Journal of Applied and Natural Science
17(1), 398 - 406 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effect of isolated active compounds of *Fragaria X ananassa* and *Prunus amigdalus* on induced hepatic steatosis in male albino rabbits

Rana Suhail Al-Juwary*
Department of Biology, College of Education for Girls, University of Mosul, Iraq
Muntaha Mahmood Al-Kattan
Department of Biology, College of Science, University of Mosul, Iraq

*Corresponding author. Email: ranasuhail@uomosul.edu.iq

Article Info
https://doi.org/10.31018/jans.v17i1.6274
Received: October 20, 2024
Revised: March 05, 2025
Accepted: March 12, 2025

How to Cite
Al-Juwary, R.S. and Al-Kattan, M. M. (2025). Effect of isolated active compounds of *Fragaria X ananassa* and *Prunus amigdalus* on induced hepatic steatosis in male albino rabbits. *Journal of Applied and Natural Science*, 17(1), 398 - 406. https://doi.org/10.31018/jans.v17i1.6274

Abstract
This study investigated the effects of *Fragaria X ananassa* polyphenols, *Prunus amigdalus* derived omega-3, and Omega3 and Vitamin E on Triton-induced fatty liver disease in 100 male rabbits divided into 10 groups. Group 1 to Group 10 as follows: control (1), rabbits given Triton (2), rabbits given polyphenols extracted from strawberry(3), rabbits given polyphenols extracted from strawberry with Triton(4), rabbits given Omega3 extracted from almond(5), rabbits given omega-3 extracted from almond with Triton(6), rabbits given polyphenols extracted from strawberry and omega3 extracted from almond(7), rabbits given polyphenols extracted from strawberry and omega-3 extracted from almond with Triton(8), rabbits given omega3, Vit.E (9) and rabbits given omega3, Vit.E with Triton (10) Treatments were administered daily for four months. Results showed a significant reduction in Hemoglobin Hb (6.42±0.16), Red Blood Cells RBCs(3.52±0.08), Packed Cell Volume PCV (2.81±0.08) and Total Antioxidant Capacity TAC (3.62±0.13) in the Triton group compared to other treated groups. Conversely, treated groups showed improved hematological parameters levels, but White Blood Cells WBCs (14.77±0.04) increased in Triton group with a probability (P≤0.01) .

Keyword: *Fragaria X ananassa* , Hepatic steatosis, Packed Cell Volume (PCV), *Prunus amigdalus* , Red Blood Cells (RBCs), Total Antioxidant Capacity (TAC) , Triton, White Blood Cells (WBCs)

INTRODUCTION

Hepatic steatosis is the second most prevalent liver condition following viral hepatitis. Its occurrence is rising sharply, presenting a major health risk. Hepatic steatosis encompasses various pathological issues, including cirrhosis and liver cancer, and can progress to steatohepatitis. Fortunately, hepatic steatosis is typically regarded as a reversible disease, as it is often possible to return the liver to its normal condition (Khan and Khan,2022).

About 25% of people worldwide suffer from hepatic steatosis, a serious public health concern (Teng *et al*., 2022). Fatty liver disease can advance from straightforward hepatic fat buildup to more serious disorders like steatohepatitis in nonalcoholics, cirrhosis, and potentially liver cancer. It also raises the risk of developing diabetes and cardiovascular diseases, contributing to a high overall mortality rate (Ghazanfar *et al*.,2024) . While insulin resistance, abdominal obesity, physical inactivity, and related inflammation are thought to be the main contributors to Nonalcoholic Fatty Liver Disease (NAFLD) development, understanding of the causes of this disease and the mechanisms underlying its development remains incomplete, limiting the available therapeutic option (Jeong, 2020) .

Although only a small percentage (5%) of hepatic steatosis patients advance to cirrhosis, NAFLD is recognized as the most common reason for long-term liver damage, largely due to the widespread increase in obesity (Han *et al*., 2022). The liver stores energy as glycogen and triglycerides, but in situations of obesity, overnutrition, or liver-damaging medications, excess fat builds up in liver cells from prolonged fat storage, resulting in the creation of NAFLD (Heeren and Scheja, 2021).

Regarding liver diseases, oxidative stress-induced loss of liver function encompasses various conditions, including hepatotoxicity (Ramachandran and Jaeschke, 2018), liver ischemia-reperfusion injury (LIRI) (Forman

This work is licensed under Attribution-Non Commercial 4.0 International (CC BY-NC 4.0). © : Author (s). Publishing rights @ ANSF.

and Zhang, 2021), NAFLD (Gonzalez *et al*., 2020), and hepatocellular carcinoma (Li *et al*., 2023). These conditions are linked to excessive free radical production, causing systemic imbalance and disrupting inter-organ communication, which can potentially result in death (Asrani *et al*., 2019).

Natural products have a long history of medicinal use and are still widely used in alternative therapies (Chaachouay and Zidane, 2024). Extracts from natural products are multi-component and possess properties that enable them to target multiple proteins. In light of these properties, extracts derived from natural products exhibit various physiological activities such as anti-inflammatory, antioxidant, and immunomodulatory effects. These inherent attributes make them invaluable in the prevention and treatment of a variety of conditions, ranging from tumors and cardiovascular diseases to metabolic disorders and respiratory diseases (Wang *et al*., 2021). Thus, natural products are emerging as a safer and more effective alternative to pharmaceutical agents (Villarroel-Vicente *et al*., 2021). Several studies have investigated natural compounds as alternatives to pharmaceutical agents as metabolic regulators.

