and sends it into the blood when there is sudden or long-term inflammation. CRP is one of the most important inflammatory indicators. Its level goes up extremely quickly when there is tissue injury, an infection, or exposure to toxins or oxidising agents [45,46].

The research found that the rats that only had H<sub>2</sub>O<sub>2</sub> had far higher CRP levels ( $59.60 \pm 15.75$  mg/L) than the control group. This clearly shows that hydrogen peroxide caused an acute inflammatory condition. This inflammatory reaction happens because H<sub>2</sub>O<sub>2</sub> may cause oxidative stress within cells, which causes reactive oxygen species (ROS) to be released, which hurts the cell membrane and other important parts. This makes the liver produce cytokines like IL-6 and TNF-alpha, which in turn start making CRP. This response is a way for the body to defend itself but may lead to several long-term and inflammatory disorders, such as heart, kidney, and liver problems if it keeps happening [47,48]. As shown in the case study, a high

level of CRP is a clear sign of inflammation, which is caused by being around hydrogen peroxide.

Glutathione is one of the most powerful antioxidants that the body makes. It is particularly critical for keeping cells from becoming damaged by oxidative stress [49]. Glutamate, cysteine, and glycine are the three amino acids that make up GSH [50]. It not only gets rid of free radicals, but it also helps other antioxidants, such as vitamins C and E, grow again. It also helps the liver get rid of toxins by adhering to them and making it simpler for the liver to get rid of them [51]. The mitochondria are where cells keep most of their GSH. It protects cells against ROS. Cells create these items themselves during metabolic activities, or they encounter them when they meet molecules like  $H_2O_2$ . The results of this study reveal that oxidative stress made the body's antioxidant defence system weaker. The positive control group, which only drank  $H_2O_2$ , experienced a big decline in GSH levels ( $1.626 \pm 0.27 \mu\text{mol/L}$ ), which shows this. This isn't true since  $H_2O_2$  inhibits the production of ROS, which makes the cell's GSH stores very weak as it attempts to fight off these free radicals. When the glutathione system is full, cells must obey the rules of compulsive oxidation, which damages tissue and kills cells.

**Lipid peroxidation:** This is a harmful process that happens when free radicals break down the polyunsaturated fatty acids in cell membranes to make lipid hydroperoxides. The last products are then broken down even further into a huge number of very different products, such as low molecular mass hydrocarbons, aldehydes and hydroxyl aldehydes, fatty acids, ketones, alkenols, and alkanals, with MDA being the primary one. MDA is one of the best indicators of lipid peroxidation. ROS break down unsaturated fatty acids in cell membranes, which leads to this substance [18]. The amount of MDA in the body shows how much oxidative damage has been done to cell membranes. It is often employed as a sign of oxidative stress that is linked to numerous illnesses, such as heart, liver, kidney, and neurological issues [52]. The MDA levels in the plasma or tissues are quite high, and so is the amount of oxidative damage. The antioxidants that protect the body are also lost.

In the results of the current study, the positive control group (G2) treated with hydrogen peroxide recorded a significant increase in malondialdehyde levels ( $5.95 \pm 0.46 \mu\text{mol/L}$ ), compared to the negative control group (G1) ( $1.271 \pm 0.19 \mu\text{mol/L}$ ). Such a drastic increase thus reveals that the cell membranes are damaged extensively by oxidative stress induced by hydrogen peroxide, producing free radicals  $OH$  and  $O_2^-$  and by inducing cell membranes to produce lipid peroxidation, which releases MDA, as the end product of this devastation [53, 54].

#### 4.7 Effects of Treatments on Animal Weights

The pre- and post-treatment weights of the four rat groups are shown in Table 7. The two weights were significantly different from each other ( $P \leq 0.01$ ). G1 weighed 218.80  $\pm$  6.95 g at the beginning of the trial and 195.60  $\pm$  6.04 g at the conclusion. Since G1's weight dropped significantly throughout the experiment, it's safe to assume that environmental or biological factors were to blame.

The positive control group (G2), on the other hand, started with a low weight (190.40  $\pm$  5.67 g) but ended up with a much higher weight (217.00  $\pm$  3.89 g). Hydrogen peroxide ( $H_2O_2$ ) may cause oxidative stress, leading to changes in animal body weight due to its effects on metabolic processes, cellular activities, hunger, and nutritional absorption [55,56]. One thing I may want to think about when it comes to today's study is the chance of gaining weight because of fluid retention in the body, or the osmotic process being impaired because of tissue damage or liver and kidney inflammation. This weight growth is not due to a normal process of development since in some of the samples, the rise is particularly substantial, and some studies have shown signs of harm to the body.

