



## Research article

## Fortified vegetarian milk for prevention of metabolic syndrome in rats: impact on hepatic and vascular complications



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## ABSTRACT

Metabolic syndrome (MetS) is characterized as a gathering of various metabolic disorders, for example, hyperglycemia, dyslipidemia, and obesity. The present research was conducted to prepare fortified almond milk as functional beverages and evaluate their protective effect against MetS and associated hepatic and vascular complications. Three beverages (I, II, and III) were prepared by fortification almond milk with carrot juice or powder of quinoa seeds and carrot juice or oat powder and banana juice. The sensory attributes, physicochemical properties, bioactive compounds (total phenolic, beta-carotene, tocopherols) and B-complex vitamins were determined in the beverages. In-vitro antioxidant activity of the beverages was assessed. MetS was induced in rats via feeding on high-fat high-fructose diet (HFHF). The biochemical (lipid profile, oxidative stress, liver, and kidney functions), nutritional and histopathological parameters were assessed in rats. The beverage I recorded the highest sensory attributes' scores. The physicochemical properties of the beverages revealed that acidity and viscosity of all beverages ranged from 4.55 to 4.88 and from 40 to 59, respectively. The beverage I showed the highest content of alpha-tocopherol (14.994 µg/g) and beta-carotene (104.541 µg/g), while the beverage II showed the highest content of gamma-tocopherol (0.557 µg/g), folic acid (0.806 µg/g), and total phenols (147.43 µg GAE/g). The results of animals revealed that the beverage II was the most promising in attenuation levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, malondialdehyde, and tumor necrosis factor-alpha. Also, the beverage II was the superior in the protection of the liver and heart tissues as reflected by the histopathological examination findings. So, it can be concluded that the newly prepared almond milk with quinoa seeds and carrot juice could be used as an effective functional beverage for the prevention of MetS and its complications.

## 1. Introduction

Metabolic syndrome (MetS) is characterized as a gathering of various metabolic disorders, for example, hypertension, hyperglycemia, dyslipidemia, low-grade inflammation, and obesity, which are key elements in the etiology of the syndrome (Liberati-Çizmek et al., 2019). Kaur (2014) mentioned that, based on the ethnicity, age, gender, and race of the population, the worldwide prevalence of MetS ranged between 10 to 84%. While, the International Diabetes Federation (IDF) reported that one-quarter of the world's population had MetS (O'Neill and O'Driscoll, 2015). The rate of cardiovascular diseases, type 2 diabetes (T2D), and nonalcoholic fatty liver disease (NAFLD) is increased by the increment of

MetS (Moreno-Fernández et al., 2018). The larger prevalence of MetS is common even at younger age due to the incremental consumption of ultra-processed food products that rich in calories, salt, sugars, and fats. The raised intake of such ultra-processed food products is associated with the prevalence of obesity, hyperglycemia, dyslipidemia, and hypertension (Monteiro et al., 2013, 2018, 2019; Gibney et al., 2017).

Fructose is the main source of sugars in processed foods, carbonated, and sweetened beverages in the form of high-fructose corn syrup or sucrose (Lozano et al., 2016). The utilization of fructose in handled food products and drinks is expanded to advance less satiety than different sugars, in this way increasing caloric intake, mostly through sweetened drinks (Pereira et al., 2017). Fructose is metabolized differently

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compared to glucose. Hence, it was suggested that fructose is a key factor in the development of MetS (Aydin et al., 2014). Chiavaroli et al. (2015) also confirmed the association of fructose consumption and the MetS in humans.

