The Effect of Biscuit Fortified with Milk Thistle (*Silybum marianum L.*) on Steatohepatitis Rats

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ABSTRACT

This study was conducted to investigate the effects of fortifying biscuits with Milk Thistle seeds (MTS) on steatohepatitis disease in rats. Thirty-five rats were separated into two groups of seven each. The first group was fed a basal diet and kept as the negative control group, while the other rats were fed a basal diet deficient in Methionine and Choline with some modifications, including the addition of 19% fat and 1% soybean oil, for six weeks after the induction of steatohepatitis. Rats were reclassified into four equal groups as follows: subgroup 1 served as the control positive group, and the other three treated rat subgroups were fed a high-fat diet containing 100g of biscuit fortified with 5%, 10%, and 15% MTS, respectively. Results showed that biscuits with a 5-15% addition of milk thistle were of good quality. The enrichment of flour with milk thistle led to a significant increase in the essential nutrients, protein, and fat content of the biscuit. Despite the increased fat content, the caloric value of the milk thistle cookies decreased compared to the control group. The results showed that biscuits fortified with different levels of MTS improved the body weight and liver weight, accompanied by a significant decrease in liver function levels (ALT, AST, and Total bilirubin), as well as in lipid profile. There was also a significant increase in high-density lipoprotein-cholesterol (HDL-C). Additionally, Malondialdehyde (MDA) was significantly reduced, while the antioxidant enzymes (SOD and CAT) were significantly increased ($P<0.05$). In conclusion, biscuits fortified with MTS could serve as a natural therapy against steatohepatitis in rats.

Keywords: Milk Thistle (Silybum marianum L.), Biscuit, Non Alcoholic Fatty Liver Disease, Steatohepatitis and Rats.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined by liver steatosis without a history of alcohol intake. Obesity greatly increases the prevalence of NAFLD **(Polyzos et al., 2019**). With the fast pace of modern life and changes in eating patterns, the prevalence of chronic illnesses such as obesity is quickly growing, posing worldwide public health issues **(Cheng *et al*., 2017)**. Obese patients with NAFLD are more likely to develop non-alcoholic steatohepatitis, liver cirrhosis, and even hepatocellular cancer **(Younes and Bugianesi, 2018)**. Identifying healthful, safe, and effective dietary systems and medication therapies for reducing obesity-induced NAFLD is a priority in public health **(Fan *et al*., 2017)**. Insulin resistance (IR) plays a vital part in causing obesity-associated NAFLD and is related to its etiology **(Fotbolcu and Zorlu, 2016)**. According to **Méndez *et al*. (2007)**, NAFLD is a dynamic process that happens at the junction of metabolic changes in the liver and its surroundings. Liver triglycerides accumulate in large quantities in hepatocytes, leading to liver inflammation, fibrosis, and cirrhosis because of inflammatory cytokines and oxidative stress (known as the "second strike theory" of NAFLD pathogenesis). **Chen *et al*., (2020)** estimated that oxidative stress is the primary factor influencing liver damage and the progression of NAFLD. Reactive oxygen species disrupt cellular balance, causing metabolic dysfunction and non-adaptive inflammatory reactions. Under oxidative stress, reactive oxidation products accumulate, reducing the stability of cellular proteins and ultimately resulting in hepatocyte fibrosis **(Masarone *et al*., 2022)**.

Sugary and high-fat foods are blamed for obesity in society. As a result, food producers have been attempting to change people's negative perceptions of confectionery goods by introducing ecological, pro-health products that are nutrient-dense and low in calories, such as eliminating sugar or replacing it with other sweeteners and introducing vitamins, fiber, and antioxidant compounds. Efforts are also being undertaken to replace standard flour in sweet flour-based baked items with more nutritious alternatives. Such treatments allow to enhance the finished product with bioactive components, vitamins, minerals, protein, and dietary fiber while reducing the caloric value. This is vital because it allows for the introduction of new items onto the market while also expanding the range of products available to individuals suffering from diet-related ailments and those who value a healthy lifestyle. The introduction of items that were previously unappealing or unknown to producers is an essential element of food enrichment. This mostly covers plants that are not abundant in the globe and reside only in a certain restricted area or continent, are not highly popular, or whose gastronomic potential is unknown **(Krystyjan *et al*., 2022)**.

