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Impact of Incorporating the Aqueous Extract of Hawthorn (*C. oxyanatha*) Leaves on Yogurt Properties and Its Therapeutic Effects against Oxidative Stress Induced by Carbon Tetrachloride in Rats

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Citation: Shahein, M.R.; Atwaa, E.-S.H.; Babalghith, A.O.; Alrashdi, B.M.; Radwan, H.A.; Umair, M.; Abdalmegeed, D.; Mahfouz, H.; Dahran, N.; Cacciotti, I.; et al. Impact of Incorporating the Aqueous Extract of Hawthorn (*C. oxyanatha*) Leaves on Yogurt Properties and Its Therapeutic Effects against Oxidative Stress Induced by Carbon Tetrachloride in Rats. *Fermentation* **2022**, *8*, 200. https://doi.org/10.3390/fermentation8050200

Academic Editor: Carlo Giuseppe Rizzello

Received: 22 March 2022
Accepted: 26 April 2022
Published: 29 April 2022

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Abstract: The current study aimed to evaluate the chemical, phytochemical, and sensory properties; the nutritional value; and the antioxidant properties resulting from the incorporation of yogurt fortified with the aqueous extract of Hawthorn leaves in Sprague Dawley rats. The results revealed that the yogurt containing the aqueous extract from Hawthorn leaves exhibited no significant differences in terms of its protein, fat, and ash contents compared to control samples. Moreover, the highest total phenolic content (62.00 ± 1.70) and antioxidant activity (20.60 ± 0.74%) were detected in the yogurt containing 0.4% Hawthorn leaf extract compared to the other samples. The consumption of yogurt fortified with the aqueous extract from Hawthorn leaves by rats experiencing oxidative stress resulted in a significant decrease ($p \leq 0.05$) in the triglyceride, total cholesterol, low-density lipoprotein, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, and malondialdehyde levels and a remarkable increase ($p \leq 0.05$) in the high-density lipoprotein, total protein, and albumin levels as well as in the total antioxidant potentials of serum compared to the positive control group, indicating that the extract from Hawthorn leaves can play a preventive role against oxidative stress. Collectively, our study concluded that the extract from Hawthorn leaves can provide health benefits to yogurt on the basis of its high bioactive components and can exert protective effects against oxidative stress in rats.

Keywords: yogurt; *Crataegus oxyacantha*; total phenolic; cholesterol; triglyceride; oxidative stress

1. Introduction

Oxidative stress is considered to be one of the main causes of chronic inflammation [1–3] and promotes the activation of various transcription factors, resulting in the consequent expression of genes that are related to inflammatory pathways [4,5]. Several previous studies have reported that oxidative stress could potentially lead to different forms of nephrotoxicity and renal damage, both of which have dramatic consequences, due to the pivotal role of the kidneys in excreting metabolic waste and in the chemical homeostasis balance [2,6]. The consumption of antioxidant-rich foods is considered to be pivotal for combating the oxidative stress. According to the available literature, several previous studies have reported that dairy products contain a low content of bioactive compounds, limiting their nutritional value. The addition of antioxidants, e.g., flavonoids, tannins, phenolic acids, vitamin E, and vitamin C, to foods has been proposed as an effective preventive adjuvant therapy, as antioxidants are able to synergistically interact with other reducing compounds, having anti-atherosclerotic, anti-inflammatory, and anti-carcinogenic actions [7]. The use of polyphenols has also been shown to have potent antioxidant activity, anti-inflammatory action, and the capability to inhibit the enzymes that are involved in the development of eicosanoids [7,8]. Recently, food producers have shown interest in substitution with synthetic additives (including nutritional and preservative agents, coloring, flavoring, and miscellaneous agents) [9–13] to improve the features of processed foods with natural ones [14–18]. Interestingly, the supplementary antioxidants from natural sources, such as plants, have proven to be efficient in providing protection against oxidative stress due to enzymatic and non-enzymatic elements, some of which are only synthesized in plants and can only be taken in by the body through food [19]. Moreover, foods that have been supplemented with bioactive compounds are able to enhance the immune system [20]. Yogurt is considered to be one of the most important fermented milk products and is produced by fermenting lactose to lactic acid via the action of a yogurt starter culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. The yogurt starter culture interacts with the milk protein, which improves the body texture and sensory attributes of the product. It is widely known that synthetic additives, including nutritional and preservatives additives, coloring, and flavoring agents as well as other miscellaneous agents, are used to improve the characteristics and properties of processed foods [21]. Clearly, the incorporation of plant-based additives can be used to fortify dairy products and improve the nutritional value of these products [22]. Hawthorn *Crataegus oxyacantha* L. belongs to the Rosaceae family of spiny shrubs also known as the Hawthorn species and is medicinally recognized in European Pharmacopeia, having been used for a long time in folk medicine for the treatment of different diseases such as diarrhea, asthma, and insomnia [23] as well as for the treatment of angina pectoris, hypertension, and arrhythmia. It has been also proposed as an alternative anxiolytic, antihyperlipidemic, immunomodulatory, antihyperglycemic, and antimutagenic medicine [24]. Likewise, *C. oxyacantha* contains different active constituents, including oligomeric, procyanidins flavonoids, triterpenic acids, phenolic acids, fatty acids, triterpenes, sterols, and phenol carboxylic acids [24,25]. Moreover, it has a significant hypolipidemic effect since it improves lipid profiles and enhances rheological blood flow and immune function [26–28]. Little information is available about the effects of incorporating the aqueous extract of Hawthorn (*C. oxyanatha*) leaves on yogurt properties and its therapeutic effects. Given the above information, the present study investigated the impact of incorporating the aqueous extract of Hawthorn (*C. oxyacantha*) leaves during yogurt manufacture to determine whether it fortified different proportions of yogurt in terms of its functional properties. Yogurt is one of the most important types of fermented milk in the world and is produced as a consequence of the interaction of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* with milk proteins, resulting in a product with improved texture and sensory properties [29]. Furthermore, the fortified yogurt was examined to determine its therapeutic effects as a functional food on the biomarkers of different liver and kidney functionalities, oxidative stress, and lipid profiles in rats with high oxidative stress induced by carbon tetrachloride.