There is a required change in dietary patterns. So, nutritious foods should be included in the daily dietary pattern. Almond is one of these nutritious foods in addition to its beneficial effects on cardiovascular disease and type 2 diabetes (Gulati et al., 2017). Cereals, fruits, and vegetables are rich sources of bioactive compounds, vitamins and minerals, which promote health through their beneficial effects as antioxidants, anti-inflammatory, and antimicrobial agents (Kang et al., 2011; Fujita et al., 2013; Zielinski et al., 2014; Singh et al., 2016a, 2016b). Also, Mohamed (2014) reported that several kinds of cereals, fruits, and vegetables can prevent or moderate MetS. In this context, it was hypothesized that almond milk fortified with different cereals, fruits and vegetables may be served as not only nutritious vegetarian milk but also with a beneficial effect on the prevention of MetS and the associated hepatic and vascular complications. So, the aim of the present research was preparation of fortified almond milk as functional beverages and evaluation the protective effect of these beverages in an animal model of metabolic syndrome induced in rats using high-fat high-fructose diet.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Plant materials and chemicals

Honey, stevia, almond, carrot, banana, quinoa seeds, and oat were purchased from local markets, Cairo, Egypt. All the colorimetric kits were purchased from Erba Lachema Co, Czech Republic. Rat tumor necrosis factor-alpha and insulin ELIZA kits were purchased from SinoGeneclon Biotech Co., Ltd. All the chemicals used were of high-quality analytical grade.

#### 2.1.2. Animals

Male Sprague–Dawley rats weighing 121–163 g were used in the present study. The animals were obtained from the animal house of the National Research Centre, Cairo, Egypt. The animals were kept individually in stainless steel cages under 12 h light and dark cycles ( $24 \pm 2^\circ\text{C}$  and 40–60% relative humidity). Water and food were given *ad-libitum*.

### 2.2. Methods

#### 2.2.1. Preparation of fortified almond milk

Almond (120g) was soaked in water (750mL) for 24h then blended using an electrical blender to obtain almond milk. Three functional beverages were prepared by fortification of the prepared almond milk with different ingredients. The beverage I was prepared by fortification of the almond milk with carrot juice (100g blended boiled carrot), honey (25g), and stevia (5g). The beverage II was prepared by fortification of the almond milk with carrot juice (100g blended boiled carrot), powder of quinoa seeds (40g), honey (25g), and stevia (5g). The beverage III was prepared by fortification of the almond milk with banana juice (100g banana blended in 50 mL water), oat powder (40g), honey (25g), and stevia (5g).

#### 2.2.2. Proximate and physicochemical analysis

Moisture, protein, fat, crude fiber, ash, and total solid contents were determined in the freshly prepared beverages' samples according to the methods of AOAC (1995). Total soluble solids (TSS) and acidity were determined using a hand refractometer (ATAGO, Japan) and expressed as Brix value. Acidity was measured according to the method of AOAC (1995) and expressed as percentage of citric acid. Brix/acid ratio was

calculated by dividing the total soluble solids on the total acidity value for each sample. The pH value was measured using Hanna pH-meter HI 9021 m, Germany. Viscosity was measured using Brookfield model LV rotary viscometer (USA) at room temperature and expressed as centipoises (cP) unit as described by Ibarz et al. (1994).

#### 2.2.3. Color determination

Color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) of the freshly prepared beverages' samples were determined using a spectro-colorimeter (Tristimulus color machine) with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. The instrument was standardized with white tile of Hunter Lab cooler standard (LX No. 16379):  $X = 72.26$ ,  $Y = 81.94$  and  $Z = 88.14$  ( $L^* = 92.46$ ,  $a^* = -0.86$ ,  $b^* = -0.16$ ) (Sapers and Douglas, 1987).

#### 2.2.4. Determination of B-complex vitamins

The freshly prepared beverages' samples were subjected to successive acid and enzymatic hydrolysis using hydrochloric acid and taka-diastase. The hydrolyzed sample was filtered and analyzed by HPLC (Agilent Technologies 1100 series) equipped with Diode-array and fluorescence detector and LiChrospher 5 RP Select B (250 X 4.0mm; 5  $\mu\text{m}$ ) column according to Vinas et al. (2003). The vitamins were identified and quantified by comparison with standards.