One such species, rarely identified thus far, is the seed of milk thistle (Silybum marianum L.), which belongs to the Asteraceae family and is an annual plant distributed throughout Europe, Africa, the Americas, and Asia **(Soleimani *et al*., 2019)**. The seeds of the plant are high in flavonolignans, known as silymarin **(Zhang *et al*., 2020)**. It mostly comprises chemicals such as silibinin, silychristin, silidianin, and isosilybin **(Andrzejewska *et al.*, 2015)**. Thistle seeds offer antioxidant, anti-inflammatory, antiatherosclerotic, anti-diabetic, and anti-obesity characteristics, according to studies conducted over the last decade **(Zhang *et al*., 2020 and Bhattacharya, 2020)**.

Silymarin molecules have significant antioxidant abilities, consequently they can decrease free radical production and lipid peroxidation in the context of liver damage. Silymarin has been discovered to preserve lipid hepatocyte membranes by preventing cell lysis **(Abenavoli *et al*.,2018)**. Also, MTS proteins include significant levels of important amino acids such as lysine, isoleucine, leucine, valine, and threonine, which are not found in wheat flour (WF). As a result, the use of milk thistle seed flour (MTSF) in cereal goods may be handy due to its nutritional and health impacts on consumer organisms **(Bortlíková *et al* .,2019)**.

AIM OF STUDY

This study was conducted to investigate the effects of fortified biscuits with Milk Thistle (Silybum marianum L.) on steatohepatitis disease in rats.

MATERIALS AND METHODS

MATERIALS:

1- **Plant:** fresh Milk Thistle (Silybum marianum L.) was obtained from the Agriculture Research Center.

2- **Ingredients** for preparing bakery products were purchased from the local market.

3- **Chemicals:** Casein, vitamins, minerals, cellulose, Methionine, and Choline were purchased from El-Gomhoria Company, Cairo, Egypt.

4- **Kits** for blood analysis were purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt.

5- **Rats:** adult male rats (Sprague Dawley strain). Thirty-five male Sprague-Dawley rats (weighing 180 ± 10 g) were obtained from the National Research Center, Dokki, Egypt. They were housed under constant conditions of room temperature and 55 ± 5% humidity, with a 12-hour light/12-hour dark cycle. All rats had continuous access to feed and water and were acclimated to laboratory conditions for 1 week.

METHODS:

The Biscuit Preparation:

The preparation of the dough and the baking of biscuits were carried out according to **(Krystyjan *et al*., 2018)**. The amount of ingredients used in the basic recipe (control biscuits) was as follows: 100 g of wheat flour, 17.8 g of sugar, 11.5 g of potato starch, 24.1 g of margarine, 4.6 g of eggs, 29.9 g of milk (cont. 1.5%) and 0.3 g of salt and 3.3 g of baking powder. In the recipe of fortified biscuits, wheat flour was replaced by ground milk thistle seeds (MTS) in variable amounts, i.e., 5, 10, 15 and 20%.

Sensory Analysis of Biscuits

A five-point evaluation scale with quality descriptors and weighting coefficients: shape (0.5), color (0.5), surface (0.75), consistency (0.75), fracture ability (0.5), smell (0.5) and taste (1.5) were used **(PN-ISO, 1998)**. The overall acceptance of fresh biscuits will be assessed based on the total score: <2.9 unacceptable, 3.0–3.5 acceptable, 3.51–4.5 good, and 4.51–5.0 very good, according to **(Gambuś *et al*., 2009)**. (MT) in variable amounts, i.e., 5, 10, 15 and 20% (samples MTS5, MTS10, MTS15 and MTS20, respectively). Each repetition consisted of cookies that were made from separate batches of dough.

The Chemical Composition of Biscuits

The analysis was carried out according to the Association of Official Analytical Chemists International methods (AOAC, 2006). The Kjeldahl procedure (using the Büchi B324 extraction system) with nitrogen was used to determine the total protein content (protein conversion factor was 6.25, method number 950.36). Analysis of the total dietary fiber (DF) and its fractions: soluble (SDF) and insoluble (IDF) was carried out by the enzymatic–gravimetric method (method number 991.43). The Soxhlet method (using the Büchi B811 extraction system) (method no. 935.38) was used to determine the fat content. The ash content was examined by carbonization (method number 923.03). The content of total carbohydrates was by difference. The caloric value = (4 × protein) + (9 × fat) + (total carbohydrates–total dietary fiber) × 4 (FAO, 2002). The result is the average of three repetitions.