2. Materials and Methods

2.1. Materials

Hawthorn (*Crataegus oxyacantha* L.) leaves were obtained from the Agricultural Research Center of Giza (Egypt), washed with tap water to eliminate toxic saponins and then dried at 45 °C for 12 h, ground into a fine powder using an electric blender, and then stored in a freezer in plastic bags until use. All of the solvents that were exploited for extraction and analyses were of analytical grade. Folin–Ciocalteu (FC), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonate) were purchased from Sigma (St. Louis, MO, USA). The kits used for the biochemical analyses of the total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, total albumin, creatinine, urea, and malondialdehyde (MDA) were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals (Cairo, Egypt). The total phenolic and radical scavenging activity of the Hawthorn leave extract were determined as described elsewhere [30] to prepare a standard basal diet consisting of 10% protein, 15% casein, 5% cellulose, 10% fat, and 65% corn starch. Fresh cow's milk (with a fat content of 3%) was provided by Zagazig University, Agriculture Faculty, Food Science Department, Dairy Technology Unit, Zagazig, Egypt. A yogurt culture containing *Lactobacillus delbruekii* subsp. *bulgaricus* EMCC1102 and *Streptococcus salivarius* subsp. *thermophilus* EMCC104 was purchased from the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2.2. Preparation of Hawthorn Leaves Aqueous Extract

The dried Hawthorn leaves were reduced to powder (0.841 mm). The aqueous extracts were obtained by dipping 10 g of Hawthorn leaves in 100 mL of hot distilled water (95 °C) and by keeping them refrigerated at 4 °C overnight. Afterwards, the Hawthorn extract was mixed at 100 rpm for 1 h in a rotary shaker, filtered using a filter paper (Whatman No. 1), lyophilized, and stored at 4 °C.

2.3. Yogurt Fortified with Aqueous Hawthorn Leaves Extract Manufacture

Fresh cow's milk (3% fat) was heated for 15 min at 80 °C and cooled to 42–45 °C before inoculation with a 3% starter culture containing *Lactobacillus delbruekii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, which was prepared by adding 2.25×10^7 cells/mL. The inoculated flask contained 250 mL of sterile culture medium. The obtained milk was then divided into three aliquots: plain yogurt (PY), yogurt loaded with 0.2% *w*/*w* (HLEY0.2), and yogurt loaded with 0.4% *w*/*w* (HLEY0.4) Hawthorn leaf aqueous extract. The three portions were maintained in plastic containers (100 mL) and incubated at 43 °C until a pH value of 4.65 was reached. When the fermentation process was complete, all of containers were immediately cooled down and kept at 4 °C in a refrigerator overnight.

2.4. Chemical Compositions, Physicochemical Analysis, and Sensory Evaluation of Yogurt Fortified with Aqueous Hawthorn Leaves Extract

For the yogurt samples, the total protein, total solid, fat, and ash contents; peroxide value; acid value; and titratable acidity were determined according to the method outlined in [31]. Their pH values were monitored using a pH meter equipped with a glass electrode (HANNA, Instrument, Portugal). Total phenolic content (TPC) (expressed as mg GAE (gallic acid equivalents)/100 g) was assessed as described elsewhere [32] with minor modifications. Briefly, 100 µL of different concentrations of the test sample was mixed with 1 mL of diluted FC reagent (1:10). After 10 min, 1 mL of 7.5% (*w*/*v*) sodium carbonate solution was added to the mixture, and the mixture was incubated in the dark for 90 min. The absorbance was recorded at 725 nm. The TPC was calculated according to the calibration curve of gallic acid $Y = 0.2808X + 0.0301$; $R^2 = 0.9983$. The antioxidant (AO) activity (%) of the prepared yogurts was assessed as described elsewhere [33], and the absorbance was noted at 517 nm using a spectrophotometer (Thermo Scientific, Wilmington,

NC, USA). The scavenging activity was calculated with the formula to determine the DPPH radical scavenging % = (Absorbance of sample—Absorbance of blank)/Absorbance of control × 100. The yogurt samples underwent a sensory evaluation to determine its flavor (45), texture (30), acidity (15), and appearance (10) following the method reported by [34]. The sensory evaluation was carried out by a team of 10 trained professional panelists. The samples were packed and coded with a 3-digit code. The encoded samples were presented to the panelists on a tray. After testing each sample, the panelists were offered plain water to cleanse their palates before moving on to the next sample.