#### 2.2.5. Determination of tocopherols and beta-carotene

The freshly prepared beverages' samples were saponified according to Lee et al. (2012) with some modifications. Sample (2g) was weighed into a saponification vessel, and then 75 mL of ethanol containing pyrogallol (6%) was added. The vessel was sonicated for 5 min, and then 25 mL of potassium hydroxide (60%) was added. The vessel was flushed with nitrogen and incubated at  $70^\circ\text{C}$  for 50 min in a shaking water bath. The mixture was cooled and tocopherols were extracted with a mixture of n-hexane and ethyl acetate (80:20) containing BHT. Tocopherols and beta-carotene were determined using an Agilent 1100 chromatographic system equipped with a fluorescence detector (excitation 292 nm, emission 325 nm) for tocopherols and DAD (470nm) for beta-carotene. Tocopherols and beta-carotene were separated on the Agilent Exlipse XDB-C18 column (5  $\mu\text{m}$ , 150 mm  $\times$  4.6 mm i.d.) using isocratic elution with a mobile phase consisting of acetonitrile: methanol (70:30).

#### 2.2.6. Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure (Zilic et al., 2012). The sample (100  $\mu\text{L}$ ) was oxidized with Folin-Ciocalteu reagent (250  $\mu\text{L}$ ). The absorbance was measured at 725 nm. The total phenolic content was calculated by the gallic acid calibration curve and expressed as  $\mu\text{g}$  of gallic acid equivalent ( $\mu\text{g}$  GAE) per g of sample. The calibration curve was plotted by mixing 1 mL aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450  $\mu\text{g}/\text{mL}$  gallic acid solutions with Folin Ciocalteu reagent.

#### 2.2.7. Determination of antioxidant activity

The antioxidant activity of the freshly prepared beverages' samples was measured by 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay.

**2.2.7.1. Determination of DPPH radical scavenging activity.** Free radical scavenging capacity was assessed using DPPH according to Hwang and Do Thi (2014). Sample (50  $\mu\text{L}$ ) was mixed with the DPPH solution (3.95 mL) for 1 h in the dark. The absorbance was measured at 517 nm. Percent inhibition of the DPPH free radical was calculated by Eq. (1)

$$\text{Inhibition (\%)} = 100 \times [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \quad (1)$$

where:

$A_{\text{control}}$  is the absorbance of the control (reagent without the tested sample).

$A_{\text{sample}}$  is the absorbance of the tested sample.

Trolox (within a concentration range from 10 to 150  $\mu\text{mol/L}$ ) was used to prepare the standard curve. Results were expressed as  $\mu\text{g}$  Trolox equivalents (TE)/g sample.

**2.2.7.2. Determination of ABTS radical scavenging activity.** An equal volume of the aqueous solution of ABTS (7 mM) and potassium persulfate (2.45 mM) were mixed in the dark for 16 h at room temperature (25 °C) to obtain the ABTS reagent according to Hwang and Do Thi (2014). The ABTS solution (1mL) was diluted with 60 mL of ethanol: water (50:50, v/v). Sample (50  $\mu\text{L}$ ) was mixed with the diluted ABTS solution (3.95 mL) for 1 h in the dark. The absorbance was measured at 734 nm. Percent inhibition of the ABTS free radical was calculated by Eq. (2)

$$\text{Inhibition (\%)} = 100 \times [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \quad (2)$$

where:

$A_{\text{control}}$  is the absorbance of the control (reagent without the tested sample).

$A_{\text{sample}}$  is the absorbance of the tested sample.

Trolox (within a concentration range from 10 to 550  $\mu\text{mol/L}$ ) was used to prepare the standard curve. Results were expressed as  $\mu\text{g}$  Trolox equivalents (TE)/g sample).

### 2.2.8. Sensory evaluation

Prior to being enlisted in the descriptive panel, trained members from the staff members of the food technology department, National Research Centre, Dokki, Cairo, Egypt were informed about the study to enable them to make an informed decision. Sensory attributes (taste, odor, color, mouthfeel, and appearance) of the freshly prepared beverages' samples were evaluated according to Meilgaard et al. (2007) by 10-members. Each member was provided with the three samples individually in unlabeled transparent cups under white light and asked to record a score from 1-10 to each sensory attribute (taste, odor, color, mouthfeel, and appearance) in the designed provided sheet and asked to cleanse the palate with water before tasting the other samples. A sensory score of 5 or above for an individual attribute was considered accepted while that below five was deemed as unacceptable. The general acceptability of each beverage was calculated from the recorded scores.