Total Polyphenol Content and Antioxidant Activity of Biscuits

The sample of biscuits was ground (0.6 g) and extracted for 2 h, at a temperature of 23 °C, using 30 mL of 80% ethanol. Then the sample was centrifuged (MPW-350 centrifuge) at 2500× g for 15 min. The extract was stored in a freezer at a temperature of −20 °C. The content of total polyphenols was determined according to **Singleton et al., (1999)** using the spectrophotometric method, and the flavonoid content were carried out according **to El Hariri *et al*., (1991)** using the spectrophotometric method.

Model of Steatohepatitis:

Rats were fed on a basal diet deficient in methionine and choline (MCDD) for 6–8 weeks according to **Corbin and Zeisel (2012)** with some modifications, including adding 19% fat and 1% soybean oil. Liver functions were significantly increased after 2 weeks of diet and increase progressively **(Itagaki *et al*., 2013)** that were confirmed by taking random blood samples from the eye of a rat.

Experimental Design

The experimental animals were done using (n=35) male rats, with body weight 180 ± 10 g. The rats were housed in cages under hygienic conditions in a temperature-controlled room at 25°C. Basal diets were semi-synthetic and nutritionally adequate (AIN-93 M). Vitamin mixtures and mineral mixtures to meet recommended nutrient levels for rats were prepared as described by **Reeves *et al. (1993).*** After a period of adaptation on a basal diet (one week), the animals were randomly divided into two main groups as follows:

The first main group (n = 7) was fed on a basal diet (Negative Control group (Ve).

The second main group (n = 28) was fed on a basal diet deficient in methionine and choline (MCDD) for 6 weeks according to **Corbin and Zeisel (2012)** with some modifications, including adding 19% fat and 1% soybean oil to induce steatohepatitis. After the rats were divided into four subgroups (n = 7 rats for each) as follows:

- Group (1): twenty-eight rats fed on a high-fat diet as the positive control group (+ve).
- Group (2): fed on high-fat diet containing 100 g control sample of biscuit fortified with 5% milk thistle seeds.
- Group (3): fed on high-fat diet containing 100 g control sample of biscuit fortified with 10% milk thistle seeds.
- Group (4): fed on high-fat diet containing 100 g control sample of biscuit fortified with 15% milk thistle seeds.

Nutritional evaluation:

The biological evaluation of the diet was carried out by determining feed intake, body weight gain percent (BWG %), and feed efficiency ratio (FER) according to Chapman (1959) using the following equation:

$$\textbf{BWG}\ \% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER = Weight gain (g) / Feed intake (g)

At the end of the experimental period (42 days), rats were fasted overnight, then the blood was collected under slight ether anesthesia. The serum was separated by centrifugation at 3000 rpm for 15 min. The obtained serum was used immediately for routine laboratory investigation.

Biochemical Analysis:

Serum Lipid Profile:

According to **Allain,(1974), Fassati and Prencipe, (1982)** and **Albers *et al*., (1983)**, the serum total cholesterol (TC), triglycerides (TG), and cholesterol contents of high-density lipoprotein (HDL-c) were measured, respectively. Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according to **(Friedewald *et al*., 1972)**.

LDL-c = TC- [HDL-c + (TG/5)]   VLDL-c = TG/5

Liver Function:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to **Bergmeyer et al. (1978)**. Serum total bilirubin was measured according to **(Principato et al., 1985).**

Oxidative and Antioxidant Biomarkers:

Following the methodology of **Draper and Hadley (1990)**, the plasma level of malondialdehyde (MDA) was calculated to measure lipid peroxidation. Superoxide dismutase (SOD) activity was evaluated by Spitz and Oberley (1989). Catalase (CAT) was measured by **Aebi (1984).**

Statistical analysis:

Statistical analysis was performed using the SPSS computer program (GraphPad Software Inc, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple tests were done. $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table (1) showed that sensory parameters of unfortified biscuits and biscuits fortified with different percentages (5, 10, 15, and 20%) of MTS were evaluated for appearance, texture, color, flavor, taste, and overall acceptability. The sensory analysis revealed that the control sample received the highest rating, with a score of 8.63. Biscuits prepared by partially replacing WF with MTS at levels of 5, 10, 15, and 20% exhibited good sensory properties. The taste and overall acceptability of biscuits fortified with 15% MTS were preferred by the panelists compared to the control. Furthermore, biscuits with 5, 10, and 15% ground milk thistle seed content had similar taste notes to the control sample. However, beyond this level, the quality parameters of the product slightly deteriorated. These findings are consistent with those reported by **Krystyjan et al. (2022)**, who demonstrated that biscuits with a 5–15% addition of milk thistle were of good quality.