2.5. Animal Experimental Design

Forty-eight male adult Sprague Dawley albino strain rats weighing 180–220 g were purchased from the Agricultural Research Center of Giza, Giza, Egypt and housed in wire cages in a 25 °C environment. After acclimatization on a basal diet for seven days, they were divided into four groups (twelve rats for every group) as follows: Twelve rats represented the normal control group and were fed a standard diet as a negative control (group 1). Thirty six rats were intraperitoneally injected with a single dose of carbon tetrachloride at a rate of 2 mL/kg body weight (oxidative stress rats), as described elsewhere [35]. The oxidative stress rats were then organized into three groups (twelve rats in each group) as follows: oxidative stress rats as a positive control (Group 2), oxidative stress rats fed a basal diet with plain yogurt (10 g/day) with a epigastric tube (Group 3), and oxidative stress rats fed a basal diet with yogurt fortified with 0.4% Hawthorn leaf aqueous extract (10 g/day) with a epigastric tube (Group 4).

2.6. Biochemical Analysis

At the end of the 5-week trial period, the rats were lightly anesthetized with diethyl ether. Blood samples were collected from the hepatic portal vein and were submitted to centrifugation to separate the serum at 3000 rpm (1.811× *g*) for 15 min and stored at −40 °C. After that, the biochemical parameters were determined using commercially available kits and related methods: total cholesterol (mg/dL) was evaluated using the enzymatic colorimeter method [36]; total triglycerides (mg/dL) were measured by following the method outlined in [37]; high-density lipoprotein (HDL, mg/dL) was measured by following the method outlined in [38]; and low-density lipoprotein (LDL, mg/dL) was measured using the Friedewald formula (LDL-cholesterol = Total cholesterol-HDL-cholesterol-(Triglycerides/5)) [39]. The alanine aminotransferase (ALT, U/L) and aspartate aminotransferase (AST, U/L) enzymes were evaluated by applying the methods described by Bergmeyer and Harder, 1986 [40]. The total albumin (g/dL), total protein (g/dL), and malondialdehyde (MDA, µmol/L) were measured on the basis of procedures reported elsewhere [41]. Moreover, the serum creatinine (mg/dL) and serum urea (mg/dL) levels were measured as described elsewhere [42,43]. The total antioxidant potentials (mM/L) of the serum were also determined [44].

2.7. Histopathological Examination

Specimens from the livers and kidneys of the rat were directly collected and weighed after r the rats were sacrificed at the end of the examined period, fixed in formalin (10%), dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin wax. Thick sections were obtained and stained with hematoxylin and eosin [45].

2.8. Statistical Analysis

Statistical data analysis was performed using the one-way ANOVA test associated with the Duncan test, which was carried out using CoStat software (Monterey, CA, USA, version 6.4). Significant difference was determined at $p \leq 0.05$.

3. Results and Discussion

3.1. Total Phenolic Content and Radical Scavenging Activity of Hawthorn Leaves Extract

The analyses performed on the Hawthorn leaf extract revealed a TFC of 340 ± 6.53 mg GAE/100 g and a DPPH radical scavenging activity of 95.40 ± 1.63%. A previous study [46] reported a total phenolic content of 343.50 mg GAE/g in the case of ethyl acetate extract from Hawthorn leaves. Another previous report [47] revealed a TPC for the dry extract from Hawthorn leaves and berry extracts that ranged from 77.4 to 94.2 mg GAE/g, showing evidence of a higher phenolic content in the case of the leaves with respect to the berries as well as a more effective DPPH radical scavenger activity (IC50 = 29.7 µg/g vs. IC50 = 111.9 µg/g, for leaves and berries extracts, respectively).

3.2. Chemical Compositions, Physicochemical, and Sensory Characteristics of Yogurt Fortified with Aqueous Hawthorn Leaves Extract

The data presented in Table 1 reveal that the total solids, protein, and fat contents of the yogurt did not seem to be affected by the fortification from the Hawthorn leaf extract with the two ratios (HLEY0.2 and HLEY0.4). This result agrees with a previous study [48] that found that fortifying yogurt with different types of herbal extracts did not affect the total solids, protein, and fat contents of the resultant yogurt. On the other hand, comparing the pH values of the PY, HLEY0.2, and HLEY0.4 samples in Table 1, a significantly lower pH value ($p \leq 0.05$) was detected in PY compared to (HLEY0.2) and (HLEY0.4), which might be attributed to the effect of aqueous extract from Hawthorn leaves on the growth of microorganism and pH values. On the contrary, a previous study evidenced slightly higher pH values in plain yogurt than those in essential oil-treated yogurts [49]. The titratable acidity values in PY significantly ($p \leq 0.05$) increased compared to (HLEY0.2) and (HLEY0.4) due to the ability of Hawthorn leaf extract to inhibit/decrease bacteria growth in the yogurt. These results agree with those reported in a previous study [50], which indicated that the addition of Cinnamon herb extract addition influenced the titratable acidity in yogurt, leading to a slight pH increase. Furthermore, adding Hawthorn leaf extract to the yogurt in the present study increased its TPC and AO activity as a function of the concentration of the Hawthorn lead extract. This experimental evidence is in agreement with the findings reported in a previous study [51] that demonstrated how an increasing the content of Hawthorn extract in yogurt samples was positively correlated with the TPC and AO. Additionally, the data collected in Table 1 show that the yogurt containing Hawthorn leaf extract had lower peroxide and acid values than the plain yogurt. The obtained results confirm previous investigations on the presence of natural antioxidants [47,52]. The results in Figure 1 are related to the results of the sensory evaluation conducted on PY, HLEY0.2, and HLEY0.4, and all three yogurt samples demonstrate acceptable sensory features. However, PY was more acceptable compared to the other two treatments, with an overall less desirable flavor being achieved in HLEY0.2 and HLEY0.4. These results confirm those that were reported previously [53], which showed that yogurt supplemented with Cinnamon herb extract had a more undesirable flavor profile overall compared to plain yogurt. Furthermore, a previous study [54] found that the addition of Hawthorn leaf powder reduced the organoleptic properties of cupcakes. In addition, another study [55] found that adding olive leaf extract at the concentrations of 0.2 and 0.4% had an unfavorable effect on the organoleptic properties of yogurt samples. In addition, another previous report [56] found that adding walnut leaf extract to yogurt reduced the organoleptic properties of the product. A possible explanation for this finding might be attributed to the fact that most herbs contain a distinctive richness and a diverse population of metabolites that is responsible for their taste and flavor [53].