In light of the rising number of men and women experiencing hepatic steatosis, The present study aimed to conduct this investigation to determine the natural therapy approaches that lower the risk of experimental hepatic steatosis with Triton by responding to the following queries: i). Do polyphenols extracted from strawberries reduce the risk of experimental hepatic steatosis in rabbits?, ii). Does Omega-3 extracted from almonds reduce the risk of experimental hepatic steatosis in rabbits? iii). Can Omega-3,Vit. E be considered the optimal treatment for hepatic steatosis?

MATERIALS AND METHODS

Ethical approval

Mosul University, Veterinary Medicine College, Institutional Animal Ethics Committee (IACUC) approved the research vide no. UM.VET.2023.064.

Procurement and acclimatization of rabbits

This study involved 100 male rabbits (*Oryctolagus caniculus* Albino New Zealand rabbits) obtained from local markets in the Kokjali area of Mosul. Veterinarians periodically examined them to ensure their health and absence of sickness. Cages constructed locally were utilized to house the rabbits. Ten groups (Groups 1 to 10) were formed, each with ten rabbits. The temperature was maintained between 26 and 28°C, with a 14-hour daily light cycle. The flooring of the cages was covered with coarse sawdust to absorb moisture with good ventilation. To keep the cages clean and free of pollution and disease, sawdust was changed daily, and continual sterilization was performed.

Rabbits underwent a one-month acclimation period to adjust to their new environment and the standard rabbit feed with equal quantities and in a fixed sequence, with regular water in special containers fixed to the cage to prevent water leakage. Throughout the treatment period, the rabbits had easy access to food and water with green feed.

Material used

Strawberry fruits

Fragaria X ananassa fruits were collected from the nurseries of Agriculture and Forestry College / Mosul University in February 2024, washed with tap water to get rid of dust with the addition of a little vinegar, then dried with a piece of cloth and left for half an hour to dry well at room temperature, then cut into thin slices and placed on special paper for drying, then placed in an electric oven with a warm air current at a temperature of (27-30°C) and turned from time to time for 24 hours, then ground to obtain strawberry powder and placed in a glass container away from moisture and sunlight until needed.

Almonds

The *Prunus amigdalus* were sourced from the Choman/Haj Omran area in Erbil Governorate. They were ground into powder and stored in a glass container until needed.

Preparation of plant extracts using a Soxhlet

Plant extracts were prepared following the method mentioned by (Al-Lahibi, 2022; Zidane, 2023), based on the characteristics of compounds extracted from the plants as well as the kind of solvent utilized throughout the procedure of separation and by utilizing the sequence of solvents system extraction, as ethyl acetate was used in the extraction process for strawberry, while petroleum ether was used for almonds. Then, a Rotary Vacuum Evaporator (RVE) was used to concentrate the extracts at 20 °C lower For about 48 hours than the boiling point of each solvent used.

Separation and purification of polyphenols from strawberry fruits

Phenolic compounds are typically not present in their free form but are bound to sugars as glycosides within plants. To obtain pure phenols and accurately identify them, an acid hydrolysis process is necessary to break the chemical bond, releasing the phenols (Al-Daody, 1998). Using this process, phenols were isolated and purified from strawberry, and polyphenols were identified using High Performance Liquid Chromatography (HPLC) technology, following the method described earlier (Harborne,1998: Behbahani *et al.*, 2011:Al-Lahibi,2022).

Identification of standard phenolic compounds by High Performance Liquid Chromatography (HPLC)

Through the chromatographic analytical charts obtained, the retention time of each of the standard compounds was determined for six compounds (Gallic acid, Quercetin, Caffeine, Kaempferol, L-Rutin, Apigenin), including Gallic acid with a standard retention time of 4.028 min, Quercetin with a standard retention time of 4.839 min, Caffeine with a standard retention time of 5.790 min, Kaempferol with a standard retention time of 7.925 min, L-Rutin with a standard retention time of 2.252 min, and Apigenin with a standard retention time of 3.044 min at a wavelength of 280 nm.

The concentration of the phenolic compound was separated as follows (Robert *et al*.,2003)

$$\text{(ppm/ml) Conc. of sample} = \frac{Hieght\ of\ sample}{Hieght\ of\ standard} \times \text{concentration of standard} \quad \text{Eq.1}$$

Figure 1 displays the phenolic components of strawberry fruits in an ethyl acetate extract. It should be noted that all reference concentrations of phenolic compounds employed in research were 1000 ppm.

Fatty acid extraction from almonds using saponification

Saponification procedure was performed to extract free fatty acids(Omega-3) following the method described by (Al-Jaghfi, 2022). The Omega-3 fatty acids were then diagnosed and purified using HPLC technology according to the technique outlined (Al-Daody, 1998).

Experimental design

This was a preliminary study to determine the optimal and most effective dose of polyphenols extracted from strawberries and Omega-3 extracted from almonds daily. 100 male rabbits were used in this study and randomly divided into 10 groups (Group I to Group 10) ; each group included 10 rabbits. Triton was acquired from the central store of the University of Mosul, and a Triton solution was prepared with a concentration of 300 mg/kg of body mass.

First group ,Control was given water to equalize the stress of holding the rabbit (Verwer *et al*., 2009).

Second group was injected with Triton in a dosage of 300 mg\kg of body mass and injection was performed subperitoneally for these rabbits.

Third group was dosed with polyphenols extracted from strawberry in a dosage of 0.2 ml\kg of body mass.

Fourth group was dosed with polyphenols extracted from strawberry in a dosage of 0.2 ml\kg of body mass, with injection of Triton in a dosage of 300 mg\kg of body mass.