Therefore, the amount of time an animal is subjected to oxidative stress, how effective its antioxidant response is, and the intensity of the stress all determine how  $H_2O_2$  affects its weight .

While previous research has shown that long-term exposure to hydrogen peroxide might cause weight loss via mechanisms such as decreased hunger, decreased absorption, and increased cellular energy expenditure to offset oxidative damage, the present study's findings contradict these findings [20, 33]. Just as  $H_2O_2$  stimulates the production of inflammatory cytokines, which then promote protein and skeletal muscle breakdown, oxidative toxicity often presents as cachexia [57].

Research shows that obesity and oxidative stress affect the secretory system of adipose tissue [58]. One sign of this is that the body makes less adiponectin, an anti-inflammatory cytokine that is important for managing glucose and lipid metabolism and keeping lipids from building up in blood vessels [59]. Oxidative stress, which may be caused by things like hydrogen peroxide, can cause the endoplasmic reticulum (ER) to stop working and proteins that are not folded correctly to accumulate within cells. Adiponectin can't be released because of this. This problem becomes a lot worse when there are more free fatty acids in the body.

In the current study, the hydrogen peroxide-treated group (G2) showed increased triglycerides, total cholesterol, and LDL, and decreased HDL, which is fully consistent with this mechanism: with decreased adiponectin levels, its effectiveness in promoting fatty acid oxidation and transport to muscle is reduced, and its resistance to inflammation is reduced, thus increasing the risk of lipid accumulation in the blood and tissues. Also, this deficiency leads to reduced nitric oxide (NO) production from the vascular endothelium, which weakens protection against inflammation and oxidation [60].

Groups G3 (macadamia oil alone) and G4 (macadamia oil + hydrogen peroxide) began with rather high weights ( $257.00 \pm 4.81$  g and  $251.00 \pm 3.00$  g, respectively) and ended with very comparable weights ( $217.20 \pm 3.59$  g and  $212.80 \pm 3.31$  g, respectively). This weight stability suggests that macadamia oil protects against hydrogen peroxide-related changes in metabolism and appetite, and nutrient absorption because it contains natural antioxidants and healthy fatty acids [60]. The initial and end weights of the groups were very different ( $P = 0.0001$  and  $P = 0.0084$ , respectively). Oxidative stress is directly linked to the disorganisation of autophagy, one of the most critical processes in the cell. Autophagy keeps cells in a state of homeostasis and metabolic stability.

An "obesogenic environment" makes people gain weight and store fat. It happens when oxidative stress and poor autophagy activity interact [61]. Researchers collected samples of visceral fat from obese people and discovered that metformin treatment reduced signs of inflammation and oxidative stress in people with type 2 diabetes. It also made intracellular autophagy flow better [62]. Our research found that macadamia oil may help preserve cells by keeping autophagy running and improving the balance of redox reactions within cells. This is helpful for things like blood lipids, liver and renal function, and the health of the tissues that are being targeted [63].

#### **4.8 Histological study**

The histological investigation showed more damaging effects of hydrogen peroxide on the liver, heart, aorta, and kidney. These were the various pathological characteristics of the liver, heart, aorta, and kidney of the rat that had been treated with hydrogen peroxide for 30 days. Long-term research has revealed that giving  $H_2O_2$  to animals causes oxidative stress, which generates strong reactive oxygen species (ROS) such as hydroxyl radicals ( $OH\bullet$ ). Most of the really bad effects of  $H_2O_2$  happen in certain tissues. Effects of  $H_2O_2$  on cells. Cells that tend to

break down DNA, membrane lipids, proteins, and enzymes, which may lead to the development of histopathological disorders [60].