Table 1. Chemical compositions and physicochemical characteristics of yogurt fortified with aqueous Hawthorn leaf extract.

Treatments	Chemical Compositions					Physicochemical Characteristics				
	Total Solids	Fat	Protein	Ash	Acidity %	pH	TPC	AO Activity	PV	AV
PY	10.24 ± 0.16^{A}	3.12 ± 0.08^{A}	3.94 ± 0.08^{A}	0.72 ± 0.02^{B}	0.82 ± 0.02^{A}	4.68 ± 0.02^{C}	18.00 ± 2.45^{C}	7.70 ± 0.57^{C}	1.20 ± 0.05^{A}	0.50 ± 0.02^{A}
HLEY 0.2	10.42 ± 0.20^{A}	3.14 ± 0.08^{A}	3.98 ± 0.13^{A}	0.76 ± 0.03^{AB}	0.76 ± 0.02^{B}	4.75 ± 0.02^{B}	38.00 ± 2.45^{B}	12.40 ± 0.49^{B}	0.90 ± 0.03^{B}	0.42 ± 0.02^{B}
HLEY 0.4	10.66 ± 0.24^{A}	3.18 ± 0.10^{A}	4.02 ± 0.16^{A}	0.80 ± 0.03^{A}	0.70 ± 0.02^{C}	4.82 ± 0.03^{A}	62.00 ± 3.27^{A}	20.60 ± 0.82^{A}	0.60 ± 0.02^{C}	0.34 ± 0.02^{C}

^{A,B,C} Letters in the same column are significantly different at ($p \leq 0.05$). Mean values ± standard deviation, $n = 3$. PY, plain yogurt; HLEY0.2, yogurt containing 0.2% Hawthorn leaf extract; HLEY0.4, yogurt containing 0.4% Hawthorn lead extract; TPC, total phenolic compounds (mg GAE/100 g); AO, antioxidant activity (%); PV, peroxide value (meq/Kg oil); AV, acid value (mg KOH/g oil).

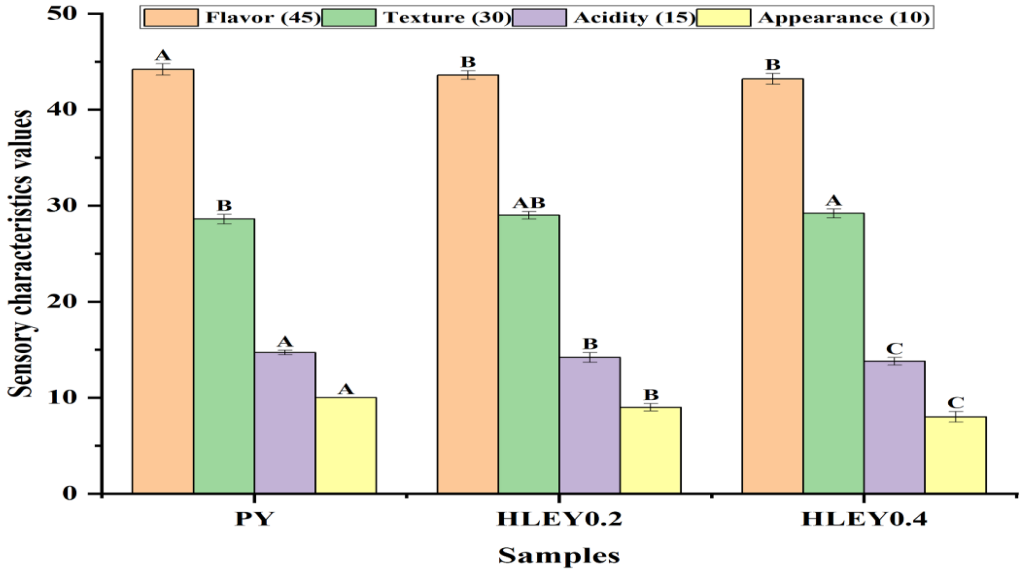

Figure 1. Sensory characteristics of yogurt fortified with aqueous Hawthorn leaf extract. Values with the different superscript letters are significantly different at ($p \leq 0.05$). Mean values ± standard deviation, $n = 10$. PY, plain yogurt; HLEY0.2, yogurt containing 0.2% Hawthorn leaf extract; HLEY0.4, yogurt containing 0.4% Hawthorn leaf extract.