The cookies with 5% milk thistle gained high consumer acceptance, but an addition greater than 20% caused a worsening in quality. At the same time, the odor, texture, and overall acceptance of the enriched biscuit with milk thistle seed flour were not significantly affected.

Most of the panelists reported that the biscuit turned brown with the addition of milk thistle flour. Moreover, they mentioned that the enriched biscuit gave a sense of the presence of remains of thin tissue on the tongue. This may be related to the presence of some seed coat of the milk thistle. These results agree with **Hyun-Jung et al. (2014)**, who reported that taste is an important characteristic in determining the acceptability of cookies. Additionally, Heinio et al. (2016) mentioned that raw materials and ingredients are key factors for flavor formation in cereal products.

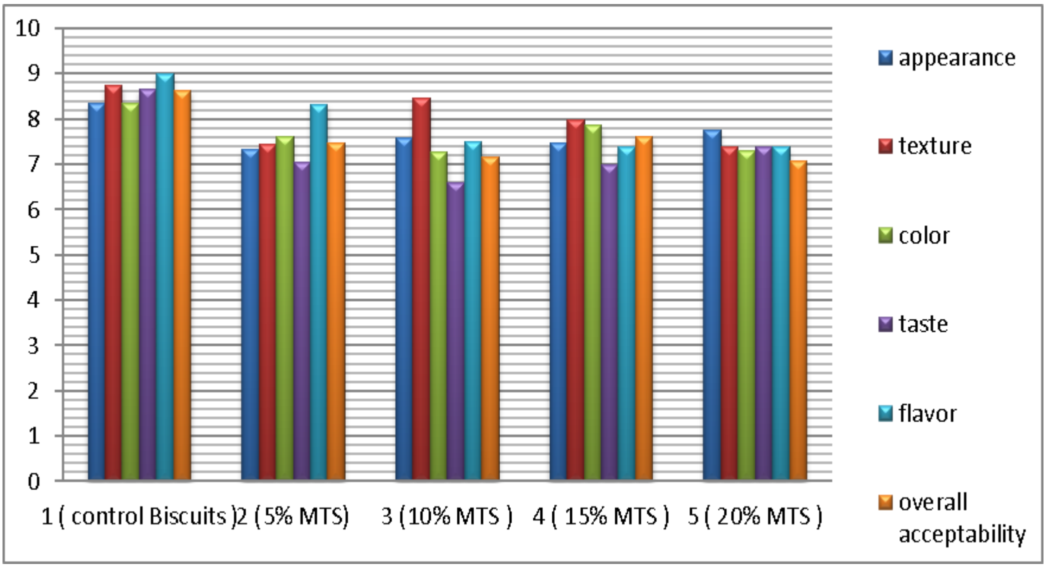

Figure (1): Sensory evaluation of Biscuits

Figure (1) shows the results of the analysis of the nutrient and dietary fiber content in the product. The addition of milk thistle to the flour resulted in a significant increase in the fat content of the biscuit, even with the lowest amount of additive used, and up to 15% fortification. Despite the higher fat content, the caloric value of the milk thistle cookies decreased compared to the control group. The content of other essential nutrients, such as protein and ash, also increased, but not as significantly as protein and fat, which increased by 10% to 15%. There was a statistically significant and consistent increase in fiber with the increasing addition of ground milk thistle seeds.

The fortification of ground milk thistle seeds led to an increase in the fat content of the biscuit. This impact is due to the difference in chemical composition between ground thistle seeds and wheat flour; the milk thistle flour contains 19.5 times more fat **(Kazazis et al., 2014 and Krystyjan et al., 2022)** proved that milk thistle seed oil has unsaturated fatty acids, with the greatest share of linoleic and oleic acid, which demonstrate beneficial impacts on human health and protect against diabetes, arteriosclerosis, and cancer. It should also be emphasized that, despite the increased content of fat, the caloric value of the milk thistle cookies decreased compared to the control.