### 2.2.9. Evaluation of the prepared beverages in metabolic syndrome rat model

**2.2.9.1. Diets.** Experimental diets were prepared as in Table 1. Salt and vitamin mixtures were prepared according to Williams and Briggs (1963) and Morcos (1967), respectively. Oil-soluble vitamins were given orally in a dose of 0.1 mL/rat per week. High-fat high-fructose diet was

**Table 1.** Composition of different diets (g per 100 g).

Ingredients	Balanced	High-fat high-fructose
Casein	12*	12*
Oil	10	-
Salt	3.5	3.5
Vitamin	1	1
Cellulose	5	-
Fat	-	25
Fructose	-	30
Sucrose	23	-
Starch	45.5	28.5

\*12 g casein has been estimated to contain 10 g protein using AOAC (1995).

prepared according to Gancheva et al. (2017) to induce metabolic syndrome model in rats.

**2.2.9.2. Design of the animal's experiment.** Thirty male rats were divided into five groups, each comprised of six rats. Group one was served as normal control, given daily oral dose (5ml) of distilled water, and fed on a balanced diet all over the experimental period (2 months). Group two was served as metabolic syndrome model control, given daily oral dose (5ml) of distilled water, and fed on high-fat high-fructose diet for two months. Groups from three to five were given daily oral dose (5ml) of beverage I, II, or III and fed on high-fat high-fructose diet for two months. At the end of the experiment blood samples were collected from all animals' groups after an overnight fast. Plasma samples were separated and used for biochemical analysis. All the colorimetric determinations were carried out using UVPC spectrophotometer (Jasco V-730, serial No. A 112361798, Japan). Plasma malondialdehyde (MDA) was determined according to Satoh (1978) as an indicator of lipid peroxidation, plasma catalase was determined according to Aebi (1984) as an antioxidant biomarker, plasma tumor necrosis factor-alpha (TNF-alpha) was determined according to Stepaniak et al. (1995) as an inflammatory marker, plasma creatinine was determined according to Houot (1985) and plasma urea was determined according to Fawcett and Scott (1960) as an indicator of kidney functions. The activity of aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Reitman and Frankel (1957) as an indicator of liver functions. Plasma total protein was determined according to Rheinhold (1953) and albumin was determined according to Doumas et al. (1971). Plasma total cholesterol (T-Ch) was determined according to Watson (1960), high-density lipoprotein cholesterol (HDL-Ch) was determined according to Burstein et al. (1970), low-density lipoprotein cholesterol (LDL-Ch) was determined according to Schriewer et al. (1984) and triglycerides (TG) was determined according to Megraw et al. (1979). T-Ch/HDL-Ch ratio and TGs/HDL-Ch ratio were calculated. Plasma insulin was determined using Eliza kit (Turkington et al., 1982) and blood glucose was determined according to Trinder (1969). Insulin resistance estimation was carried out using the homeostasis model assessment method, HOMA-IR, and calculated according to Matthews et al. (1985) and Cacho et al. (2008) using Eq. (3)

$$\text{Plasma glucose in the fasting state (mg/dL)} \times \text{fasting plasma insulin (IU mg/L)} \text{ divided by } 405 \quad (3)$$

After blood withdrawing, the heart and liver were immediately removed from each rat, weighed and impressed in 10% formalin solution for the histopathological examinations. This study has been carried out as a part of the internal project (number: 11040113) according to the policies of Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, and followed the National Institutes of Health (NIH) Guidelines applied for the use and care of Laboratory Animals, 8<sup>th</sup> edition (NIH Publication no. 85-23, revised 1985).