3.3. Final Weight and Body Weight Gain in All Groups of Rats

As shown in Table 2, the negative control (Group 1) achieved the highest weight parameter values, whereas the treatments (Groups 3 and 4) had a remarkable ($p \leq 0.05$) effect on the final weight and on body weight gain. The best results were achieved in the case of Group 4, of the group in which the rats were yogurt fortified with 0.4% aqueous Hawthorn leaf extract, reaching higher final weight (275.48 g) and body weight gain (21.09%) values compared to the positive control group (final weight of 265.40 g and body weight gain of 15.49%). This improvement may be able to be ascribed to the high vitamin, mineral, and antioxidant contents in Hawthorn, which may prevent damage to body cells caused by free radicals [23,24]. Accordingly, a previous study [57] found that the addition of Hawthorn leaf extract in rat diets promoted a significant increase in body weight gain and enhanced their nutritional status.

Table 2. Final weight (g) and body weight gain (%) in rats treated with yogurt fortified with 0.4% aqueous Hawthorn leaf extract.

Groups	Initial Weight (g)	Final Weight (g)	Body Weight Gain (BWG (%))
Group (1)	227.40 ± 2.49^{A}	287.07 ± 1.72^{A}	26.25 ± 1.18^{A}
Group (2)	229.80 ± 1.54^{A}	265.40 ± 1.66^{D}	15.49 ± 0.30^{D}
Group (3)	228.60 ± 1.41^{A}	271.20 ± 2.62^{C}	18.63 ± 0.67^{C}
Group (4)	227.50 ± 1.78^{A}	275.48 ± 2.14^{B}	21.09 ± 0.56^{B}

(BWG %) = Final weight − Initial weight ÷ Initial Weight × 100. Group (1) non-treated rats (negative control). Group (2) treated oxidative stress rats (positive control). Group (3) oxidative stress rats fed a basal diet with plain yogurt (10 g/day). Group (4) oxidative stress rats fed a basal diet with yogurt fortified with 0.4% aqueous Hawthorn leaf extract (10 g/day). ^{A,B,C,D} Letters in the same column are significantly different at ($p \leq 0.05$). Mean values of ± standard deviation.

3.4. Effect of Yogurt Fortified with Hawthorn Leaves Extract on the Serum Lipid Profile, Liver Function Parameters, Kidney Function Parameters, Liver Weight, Kidney Weight, and Total Antioxidant Potentials of Serum of Oxidative Stress Rats

The detected lipid profiles for all of the investigated groups are compared and shown in Figure 2a. It should be noticed that the non-treated rats (negative control) showed lower total cholesterol triglycerides and LDL contents (67.40, 77.50, and 15.70 mg/dL, respectively) and a higher HDL content (36.20 mg/dL) compared to the other groups. Among the oxidative stress rat groups, Group 4, which was treated with yogurt fortified with 0.4% aqueous Hawthorn leaf extract, presented with lower total cholesterol (72.50 mg/dL) as well as significantly ($p \leq 0.05$) lower triglyceride and LDL contents (79.20 and 22.96 mg/dL, respectively) compared to the positive control group (total cholesterol, triglycerides, and LDL of 135.20, 119.20, and 85.96 mg/dL, respectively). Concerning the HDL content, the positive control group showed the lowest value (25.40 mg/dL) compared to the rats who had been fed a normal diet and yogurt fortified with 0.4% aqueous Hawthorn leaf extract, with a significant increase (33.70 mg/dL) being recorded. Clearly, comparing the present results, the treatment with yogurt fortified with 0.4% aqueous Hawthorn leaf extract resulted in a significant reduction in the total cholesterol, triglyceride, and LDL levels with respect to the positive control. It is noteworthy to state that *C. oxyacantha* Hawthorn demonstrates remarkable hypolipidemic and hypocholesterolemic effects [57,58] through the reduction of ApoB synthesis, total cholesterol, and LDL, and these affects are associated with a significant increase in the HDL [26]. Meanwhile, the positive control condition, in which oxidative stress was induced by carbon tetrachloride, demonstrated a consequent increase in the formation of reactive oxygen species and was able to promote the oxidation of pivotal cellular components (e.g., membrane lipids, proteins, and DNA), leading to cellular damage [59]. The reported beneficial hypolipidemic effect of the Hawthorn leaf extract on the lipid profile may be due to phenolic compounds the natural antioxidants that are present in Hawthorn leaves [25,28]. This hypolipidemic action can also be ascribed to the lipid metabolism modulation caused by phenolics such as chlorogenic acid and flavonoids such as quercetin, leading to a decrease in the total cholesterol, triglycerides, and LDL and was not associated with the increase in HDL levels because it up-regulated hepatic peroxisome proliferator-activated receptor (PPAR-α) expression [60].