Fifth group was dosed with Omega-3 extracted from almonds in a dosage of 0.14 ml\kg of body mass.

Sixth group was dosed with Omega-3 extracted from almonds in a dosage of 0.14 ml\kg of body mass with injection of Triton in a dosage of 300 mg\kg of body mass.

Seventh group was dosed with polyphenols extracted from strawberry in a dosage of 0.2 ml\kg of body mass and Omega-3 extracted from almonds in a dosage of 0.14 ml\kg of body mass.

Eighth group was dosed with polyphenols extracted from strawberry in a dosage of 0.2 ml\kg of body mass and Omega-3 extracted from almonds in a dosage of 0.14 ml\kg of body mass with injection of Triton in a dosage of 300 mg\kg of body mass.

Ninth group was dosed with Vit. E in a dosage of 0.2 ml\kg of body mass and Omega-3 in a dosage of 0.14 ml\kg of body mass.

Tenth group was dosed with Vit. E in a dosage of 0.2 ml\kg of body mass and Omega-3 in a dosage of 0.14 ml\kg of body mass with injection of Triton in a dosage of 300 mg\kg of body mass.

Sample collection

After 4 months of treatment, blood samples were collected from rabbits by performing a cardiac puncture with a 6 ml medical syringe. Blood was put into tubes without anticoagulant ingredients and sealed with tight yellow caps. The tubes were then kept at room temperature for 20 min. or until blood clotted (Wilson *et al*., 1972).

Hematological Tests

Blood tests were performed on the treated rabbits and the control group, including Haemoglobin (Hb), Red Blood Cell (RBCs) count, Packed Cell Volume (PCV) and White Blood Cell (WBCs) count. Blood samples were analyzed using a Complete Blood Count (CBC) for the veterinary device from GP animal Getein Animal Medical (BHA-5000Vet) of German origin.

Determination of Total Antioxidant Capacity (TAC)

The concentration of TAC in rabbit serum was estimated using a ready-made analysis kit from Bioassay Technology Laboratory (BT LAB) using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Statistical analysis

The statistical analysis was made to find the mean and standard deviation using the simple experimental system and the Complete Randomized Design (CRD). The comparison involving treated rabbit groups and control group, Applied Duncan's Multiple Range Test was donewith the aid of the Statistical Analysis System (SAS) program, as the coefficients took different significant letters at the probability level ($P \leq 0.01$) (Antar and Al-Wakaa, 2017). The graphs were drawn using the Excel 2010 statistical program.

RESULTS

Omega-3 concentration in almonds

Unsaturated fatty acids were easily broken down by the solvent used (petroleum ether). Chromatographic analysis (HPLC) charts were obtained, and the retention time of fatty acid (Omega-3) in the study sample was determined and compared to the retention time of the standard sample. The diagnosis showed that the Omega-3 of the sample was almost identical to the standard fatty acid, as shown in Figure 2,). Omega-3 appeared with a standard retention time of 2.493 minutes, while the retention time of Omega-3 in the study sample was 2.456 min in the petroleum ether extract at a wavelength of 254 nm. The omega-3 concentration of the study sample was found to be 40.34 mg/ml compared to concentration of standard solution 54 mg/ml.

Estimation of blood hemoglobin Hb gm/100ml

From Table 2, the study noticed a significant increase in the concentration of hemoglobin for groups (3,5,7,9), with means of 15.11±0.16, 15.01±0.11, 15.08±0.07, and 15.10±0.19 gm/100 ml, respectively, compared to the rest of the groups and at a probability level of (P≤0.01). There were no significant differences between these groups.

The mean of group (1)was (8.37±0.30) gm/100ml, while the mean of groups (4,6,8,10) were 8.08±0.07, 8.07±0.12, 8.00±0.17, and 8.04±0.12 gm/100 ml, respectively, and there were no significant differences between these groups, while the lowest mean was for group(2), which amounted to 6.42±0.16 gm/100 ml.

Estimation of red blood cell count RBCs million cells/mm^3

From Table 2, it was noticed that there was a significant increase in the number of red blood cells in the group (7), with an arithmetic mean of 7.69±0.05 million globules /mm^3 compared to the rest of the groups and at a probability level of P≤0.01, followed by the group (3) with an arithmetic mean of 7.41±0.08 million globules / mm^3, while the arithmetic mean of the group (5) was 7.25±0.22 million globules /mm^3, and the arithmetic mean of the group (9) was 6.75±0.03 million globules/

Table 1. Height and concentrations of phenolic compounds identified using HPLC technology for strawberry fruits

Numbers	Polyphenols	Standard height	Ethyl acetate extract	
			Heights	Conc. (ppm/ml)
1	Gallic acid	2323.380	78.13	33.627
2	Quercetin	1005.238	1113.54	1107.737
3	Caffeine	1328.621	1083.032	815.155
4	Kaempferol	447.562	124.587	278.368
5	L-Rutin	466.175	21.71	46.570
6	Apigenin	1839.380	94.22	51.223