This was due to the lower content of carbohydrates in thistle seeds compared to wheat flour. The nutrient content of the raw materials used in baking has a significant impact on the physicochemical and health-promoting properties of the final product. Carbohydrates and fats control the flavor, color, and texture of biscuits **(Krystyjan et al., 2015 and Krystyjan et al., 2022).**

Table (1): The chemical composition of unfortified fortified and fortified Biscuit with different levels of Milk Thistle (g/100g)

Nutrition value	Moisture	Protein	Fat	Ash	Fiber	Carbo	Energy values (Kcal/ 100g)
Control	5.01±0.24[d]	6.50±0.02[b]	12.95±0.3[b]	1.65±0.03[a]	2.35±0.02[d]	71.54±0.15[a]	428.71
Biscuit fortified with 5 % MT	6.40±0.36[c]	7.84±0.12[a]	13.63±0.34[b]	1.45±0.01[c]	3.40±0.16[c]	67.28±0.1.16[b]	423.15
Biscuit fortified with 10 % MT	7.87±0.14[b]	8.09±0.13[a]	14.80±0.16[a]	1.55±0.02[b]	4.39±0.04[b]	63.3±1.85[b]	418.76
Biscuit fortified with 15 % MT	8.20±0.21[a]	8.08±0.10[a]	15.42±0.08[a]	1.64±0.02[a]	5.20±0.07[a]	61.46±1.37[b]	416.94

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

Table (2) shows the effect of different levels of Milk Thistle fortified biscuits on body weight gain (BWG), food intake (FI), food efficiency ratio (FER), and liver weight of steatohepatitis rats. The data showed a significant increase ($p<0.05$) in the positive control group compared to the negative control group. On the other hand, the results showed a significant decrease ($P<0.05$) in BWG, FI, FER, and liver weight in the groups treated with biscuits fortified with 5%, 10%, and 15% MT compared to the positive group. Rats that were fed biscuits fortified with 15% MT recorded the highest increase and best result when compared to the positive control group.

It is well known that high-fat diets increase body weight and visceral fat deposition, and such findings have previously been published **(Kubant et al., 2015; Negm, 2023 and Negm, 2023a).** Moreover, the treated groups of biscuits fortified with different levels of MTS significantly decreased liver weight. Furthermore, there was no significant difference among groups treated with different levels of MTS. These results are consistent with **Negm and Aboraya (2023) and Zhu et al. (2018)**, who reported that S. marianum effectively reduced mice body weight.

Table (2): The effect of different levels of Biscuit fortified with Milk Thistle on body weight gain (BWG), food intake (FI), and food efficiency ratio (FER) of steatohepatitis rats.

Parameters / Groups	BWG (g/28 day)	FI (g/d/rat)	FER	Liver weight (g)
Control (-Ve)	24.10±0.42[d]	15	0.057±0.003[d]	6.18±0.18[c]
Control (+Ve)	58.21±0.34[a]	23	0.090±0.001[a]	7.30±0.50[a]
Biscuit fortified with 5 % MT	50.25±0.17[b]	22	0.082±0.002[b]	6.79±0.25[b]
Biscuit fortified with 10 % MT	45.65±0.25[c]	21	0.078±0.004[bc]	6.49±0.02[b]
Biscuit fortified with 15 % MT	42.84±0.13[c]	20	0.077±0.004[c]	6.42±0.01[bc]

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

The data in **Table (3)** show the effect of different levels of biscuits fortified with MT on triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and very low-density lipoprotein cholesterol (VLDL) in steatohepatitis rats. The results significantly increased (P<0.05) in the lipid profile in the positive control group, while decreased in HDL level compared to the negative control group. These findings are consistent with **Negm et al., (2024) and Lemus-Conejo et al., (2020)** who observed that NAFLD rats had a significant increase in the serum levels of TC, TG, LDL, and VLDL but lowered HDL level in the (+ve) group as compared to the (-ve) groups. This may be attributed to lipolysis in adipose tissue which, in turn, causes hyperlipidemia **(Liu et al., 2019 and Negm et al., 2023b).**

Table (3): The effect of different levels of Biscuit fortified with Milk Thistle on lipid profile of steatohepatitis rats.