Concerning the liver function parameters, as illustrated in Figure 2b, a significant increase in the AST and ALT and a decrease in the total albumin were revealed in the untreated group (positive control, Group 2) compared to the negative control group (Group 1). On the other hand, the group treated that received the yogurt that had been fortified with 0.4% aqueous Hawthorn leaf extract (Group 4) presented a significant increase in the total albumin and a decrease in AST and ALT compared to the positive control group, which is consistent with results that have been described elsewhere [57]. This decrease in the values of aminotransferase enzymes and the restoration of some vital functions by the hepatocytes can be ascribed to the high contents of phenolic and bioactive components in Hawthorn leaves, which preserve the plasma membrane in hepatocytes and protect it from the rupture and the exit of the cytosol that is loaded with these enzymes [25,28].

Regarding the kidney function parameters, as shown in Figure 2c, a significant increase in creatinine and urea ($p \leq 0.05$) was revealed in the positive control group compared to in the normal control group (negative group), whereas the group treated with yogurt fortified with 0.4% aqueous Hawthorn leaf extract showed a significant decrease in creatinine and urea. This conspicuous change could be partially attributed to the bioactive components present within Hawthorn leaves, such as phenolic compounds, flavonoids, minerals, and vitamins [24], as these may indirectly reduce uric acid levels, keeping the kidneys safe from the damage that can potentially be caused by oxidative stress. These bioactive components act as superoxide scavengers, resulting in the suppression of reactive oxygen species; thus, in the body cells, oxidative stress and inflammation are reduced. Moreover, they can inhibit the formation of uric acid through the direct uricosuric potential or increase the glomerular filtration rate, resulting in a consequent decrease in the uric acid levels in the blood [61,62]. Concerning MDA, the highest mean value was achieved in the positive control group (67.77 µmol/L), whereas the lowest one was observed in the case of the negative control group (46.34 µmol/L). A significant decrease of up to 47.18 µmol/L was revealed in the rats treated with yogurt fortified with 0.4% aqueous Hawthorn leaf extract. These findings were corroborated those by Qi et al., 2019 [58], who found that supplementation with Hawthorn leaf extract was effective in decreasing the creatinine, urea, and MDA levels compared to the positive control group. It should be borne in mind that MDA plays a very negative role and is able to alter the structure and function of the cell membrane [63]. The formation of MDA and increasing its levels can lead to inhibitory actions, oxidative mechanisms, high cytotoxicity, and tumor development, as it can act as a co-carcinogenic agent [64].

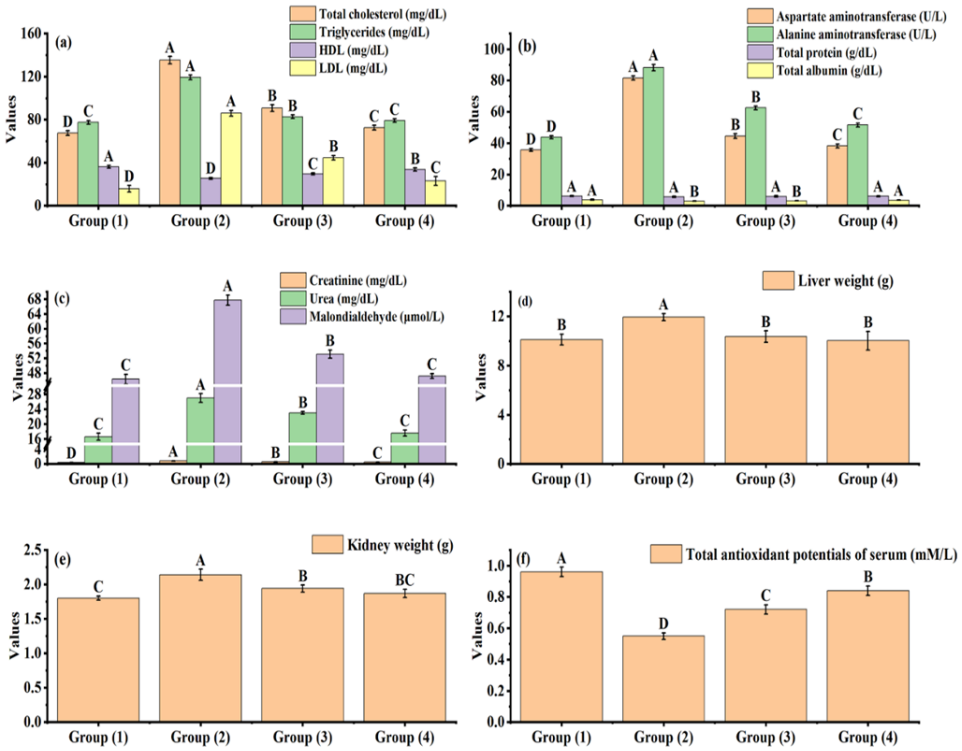

Figure 2. Effect of yogurt fortified with 0.4% aqueous Hawthorn leaf extract on serum: lipid profile (**a**), liver function parameters (**b**), kidney function parameters (**c**), liver weight (**d**), kidney weight (**e**), and total antioxidant potentials of serum (**f**) of the oxidative stress rats. Group (1) non-treated rats (negative control). Group (2) treated oxidative stress rats (positive control). Group (3) oxidative stress rats fed a basal diet with plain yogurt (10 g/day). Group (4) oxidative stress rats fed on basal diet with yogurt fortified with 0.4% aqueous Hawthorn leaf extract (10 g/day). Values with different superscript letters are significantly different at ($p \leq 0.05$). Mean values ± standard deviation.