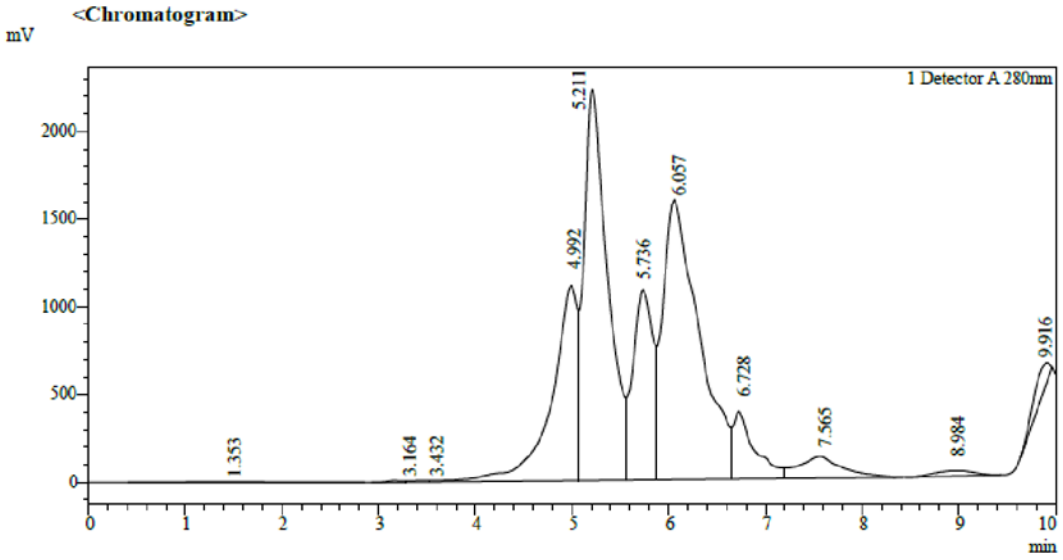

Fig. 1. *HPLC chromatography of ethyl acetate extract of strawberry fruits, characterized by distinct peaks corresponding to Gallic acid, Quercetin, Caffeine, Kaempferol, L-Rutin and Apigenin, respectively where the peak heights and concentrations (ppm/ml) are provided, which illustrates the quantitative and qualitative characteristics of the extract composition; 1.L-Rutin 2. Apigenin 3.Gallic acid 4.Quercetin 5.Caffiene 6.Kaempferol*

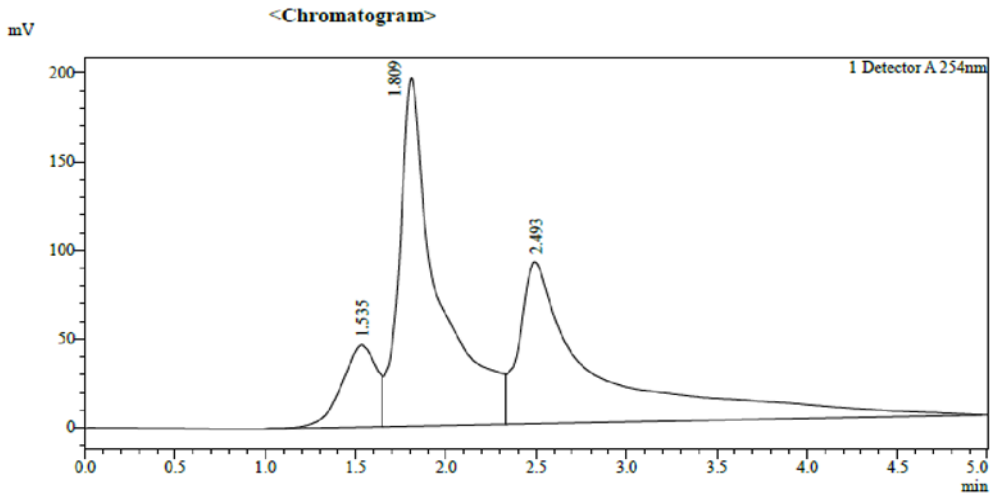

Fig. 2. *Standard curve of Omega3, as determined by High Performance Liquid Chromatography (HPLC)*

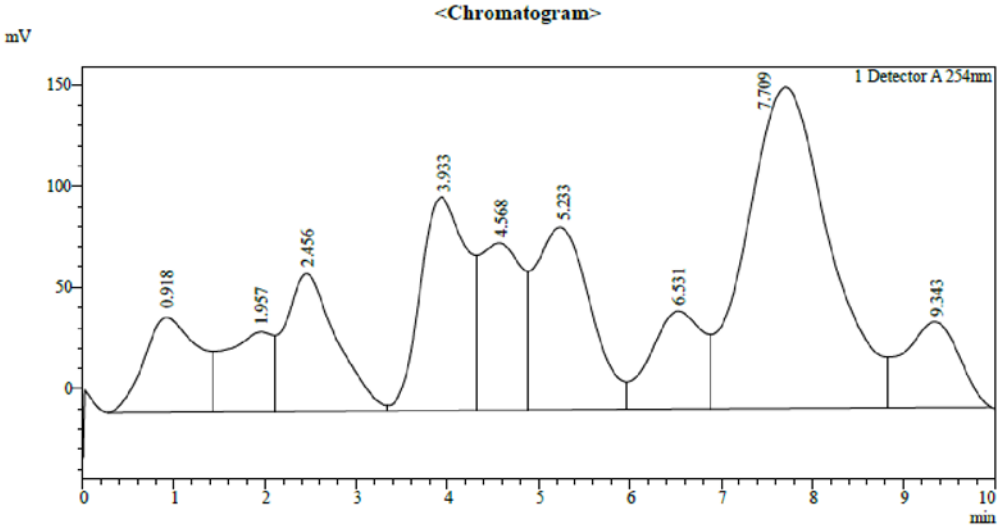

Fig. 3. *Omega3 compound of almond separated from petroleum ether extract and characterized using High Performance Liquid Chromatography (HPLC)*

mm3, while the arithmetic mean of the group (8) was 6.54±0.03 million globules/mm^3.