So, the MO supplement caused a big drop in the amount of fat that built up in the liver, which lowered the hyperlipidemia and the damage to the liver. Han *et al.* [64] also found that mice given flaxseed oil had a big decline in circular lipid droplets in their liver tissue. According to Cao *et al.* [65], medium-chain fatty acids may start a CREBH-FGF21 axis that helps reduce the development of lipids in the liver. The study showed that changing how lipids are broken down in liver cells is strongly linked to the positive effects of oleic acid on problems with autophagy [66]. This gave a reasonable explanation for the increase in fatty liver seen in the groups that were given MO (oleic acid concentration > 60%) compared to the Model [67]. Macadamia oil also has a lot of palmitoleic acid, which is a lipokine [68] that may change how the body works, including how the immune system works and how it fights inflammation [69]. Also, those who ate macadamia oil had a beneficial effect on their serum lipid profile, which was linked to a high quantity of palmitoleic acid in their blood [70]. It has also been said that palmitoleic acid is another way to calm down inflammation in various testing, such as *in vivo* and *in vitro* experiments.

Stress promotes the loss of cardiomyocytes more severely [71]. Hydrogen peroxide consistently promotes apoptosis in H9C2 cardiomyocytes, which is consistent with previously established finding, since oxidative stress, inflammation, immunity, and cell death are all tightly related [72]. Some individuals suggest that this process includes autophagy. Several mechanisms show cell death that are related to autophagy, such as apoptosis, burn-induced apoptosis, and necrosis [73]. This study's heart tissue was probably linked to hydrogen peroxide-induced autophagy. This is because autophagy may kill cells when they are exposed to anything from the outside, which indicates that autophagy can also kill cells. After treatment with MO, more cells were able to live, and the rate of apoptosis was reduced. This means that MO protects the heart by blocking oxidative stress from killing cardiomyocytes. A review of important epidemiological studies, such as the Adventist Health Study [74], found that persons who ate nuts with oils (such as MO) more often had a 37% decreased risk of heart disease [75]. People who ate nuts four times a week had an 8.3% reduced risk per weekly consumption than people who seldom or never ate nuts [76].

Hydrogen peroxide causes ROS to form, which have a direct effect on the structure and function of the kidneys, especially in the pre-renal stage. Long-term exposure to H<sub>2</sub>O<sub>2</sub> can cause an abnormal rise in the glomerular filtration rate (GFR), which is seen as the kidneys' first response

to oxidative stress [77]. The afferent arteriole's vasodilation, which is caused by less nitric oxide (NO) being available and a change in the balance between vasodilators and vasoconstrictors such as prostaglandins and angiotensin II, is what causes this rise. This widening will increase the glomeruli's filtering power, and for a brief time, the GFR will be higher than normal circumstances. It puts stress on the glomeruli and eventually destroys the glomerular basement membrane and podocyte, an important cell that helps in selective filtration. The damage lets big molecules like proteins get into the urine (proteinuria), which is an early symptom of glomerulopathy. Macadamia oil fights these free radicals and lowers lipid peroxidation, which lowers MDA levels, a sign of cell membrane damage [78]. Researchers have shown that certain parts of macadamia oil may raise levels of glutathione (GSH), one of the body's most essential natural antioxidants. This makes kidney tissue stronger and stops renal failure and fibrosis [14]. The data also show that using macadamia oil raised levels of urea, creatinine, and uric acid by lowering the glomerular filtration rate. This is a measure of how well the kidneys get rid of nitrogenous waste. But we still do not know whether the biological effects found in this research are due to the high levels of oleic and palmitoleic acid [78]. This is the reason why further research into the possible mechanism of the described action of macadamia extract and the possible participation of oleic acid or palmitoleic acid in carrying out these activities is strongly suggested.

## 5. Conclusions

In conclusion, the present study demonstrated that macadamia oil has a protective effect on several biochemical biomarkers in male rats exposed to oxidative stress induced by hydrogen peroxide. Treatment with macadamia oil resulted in a significant reduction in glucose levels and liver acidity-related enzymes (AST and ALT), heart enzymes (LDH, Troponin I, and CK-BM), improved (HDL) and reduced (LDL) blood lipids, lower CRP, higher blood GSH, and lower MDA. Moreover, body weight remained relatively stable, suggesting the safety of macadamia oil administration under the experimental conditions. Collectively, these findings highlight the potential of macadamia oil as a natural dietary antioxidant capable of mitigating oxidative stress related biochemical disturbances and protecting vital organs. Future studies should focus on elucidating the molecular mechanisms underlying these protective effects, exploring dose-dependent responses, and evaluating the long-term impact of macadamia oil consumption. Furthermore, clinical investigations are warranted to assess its therapeutic and preventive potential in oxidative stress associated disorders in humans.