Parameters / Groups	TG	TC	HDL	LDL	VLDL
	(mg / dl)				
Control (-Ve)	79.57±2.86[d]	115.41±3.37[d]	60.45±2.63[a]	39.05±0.17[d]	15.91± 0.57[d]
Control (+Ve)	115.56±3.76[a]	151.85±3.10[a]	50.48±1.89[c]	78.26±0.46[a]	23.11±0.75[a]
Biscuit fortified with 5 % MT	103.34±2.56[b]	136.48±2.56[b]	54.23±1.27[b]	61.58±0.78[b]	20.67± 0.51[b]
Biscuit fortified with 10 % MT	88.78±1.78[c]	126.10±3.93[c]	56.86±2.39[b]	51.48±1.18[c]	17.76± 0.36[c]
Biscuit fortified with 15 % MT	81.64±2.24[d]	117.51±4.68[d]	62.61±2.82[a]	38.57±1.41[d]	16.33±0.45[cd]

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

Contrast, the groups treated with biscuits fortified with different levels of milk thistle (5, 10, and 15%) induced a significant decrease (P<0.05) in serum TC, LDL, and VLDL levels, but an increase in HDL levels compared to the positive control group (+Ve) as recorded in **Table 3**. The best result and the lowest level of lipid profile were observed in the supplementation diet with 15% biscuits fortified with milk thistle compared to the +Ve group. These results are consistent with **Negm and Aboraya (2023)**, who found that treatment with milk thistle seeds at 5% significantly suppressed the elevation of lipid levels and increased HDL levels. **Ghanem et al. (2022)** also demonstrated that treatment with milk thistle improved lipid profile. In a similar study, **Jiang et al. (2022)** showed that silymarin may considerably decrease TC, TG, and LDL levels while increasing the levels of HDL in mice. **Ahmed (2021)** showed that feeding female rats a baseline diet supplemented with milk thistle seeds at 10% and 15% improved their lipid profile in female rats within toxified liver.

The effect of different levels of biscuit fortified with Milk Thistle on liver functions of steatohepatitis rats was recorded in **Table (4)**. The levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Total bilirubin were compared between the positive control group and the negative control group. The results showed that the positive control group had significantly higher levels of AST, ALT, and Total bilirubin compared to the negative control group. However, all treated rats showed a significant decrease ($p<0.05$) in these levels compared to the positive control group. This led to a decrease in AST, ALT, and total bilirubin compared to the positive control group. These findings are consistent with the observations made by **Negm et al. (2024)**, who found a significant increase in the serum levels of ALT, AST, and ALP in NAFLD rats.

On the other hand, the group receiving 15% MT recorded the greatest reduction in AST, ALT, and total bilirubin compared to the positive control group, due to polyphenols including silymarin exerting a beneficial effect on the health of animals through reducing oxidative stress and inflammation (Negm and Aboraya, 2023). These results agree with El **Hassanen et al., (2021)** that feeding milk thistle herb improved liver functions. Also, **Kumar and Khanna, (2018)** and **Amin et al., (2019)** reported that administration with different levels of dried milk thistle (20% - 40%) had a significant decrease in their liver function, compared with the positive control group.

Table (4): The effect of different levels of Biscuit fortified with Milk Thistle on liver functions of steatohepatitis rats.

Parameters / Groups	AST (μ /L)	ALT (μ /L)	Total bilirubin mg/dL
Control (-Ve)	24.25±1.85[c]	64.35±4.25[b]	0.65±0.05[c]
Control (+Ve)	32.04±2.15[a]	98.41±0.80[a]	0.97±0.10[a]
Biscuit fortified with 5 % MT	28.58± 0.73[b]	66.75±2.75[b]	0.80±0.02[b]
Biscuit fortified with 10 % MT	26.48±0.73b[c]	58.21±1.38[c]	0.77±0.01[b]
Biscuit fortified with 15 % MT	25.45±0.56[c]	50.91±2.51[d]	0.74±0.01[bc]

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

Results **in Table (5)** revealed that the (+Ve group) showed a significant (P<0.05) elevation in serum levels of malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) compared to the (-Ve group). On the other hand, feeding rats a diet supplemented with 5%, 10%, and 15% of biscuit fortified with Milk Thistle (MTS) induced a significant decrease (P<0.05) in MDA levels, but an increase in CAT and SOD levels compared to the (+Ve group). The group receiving 15% of biscuit fortified with MTS showed the lowest reduction in serum levels of MDA, but an increase in CAT and SOD levels compared to the (+Ve group). The best result of antioxidant enzymes was recorded for the biscuit fortified with 15% MTS, as indicated by the ratios of MDA, CAT, and SOD.