The arithmetic mean of the group (1,10) was (6.42±0.23) and (6.39±0.04) million globules/mm^3, respectively, and there were no significant differences between these two groups. The arithmetic mean of group (4,6) was (6.09±0.02) and (6.08±0.03) million globules/mm^3, respectively, and there were no significant differences between these two groups, while the lowest arithmetic mean was for group (2), which was (3.52±0.08) million globules/mm^3.

Estimation of Packed Cell Volume (PCV) %

From the Table (2), we notice a significant increase in PCV% in the groups (7,9) with arithmetic averages of (6.08±0.03) and (6.08±0.03)% compared to the rest of the groups and at a probability level of (P≤0.01) respectively, and there were no significant differences between these two groups.

While the arithmetic mean of the groups (3,5) reached arithmetic means of (5.89±0.06) and (5.92±0.05)% respectively. There were no significant differences between these two groups. In contrast, the arithmetic mean of groups (1,8) was (3.79±0.06) and (3.78±0.05)% respectively. There were no significant differences between these two groups, and the arithmetic mean of the groups (4,6,10) was (3.69±0.05) and (3.68±0.05) and (3.65±0.03)%, respectively, and there were no significant differences between these groups. The arithmetic mean for the group (2) was the lowest (2.81±0.08)%.

Estimation of White blood cell count (WBCs) thousand cells/mm^3

Table 2. shows a significant increase in the number of white blood cells in the group(2) with an arithmetic mean of (14.77±0.04) thousand cells/mm^3 compared to the rest of the groups and at a probability level of (P≤0.01), followed by the group (3) with an arithmetic mean of (13.25±0.08) thousand cells/mm^3. In contrast,

Table 2. Effect of treatment with polyphenols extracted from strawberries, Omega-3 extracted from almonds, and Omega -3 with Vit. E on (Hb, RBCs, P.C.V, WBCs) in serum of healthy male New Zealand white rabbits and those with experimentally induced liver steatosis.

Groups Parameters	Control (1)	Triton (2)	Polyphenols (3)	Polyphenols with Triton (4)	Omega-3 (5)	Omega-3 with Triton (6)	Polyphenols & Omega-3 (7)	Ph & Omega-3 with Triton (8)	Omega-3,Vit.E (9)	Omega-3,Vit.E with Triton (10)
Hb	8.37± 0.30a	6.42± 0.16d	15.11± 0.16a	8.08± 0.07c	15.01± 0.11a	8.07± 0.12c	15.08±0. 07a	8.01± 0.17c	15.10± 0.19a	8.04± 0.12c
RBCs	6.42± 0.23f	3.52± 0.08h	7.41± 0.08b	6.09± 0.02g	7.25± 0.22c	6.08± 0.03g	7.69± 0.05a	6.54± 0.03e	6.75± 0.03d	6.39± 0.04f
P.C.V	3.79± 0.06c	2.81± 0.08e	5.89± 0.06b	3.69± 0.05d	5.92± 0.05b	3.68± 0.05d	6.08± 0.03a	3.78± 0.05c	6.08± 0.03a	3.65± 0.03d
WBCs	12.40± 0.10f	14.77±0. 04a	13.25±0. 08b	12.56±0.0 4e	11.61±0. 05h	12.60±0. 02d	13.06±0. 37c	12.75±0. 03d	12.77±0. 04g	12.10±0. 02d

Values are expressed as mean (±) standard deviation and number of rabbits/group = 10. ; Fig. paired with different letters indicate a significant difference at the probability level (P≤0.01).

the arithmetic mean of the group (7) was (13.06±0.37) thousand cells/mm3, while the arithmetic mean of the group (6,8,10) was (12.60±0.02),(12.75±0.03)and (12.10±0.02)thousand cells/mm3 respectively, and there were no significant differences between these groups. In contrast, the arithmetic mean of the group (4) was 12.56±0.04 thousand cells/mm^3 . The arithmetic mean of the group (1) was (12.40±0.10) thousand cells/mm^3.The arithmetic mean of group (9) was (12.77±0.04) thousand cells/mm^3, While the lowest arithmetic mean was for group (5) and reached (11.61±0.05) thousand cells/mm^3.

Estimation of Total antioxidant capacity (TAC) concentration IU/ml

Table 3 shows a significant increase in the concentration of TAC in group (9) with an arithmetic mean of (8.31±0.02) IU/ml compared to the rest of the groups and at a probability level of (P≤0.01), followed by the group (7) with an arithmetic mean (8.14±0.02) IU/ml, then the group (3,5) with arithmetic means (7.66±0.03) and (7.67±0.03) IU/ml respectively, and there were no significant differences between these two groups. The arithmetic mean of the group (1,8,10) were 6.78±0.05, 6.77±0.04, and 6.82±0.03 IU/ml, respectively. There were no significant differences between these groups. The arithmetic means of the group (4,6)were 6.57±0.09 and 6.53±0.04 IU/ml, respectively. There were no significant differences between these two groups, while the lowest arithmetic mean was for the group (2) with an arithmetic mean of 3.62±0.13 IU/ml.

DISCUSSION

The significant increase in hemoglobin concentration, red blood cell count, and red blood cell volume in the group (7), as well as group (5) as in Table (2), may be attributed to the role of polyphenols in activating the enzyme ferrochelatase. This enzyme facilitates the binding of ferrous ion (Fe^{2}□) to protoporphyrin at the final stage of heme synthesis in red blood cells (Poli *et al*., 2021; Aksu *et al*., 2012).