## Ethics approval

The Institutional Animal Ethics Committee gave its approval to the experimental methods and the use of animals (number UM. VET.360, date 29/4/2024), and all procedures followed the moral principles set forth by Mosul University.

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## تقدير بعض المؤشرات الكيموحيوية في ذكور الجرذان المعرضة للكرب التأكسدي والمعالجة بزيت المكاديميا

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قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل – العراق.

### المستخلص

يُعدّ الإجهاد التأكسدي عاملاً رئيسياً في تلف الخلايا وتفاقم العديد من الأمراض التي تصيب الأعضاء الحيوية مثل القلب، الكبد، والكلية. وقد تُساهم مضادات الأكسدة الغذائية، مثل بعض الزيوت، في تقليل مستويات المؤشرات الحيوية المرتبطة بالإجهاد التأكسدي، مما يساعد في إبطاء تقدم هذه الأمراض، يهدف البحث الى دراسة التأثير الوقائي لزيت المكاديميا ضد الإجهاد التأكسدي الناتج عن بيروكسيد الهيدروجين. استخدم لهذه الدراسة عشرون من ذكور الجرذان البيضاء وقد قسمت بشكل عشوائي الى خمس مجاميع فرعية وكل مجموعة مكونة من خمسة حيوانات وتراوحت فترة التجربة اربعة اسابيع، المجموعة 1 (الضابطة السلبية): أعطيت الغذاء وماء شرب العادي، المجموعة 2 (السيطرة الموجبة): أعطيت الغذاء وماء الشرب الحاوي على 0.5% H<sub>2</sub>O<sub>2</sub> ، المجموعة 3 (زيت المكاديميا فقط): أعطيت الغذاء وماء شرب عادي مع جرعة يومية فموية من زيت المكاديميا، المجموعة 4 (H<sub>2</sub>O<sub>2</sub> + زيت المكاديميا): أعطيت الغذاء وماء شرب يحتوي على 0.5% H<sub>2</sub>O<sub>2</sub> بالإضافة إلى جرعة يومية فموية من زيت المكاديميا. تم قياس عدة مؤشرات كيموحيوية في مصل دم الحيوانات، مثل: الكلوكون، حامض اليوريك، اليوريا، الكرياتينين، إنزيمات الكبد (ALT ، AST )، LDH ، التروبونين القلبي i ، CK-BM ، الكوليسترول الكلي ، الدهون الثلاثية ، HDL ، LDL ، C-RP ، الكلوتاتايون المختزل والمالون ثنائي الالديهيد ، كما تم إجراء تقييم نسيجي لأعضاء الكبد، الكلى ، القلب والشريان الاورطي، بالإضافة الى قياس اوزان الحيوانات. أظهرت مجموعة بيروكسيد الهيدروجين (المجموعة 2) ارتفاعاً كبيراً في معظم المؤشرات البيوكيميائية. حيث ارتفع كل من الكلوكون، اليوريا، الكرياتينين، حمض اليوريك، AST ، ALT ، LDH ، تروبونين I ، CK-BM . كذلك زادت مستويات الدهون في الدم ( الكوليسترول الكلي ، الدهون الثلاثية ، LDL ) بينما انخفض HDL وارتفع مستوى MDA ، CRP ، في حين انخفض GSH ، كما لوحظ أن المجموعة الضابطة الإيجابية اكتسبت وزناً أكبر من المجموعة الضابطة السلبية رغم بدايتها بوزن أقل. أما المجموعتان المعالجتان بزيت المكاديميا (المجموعة 3 و4)، فقد أظهرتا تحسناً ملحوظاً، حيث عادت معظم المؤشرات إلى مستوياتها الطبيعية أو قريبة منها. تشير النتائج إلى أن زيت المكاديميا يمتلك خصائص وقائية فعالة ضد الإجهاد التأكسدي الناتج عن بيروكسيد الهيدروجين، من خلال خصائصه المضادة للالتهاب، ودعمه لنظام مضادات الأكسدة، مما يساهم في الحفاظ على التوازن الأيضي وتقليل التلف الخلوي والنسيجي.

**الكلمات المفتاحية:** الإجهاد التأكسدي، بيروكسيد الهيدروجين، زيت المكاديميا، مضادات الأكسدة، الجرذان.