When comparing the liver and kidney weights, the results of which are shown in Figure 2d,e, respectively, a significant ($p \leq 0.05$) increase in the liver and kidney weights of the positive control group was revealed compared to the other groups. This experimental evidence suggests that the liver weight was affected by oxidative stress, which caused spindle cell proliferation, collagen fibers admixed with erythrocytes, and inflammatory cells among the blood inside the bile duct lumen in the liver, something that is evident in the histological sections (Figure 3). Additionally, these results indicate the influence of oxidative stress on the kidney weight, causing the degeneration of collected tubules and desquamated lining epithelium (Figure 4b, histological section). It seems that Hawthorn leaf extract has the ability to improve liver and kidney function (Groups 3 and 4) due to its antioxidant content, which is in harmony with what has been reported elsewhere [58,65]. As shown in Figure 2f, the oxidative stress rat groups (2, 3, and 4) presented significantly lower serum antioxidant potential values compared to the negative control (Group 1), whereas the serum antioxidant potential in Group 4 (yogurt fortified with Hawthorn leaf extract) was significantly ($p \leq 0.05$) higher than it was in the positive control (Group 2). This experimental evidence confirms that the yogurt fortified with Hawthorn leaf extract has antioxidative and beneficial effects when the liver and kidneys are recovering from carbon tetrachloride injury [57,66].

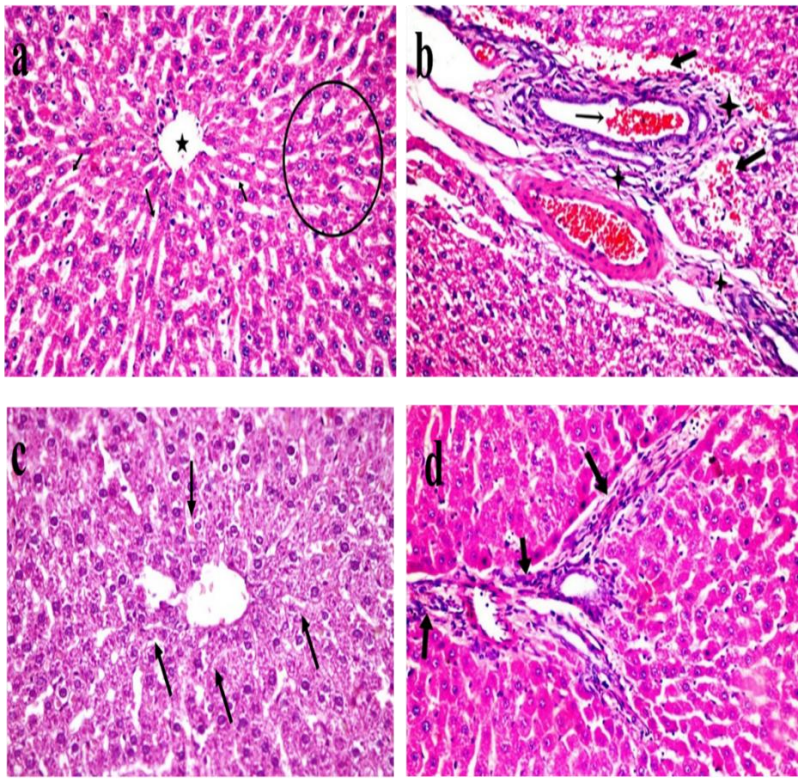

Figure 3. Representative photomicrographs of rat livers. (**a**) Group 1, negative control: normal hepatic parenchymal structures of the central vein (star), hepatic cords (circle), and sinusoids (arrows) containing Kupffer cells. (**b**) Group 2, positive control: portal trade fibrosis (stars) represented by spindle cells proliferation, collagen fibers ad-mixed with erythrocytes, and inflammatory cells (thick arrows) among the blood inside the bile duct lumen (thin arrow). (**c**) Group 3, plain yogurt (10 g/day): massive distribution of slightly vacuolated hepatocytes (arrows). (**d**) Group 4, yogurt with 0.4% aqueous Hawthorn leaf extract (10 g/day): interlobular fibrous bridge (arrows) with normal hepatic cords and sinusoids. Hematoxylin and eosin staining, magnification ×400.