Silymarin acts as a strong antioxidant agent and has a high ability to scavenge ROS **(Khazaei et al., 2022)**. These results are consistent with **Negm and Aboraya (2023)**, who found that treatment with milk thistle seeds at 5% significantly decreased serum levels of MDA while increasing the levels of SOD in treated groups (p ≤ 0.05) compared to PCOS. This could be due to its anti-inflammatory and antioxidant properties. The mixture group showed the best results in all parameters. Additionally, **Ghanem et al. (2022)** showed that doses of milk thistle extract (20 and 30 g/day) significantly boosted antioxidant enzymes (SOD and CAT) but decreased the level of MDA. In a similar study, **Abd Elalal et al. (2022)** and **Ahmed (2021)** suggested that treatment with milk thistle (rich in silymarin) may contribute to an increase in antioxidant enzymes while inhibiting MDA.

Table (5): The effect of different levels of Biscuit fortified with Milk Thistle on oxidative stress and antioxidant enzymes of steatohepatitis rats

Parameters / Groups	MDA nmol/ml	CAT U/ml	SOD U/ml
Control (-Ve)	0.62±0.45[d]	50.74±0.38[a]	2.86±0.21[a]
Control (+Ve)	98.04±1.69[a]	22.39±0.05[d]	1.63±0.26[d]
Biscuit fortified with 5 % MT	0.85±1.82[b]	38.92±0.27[c]	2.35±0.30[c]
Biscuit fortified with 10 % MT	0.76±1.44[c]	42.88±0.29[b]	2.58±0.18[b]
Biscuit fortified with 15 % MT	0.65±1.82[d]	45.64±0.33[b]	2.69±0.20[b]

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

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تأثير البسكويت المدعم بشوكة الحليب على الفئران المصابة بإلتهاب الكبد الدهني

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الملخص العربي

اجريت هذه الدراسة لمعرفة في تأثير البسكويت المدعم ببذور شوك الحليب على مرض التهاب الكبد الدهني في الفئران. تم تقسيم خمسة وثلاثين فأرًا إلى مجموعتين كل منهما ٧ فئران تم تغذية المجموعة الأولى على نظام غذائي أساسي وتم الاحتفاظ بها كمجموعة ضابطة سالبة، بينما تم تغذية الفئران الأخرى على نظام غذائي أساسي يفتقر إلى الميثيونين والكولين مع بعض التعديلات بما في ذلك إضافة (١٩٪ دهون و ١٪ زيت فول الصويا) لمدة ٦ أسابيع، لإحداث التهاب الكبد الدهني. تم إعادة تصنيف الفئران إلى ٤ مجموعات متساوية على النحو التالي: المجموعة الفرعية ١ خصصت كمجموعة ضابطة موجبة و٣ مجموعات فرعية من الفئران المعالجة تم تغذيتها على نظام غذائي عالي الدهون يحتوي على ١٠٠ جرام (عينة ضابطة من البسكويت المدعم بـ (٥ ، ١٠، ١٥٪ MTS) على التوالي. أظهرت النتائج أن البسكويت المضاف إليه ٥– ١٥٪ من شوكة الحليب كان جيد. أدى إثراء الدقيق بشوك الحليب إلى زيادة كبيرة في العناصر الغذائية الأساسية ومحتوى البروتين والدهون في البسكويت. على الرغم من زيادة محتوى الدهون، انخفضت السعرات الحرارية لبسكويت شوكة الحليب مقارنة بالمجموعة الضابطة. أوضحت النتائج أن البسكويت المدعم بمستويات مختلفة من شوكة الحليب قد حسنت من وزن الجسم ووزن الكبد مصحوبًا بانخفاض كبير في مستويات وظائف الكبد (ALT و AST والبيليروبين الكلي)، وكذلك في صورة الدهون، في حين تم تسجيل زيادة كبيرة في البروتين الدهني عالي الكثافة (HDL–C). بالإضافة إلى ذلك، انخفض بشكل كبير مستوى المانولدهايد (MDA) في حين ارتفعت مستويات إنزيمات مضادات الأكسدة (SOD) و (CAT) بشكل ملحوظ ($P<0.05$). الاستنتاج، يمكن أن يقدم البسكويت المدعم بشوكة الحليب علاجًا طبيعيًا ضد التهاب الكبد الدهني في الفئران.