Polyphenols exhibit antioxidant properties and enhance resistance to oxidative stress in red blood cells, preventing membrane oxidation when exposed to oxidative agents. Furthermore, specific polyphenols such as L-

Table 3. Effect of treatment with polyphenols extracted from strawberries, Omega-3 extracted from almonds, and Omega -3 with Vit. E on (TAC U\L) in serum of healthy male New Zealand white rabbits and those with experimentally induced liver steatosis

Groups Parameters	Control (1)	Triton (2)	Polyphenols (3)	Polyphenols with Triton (4)	Omega3 (5)	Omega-3 with Triton (6)	Polyphenols& Omega3 (7)	Ph & Omega3 with Triton (8)	Omega3,Vit. E (9)	Omega3,Vit.E with Triton (10)
TAC	6.78± 0.05d	3.62± 0.13f	7.66± 0.03c	6.57±0.09 e	7.67± 0.03c	6.53± 0.04e	8.14± 0.02b	6.77± 0.04 d	8.31± 0.02a	6.82± 0.03d

Values are expressed as mean (±) standard deviation and number of rabbits/group = 10; Fig. paired with different letters indicate a significant difference at the probability level (P≤0.01).

Rutin, Quercetin, and Lignan stimulate and activate the immune system (Al-Mashhadini and Al-Hayali, 2020; Rudrapal *et al*., 2022; Shakoor *et al*., 2021). The combined presence of several phenols and omega-3 increases hemoglobin concentration and packed cell volume (PCV), while protecting cellular components and nucleic acids from oxidative damage. This synergy enhances the activity of immune receptors. Furthermore, the interaction between phenols and omega-3s may improve iron absorption from the gastrointestinal tract, stimulating the bone marrow to produce new red blood cells, thus increasing hemoglobin and PCV. A similar mechanism has been observed when omega-3s are combined with vitamin E as a therapeutic agent, where it protects cell membranes from apoptosis (programmed cell death) in blood components. Research suggests that omega-3 and vitamin E are antioxidants, potentially reducing the risk of fatty liver disease (Djuricic and Calder, 2021; Ricci *et al*.,2022).

The decrease in red blood cell count, hemoglobin concentration, and packed cell volume (PCV) after Triton treatment in the group (2) shown in Table (2) may be attributed to its effect on red blood cell formation in the bone marrow. In addition, Triton may inhibit hemoglobin synthesis, accelerate red blood cell destruction, and expose cell membranes to oxidative damage, leading to increased apoptosis. This weakens the immune response and reduces hemoglobin levels and packed cell volume. Triton is a toxic substance that promotes the accumulation of free radicals, which ultimately leads to cell damage and death when used at concentrations exceeding the critical limit (El-Aila, 2009; Al-Ani and Al-Qattan, 2017; Tijjani *et al*., 2020).

Triton administration to male New Zealand white rabbits significantly increased the total white blood cell count, and this effect is likely due to the accumulation of fat in hepatocytes, which may interfere with the production of proteins responsible for regulating the immune system. As a result, the activity of white blood cells increases, leading to the release of large amounts of inflammatory cytokines, including interleukins and tumor necrosis factor-alpha (TNF-α), which promotes the production of white blood cells to repair tissue damage or eliminate infected cells (Zhang *et al*., 2019). In addition, excess free fatty acids in hepatocytes affected by NAFLD can elevate the levels of reactive oxygen species, leading to lipid peroxidation, cytokine production, and hepatitis. Increased secretion of pro-inflammatory cytokines in hepatocytes, such as monocyte chemoattractant protein-1, IL-1, IL-6, and TNF-α, further stimulates the production of white blood cells (Xia *et al*., 2024). The normalization of white blood cell count after treatment with polyphenols and omega-3 can be attributed to their antioxidant properties, which help maintain cell membrane integrity and prevent the accumulation of free radicals (Djuricic and Calder, 2021).

The increase in total antioxidant capacity (TAC) observed in rabbits treated with omega-3, vitamin E, and polyphenols extracted from strawberries and almonds in groups (3-10) as in Table (3) may be due to their antioxidant properties, which work to neutralize free radicals and protect against oxidative damage. Omega-3, an essential polyunsaturated fatty acid that humans cannot synthesize due to a deficiency in the enzyme ditashorase, works synergistically with vitamin E to reduce fat accumulation in the liver, limit free radical accumulation, and enhance total antioxidant capacity concentration (Othón-Díaz *et al*., 2023). Vitamin E also enhances total antioxidant capacity levels by increasing lipid membrane fluidity and efficient electron acceptor (Galli *et al*., 2022: Kadhim *et al*., 2023). In addition, polyphenols present in strawberries and almond compounds contributed to higher TAC levels, in contrast to group (2), which showed lower TAC levels. The mechanism of Triton is similar to that of H_2O_2 and cholesterol in that it causes oxidative stress by directly affecting enzymatic and non-enzymatic antioxidants or disrupting their synthesis (Wang *et al*., 2024).