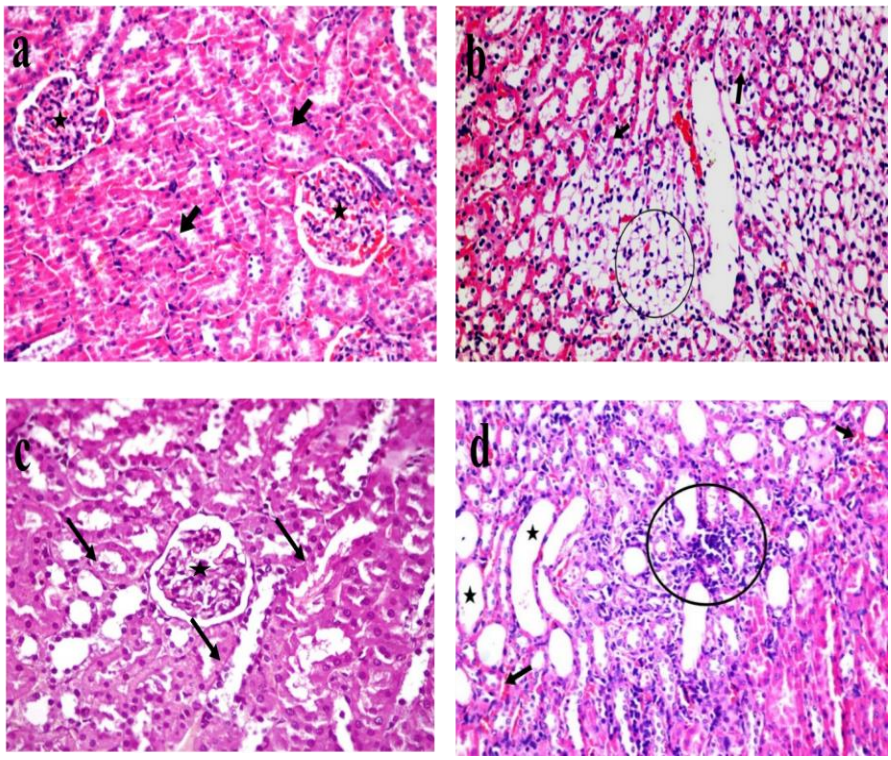

Figure 4. Representative photomicrographs of rat kidneys. (**a**) Group 1, negative control: normal renal tubules (arrows) and glomeruli (stars). (**b**) Group 2, positive control: degeneration collected tubules (circle) with desquamated lining epithelium (arrows). (**c**) Group 3, plain yogurt (10 g/day): vacuolation of the glomerular epithelium (arrow) and focally distributed degeneration of some renal tubules (star). (**d**) Group 4, yogurt with 0.4% aqueous Hawthorn leaf extract (10 g/day): focal interstitial lymphocytic infiltrations (circle), cystic dilated renal tubules (stars), and slightly congested interstitial capillaries (arrows). Hematoxylin and eosin staining, magnification × 400.

3.5. Light Microscopic Histological Study

In the present work, hepatic damage was assessed by means of histological organ examination, and the degree of histological changes in the different groups of rats were determined (Figure 3). The histological examinations of the livers in the normal rats (negative control group) showed the appearance of normally preserved normal hepatocytes (Figure 3a), whereas the liver tissue sections of the rats submitted to oxidative stress during the experiment (Group 2) were characterized by portal trade fibrosis represented by spindle cells proliferation, collagen fibers admixed with erythrocytes, and inflammatory cells among the blood inside the bile duct lumen (Figure 3b). On the other hand, the histological examination of the liver tissue sections taken from the rats treated with plain yogurt showed a mass distribution of slightly vacuolated hepatocytes (Figure 3c). The rats treated with yogurt fortified with aqueous Hawthorn leaf extract showed a consistent decrease in hepatocyte necrosis processes and a more regular liver structure. Furthermore, slight fibrosis in the portal area as well as the restoration of the presence of a fibrous bridge between the portal vein cavity was reported, and the cells and hepatic lobes were restored to normal (Figure 3d). These findings corroborate those reported elsewhere [26,67] and highlight no histological changes in the liver, kidney, and heart in the groups treated with wild plant extracts. Similarly, the renal damage was also evaluated through histological organ examinations to determine the degree of histological changes, as shown in Figure 4. Figure 4a reveals the normal appearance of renal tubules and glomeruli in the negative control group (normal). Meanwhile, the renal tissue samples from the Group 2 rats showed degeneration in the collected tubules and a desquamated lining epithelium, as shown in Figure 4b and represent positive control group. In the case of the rats treated with plain

yogurt, the kidney sections were characterized by glomerular epithelium vacuolation and the focally distributed degeneration of some of the renal tubules (Figure 4c). On the other hand, the histological examination of kidney sections taken from the rats treated with the yogurt fortified with aqueous Hawthorn leaf extract presented normal glomerular structures and slight congestion in the cellular capillaries (Figure 4d), which is in harmony with several previous reports [58].

4. Conclusions

In summary, Hawthorn (*Crataegus oxyacantha*) leaf extract might exert protective effects against oxidative stress in rats due to their high bioactive components. Clearly, it can be used in some vital food products such as yogurt. Using *Crataegus oxyacantha* leaf aqueous extract at concentrations of up to 0.4% during the manufacture of yogurt greatly affected its physicochemical, phytochemical, and sensory properties and imparted health benefits to the yogurt on the basis of its high bioactive components. The present findings also concluded that the addition of *Crataegus oxyacantha* leaf extract as a bioactive supplement to yogurt is useful to maintain good oxidative status, which was positively reflected in the general health of the oxidative stress rats.

Author Contributions: M.R.S., E.-S.H.A. and H.A.R. were involved in the conception of the research idea and the design of the methodology, and supervision, and performed data analysis and interpretation. A.O.B., M.U., D.A., H.M., N.D., I.C., B.M.A. and E.K.E. were involved in the methodology and drafted and prepared the manuscript for publication and revision. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The experimental protocol was approved by the Ethics Committee of Suez Canal University, Suez, Egypt (ref. no.: 2021047).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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