Conclusion

The present study concluded that treatment with Triton group (2) significantly contributes to developing hepatic steatosis, while polyphenols extracted from strawberries, Omega-3 extracted from almonds, and Omega-3, Vit. E in groups (3-10) was key in reducing the treatment effects with Triton and improving the antioxidant status.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Aksu, D. S., Didin, M. & Kayikci, F. (2012). The protective role of polyphenols on blood cells in rats exposed to lead. *Revista Română de Medicină de Laborator Vol*, 20 (3/4),233-243.
2. Al-Ani, Z. S. & Al-Kattan, M. (2017). Effect of Cinnamon Boiled Extract on Blood Picture and Leptin Hormone of Experimentally-Induced Diabetic White New Zealand Female Rabbits. *Rafidain Journal of Science*, *26*(2), 53-62.
3. Al-Daody, A.C. (1998) . Chemical Study on Some Iraqi Plants . PhD Thesis, Mosul University, PP.87.
4. Al-Jaghfi, Z. N. H.(2022). Chromatographic separation of some natural compounds of the seeds of the Iraqi cumin plant *Cuminum cyminuan* L. and a study of their inhibitory effect on two types of bacteria. Master's thesis, Mosul University,PP.33-38.
5. Al-Lahibi, A. H. Gh. (2022). Qualitative and quantitative separation of a number of active substances in *Cyperus rotundus* L. tubers and a study of their antibacterial effect

on some types of bacteria. Master's thesis, Mosul University.PP.44-50
6. Al-Mashhadini, T. & Al-Hayali, H. L. (2020). Biochemical and physiological study of the effect of sesame seeds on quail males exposed to thermal stress. *Indian J Public Health Res Dev*, *11*(4), 1077-1083.
7. Antar, S. H. & Al-Wakaa,A.H.A. (2017). Statistical analysis of agricultural experiments using SAS program. Central Press, Diyala University, 1st ed., , Diyala, Iraq. pp. 469
8. Asrani, S. K., Devarbhavi,H. ,Eaton,J. & Kamath,P.S. (2019). Burden of liver diseases in the world. *Journal of Hepatology*, 70(1), 151-171. https://doi.org/10.1016/j.jhep.2018.09.014
9. Behbahani, M., Shanehsazzadeh,M. & Hessami,M.J. (2011). Optimization of callus and cell suspension cultures of *Barringtonia racemosa* (Lecythidaceae family) for lycopene production. *Scientia Agricola*, 68(1): 69-76. https://doi.org/10.1590/S0103-90162011000100011
10. Chaachouay, N. & Zidane, L. (2024). Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates*, 3(1), 184-207. https://doi.org/10.3390/ddc3010011
11. Djuricic, I. & Calder, P. C. (2021). Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients*, 13(7), 2421. https://doi.org/10.3390/nu13072421
12. El-Aila, H. J. (2009). Interaction of nonionic surfactant Triton-X-100 with ionic surfactants. *Journal of Dispersion Science and Technology*, 30(9), 1277-1280. https://doi.org/10.1080/01932690902735207
13. Forman, H. J. & Zhang, H.(2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, 20(9), 689-709. https://doi.org/10.1038/s41573-021-00233-1
14. Galli, F., Bonomini, M., Bartolini, D., Zatini, L., Reboldi, G., Marcantonini, G., Gentile,G.,Sirolli, V.& Di Pietro, N. (2022).Vitamin E(alpha-tocopherol) metabolism and nutrition in chronic kidney disease. *Antioxidants*, 11(5),989. https://doi.org/10.3390/antiox11050989
15. Ghazanfar, H., Javed,N. , Qasim, A.G. S., Zacharia,G.S., Ghazanfar, A., Jyala,A. ,Shehi,A. & PatelH.(2024). Metabolic Dysfunction-Associated Steatohepatitis and Progression to Hepatocellular Carcinoma: *A Literature Review. Cancers*, 16(6), 1214. https://doi.org/10.3390/cancers16061214
16. Gonzalez, A., Huerta-Salgado, C., Orozco-Aguilar, J., Aguirre, F., Tacchi, F. & Simon ,F. (2020). Role of oxidative stress in hepatic and extrahepatic dysfunctions during nonalcoholic fatty liver disease (NAFLD). *Oxid Med Cell Longev*.; 1617805. https://doi.org/10.1155/2020/1617805
17. Han, S. K., Baik, S.K. & Kim,M.Y. (2022). Nonalcoholic fatty liver disease: Definition and subtypes. *Clinical and Molecular Hepatology*, 29(Suppl), S5. https://doi.org/10.3350%2Fcmh.2022.0424
18. Harborne, J.B. (1998) .Photochemical methods a guide to modern techniques of plant analysis. 5th edition, Chapman and Hall, London, pp.21-72 .
19. Heeren, J. & Scheja, L. (2021). Metabolic-associated fatty liver disease and lipoprotein metabolism. *Molecular Metabolism*, 50, 101238. https://doi.org/10.1016/j.molmet.2021.101238
20. Jeong, S. W. (2020). Nonalcoholic fatty liver disease: a drug revolution is coming. *Diabetes & Metabolism Journal*, 44(5), 640. https://doi.org/10.4093/dmj.2020.0115
21. Kadhim,A.A.,Al-kattan, M.M. & Abou, A. M. (2023).Analyzing and Examining Smoking Effects and Oxidative Stress Markers in Seminal Plasma in Iraqi Infertility Men. *Journal of Bionatura* :Vol.8 NO.2:1-11. http://dx.doi.org/10.21931/RB/CSS/2023.08.02.58
22. Khan, S. U. & Khan ,M. U. (2022). Molecular developments in cell models of fatty liver disease. *DYSONA-Life Science*, 3(1), 16-29. https://doi.org/10.30493/dls.2022.325915
23. Li, Y., Yu ,Y., Yang, L. & Wang, R. (2023). Insights into the Role of Oxidative Stress in Hepatocellular Carcinoma Development. *Frontiers in Bioscience-Landmark*, 28(11), 286. https://doi.org/10.31083/j.fbl2811286
24. Othón-Díaz, E. D., Fimbres-García, J. O., Flores-Sauceda, M., Silva-Espinoza, B. A., López-Martínez, L. X., Bernal-Mercado, A. T. & Ayala-Zavala, J. F. (2023). Antioxidants in oak (Quercus sp.): Potential application to reduce oxidative rancidity in foods. *Antioxidants*, 12(4), 861. https://doi.org/10.3390/antiox12040861
25. Poli, A., Schmitt, C., Moulouel, B., Mirmiran, A., Puy, H., Lefèbvre, T. & Gouya, L. (2021). Iron, heme synthesis and erythropoietic porphyrias: a complex interplay. *Metabolites*, 11(12), 798. https://doi.org/10.3390/metabo11120798
26. Ramachandran, A. & Jaeschke, H. (2018). Oxidative stress and acute hepatic injury. *Current Opinion in Toxicology*, 7, 17-21. https://doi.org/10.1016/j.cotox.2017.10.011
27. Ricci, A., Di Betto, G., Bergamini, E., Buzzetti, E., Corradini, E. & Ventura, P. (2022). Iron metabolism in the disorders of heme biosynthesis. *Metabolites*, 12(9), 819. https://doi.org/10.3390/metabo12090819
28. Robert, K. M., Daryl, K. G., Peter, A. M. & Victor, W. R. (2003). Harper's illustrated Biochemistry twenty sixth edition. 2003 by the McGraw-Hill companies Inc. Printed in London, 12-21.
29. Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi,F.M., Palai,S., Deb,P.K. & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in Pharmacology*, 13, 806470. https://doi.org/10.3389/fphar.2022.806470
30. Shakoor, H., Feehan, J., Apostolopoulos, V., Platat, C., Al Dhaheri, A. S., Ali, H. I., Ismail, L.C., Bosevski,M. & Stojanovska, L. (2021). Immunomodulatory effects of dietary polyphenols. *Nutrients*, 13(3), 728. https://doi.org/10.3390/nu13030728
31. Teng, M. L., Ng, C. H., Huang, D. Q., Chan, K. E., Tan, D. J., Lim, W. H., Tan,E. & Muthiah, M. D. (2022). Global incidence and prevalence of nonalcoholic fatty liver disease. *Clinical and molecular hepatology*, 29(Suppl), S32. https://doi.org/10.3350%2Fcmh.2022.0365
32. Tijjani, H., Adegunloye, A. P., Uba, H., Joel, E. B. & Olatunde, A. (2020). Antioxidant activities of aqueous and ethyl acetate fractions of *Daucus carota* L. seed against triton X-100 induced oxidative stress in mice. *Scientific African*, 8, e00429. https://doi.org/10.1016/j.sciaf.2020.e00429
33. Verwer, C. M., van Amerongen,G., van den Bos, R. & C.

F. Hendriksen,C. F. (2009). Handling effects on body weight and behaviour of group-housed male rabbits in a laboratory setting. *Applied Animal Behaviour Science*, 117 (1-2), 93-102. https://doi.org/10.1016/j.applanim.2008.12.004

34. Villarroel-Vicente, C., Gutiérrez-Palomo, S., Ferri, J., Cortes, D. & Cabedo, N. (2021). Natural products and analogs as preventive agents for metabolic syndrome via peroxisome proliferator-activated receptors: An overview. *European Journal of Medicinal Chemistry*, 221, 113535. https://doi.org/10.1016/j.ejmech.2021.113535
35. Wang, L., Li, S., Yao, Y., Yin, W. & Ye, T. (2021). The role of natural products in the prevention and treatment of pulmonary fibrosis: a review. *Food & function*, 12(3), 990-1007. https://doi.org/10.1039/D0FO03001E
36. Wang, X., Liang, G., Zhou, Y., Ni, B. & Zhou, X. (2024). Ameliorative effect and mechanism of ursodeoxycholic acid on hydrogen peroxide-induced hepatocyte injury. *Scientific Reports,* 14(1), 4446. https://doi.org/10.1038/s41598-024-55043-3
37. Wilson, S. , Gullan, R. & Hocker,E. (1972). Studies of the stability of 18 chemical constituent's serum. *Clin. Chem.*, 18(2): 1498-1503. https://doi.org/10.1093/clinchem/18.12.1498Xia, G., Xu, Y., Zhang, C., Li, M., Li, H., and Chen, C. (2024). High levels of serum hypersensitive C-reactive protein are associated with nonalcoholic fatty liver disease in non-obese people: a cross-sectional study. *European Journal of Medical Research*, 29(1), 496. https://doi.org/10.1186/s40001-024-02065-2
38. Xia, G., Xu, Y., Zhang, C., Li, M., Li, H. & Chen, C. (2024). High levels of serum hypersensitive C-reactive protein are associated with non-alcoholic fatty liver disease in non-obese people: a cross-sectional study. *European Journal of Medical Research*, 29(1), 496. https://doi.org/10.1186/s40001-024-02065-2
39. Zhang, H., Niu, Y., Gu, H., Lu, S., Su, W., Lin, N.,Li, X., Yang, Z., Qin, L. & Su, Q. (2019). The association between white blood cell subtypes and prevalence and incidence of nonalcoholic fatty liver disease. *European Journal of Inflammation*, 17, 2058739219834477. https://doi.org/10.1177/2058739219834477
40. Zidane, S. S. R. (2023). Isolation and identification of a number of natural products of the flowers of the Iraqi karjat plant and their impact on the growth of two types of pathogenic bacteria. Master's thesis, Mosul University, Iraq,PP.55-60.