**2.2.9.3. Histopathological examinations.** Tissue specimens from liver and heart fixed in 10% formalin were routinely processed, dehydrated in different grades of ethanol, cleared in xylene, and finally embedded in paraffin blocks. Then they were sectioned in 5-6  $\mu\text{m}$  thickness and stained with hematoxylin and eosin stain (H and E) according to Bancroft and Gamble (2008). The scores for hepatocyte ballooning were from 0 to 2 while, the scores for the non-alcoholic fatty liver disease were from 0 to 8 according to Kleiner et al. (2005).

### 2.2.10. Statistical analysis

The results of the sensory evaluation and the animal experiment were expressed as the Mean  $\pm$  SE and analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. While the results of vitamins, bioactive components, and the physicochemical results were presented as Mean  $\pm$  SD and compared by the analysis of variance (one-

way ANOVA) followed by LSD test ( $p < 0.05$ ) using SPSS software program version 16.

### 3. Results and discussion

#### 3.1. Proximate analysis, physicochemical and antioxidant properties of the prepared beverages

Proximate analysis, physicochemical parameters, color attributes, and antioxidant properties of the prepared beverages are shown in Table 2. Significant differences ( $p \leq 0.05$ ) were found in all measured parameters of the prepared beverage, except pH and acidity. From this table, it could be seen that the beverage II contained the highest protein, fat, and carbohydrate content being 5.22 %, 0.5% and 12.56%, respectively may be due to the presence of quinoa seeds powder which is considered a rich source of protein and carbohydrate (Mohamed et al., 2019). Regarding the physicochemical parameters, also the beverage II showed the highest total solids percent and viscosity which also can be attributed to the presence of quinoa seeds powder.

Color characteristic is one of the major parameters that affect the attractiveness of the final product for consumers. Data in Table 2 represent the color attributes of the beverages. Color parameters of the beverages' samples showed that the beverage II was darker than the other beverages' samples, where it had the lowest lightness ( $L^* = 65.42$ ). The lightness ( $L^*$ ) value was higher in beverage I (78.08), then beverage III (67.13). The highest redness ( $a^*$ ) value was found in beverage I (9.54) followed by beverage II (8.79) then beverage III (4.34). The highest yellowness value ( $b^*$ ) was also found in beverage I (36.84) followed by beverage II (29.93) then beverage III (15.07).

Total phenolic content and antioxidant activity (DPPH and ABTS) of the different beverages are shown in Table 2. The highest total phenolic compounds content (147.43  $\mu\text{g GAE/g}$ ) was found in the beverage II. The highest DPPH radical scavenging activity was found in the beverage II (122.19  $\mu\text{g TE/g}$ ). Also, the beverage II showed the highest ABTS radical scavenging activity (326.7  $\mu\text{g TE/g}$ ). Almond possesses potent antioxidant activity due to its content of phenolic compounds, polyunsaturated

fatty acids (PUFA), and tocopherols (Keser et al., 2014). The superiority of the beverage II in radical scavenging activity may be attributed to the antioxidant activity and phenolic compounds content of carrot and quinoa seeds as confirmed by Sharma et al. (2012) and Mohamed et al. (2019), respectively.

#### 3.2. B-complex vitamins, tocopherols and beta-carotene content of the prepared beverages

Vitamins and antioxidants (tocopherols and beta-carotene) are important in the metabolic syndrome, especially that Kodentsova et al. (2019) declared that the intake of vitamins is necessary for metabolic syndrome patients and the lack of vitamins is a risk factor for metabolic syndrome. Hence, it was important to determine the content of the beverages of these components. The beverages' contents of B1, B2, B3, B6 and B9, as water soluble vitamins, gamma-tocopherol, alpha-tocopherol and beta-carotene are presented in Table 3. The beverage I showed the highest content of vitamin B3 (22.294  $\mu\text{g/g}$ ), alpha-tocopherol (14.994  $\mu\text{g/g}$ ) and beta-carotene (104.541  $\mu\text{g/g}$ ). The beverage II showed the highest content of gamma-tocopherol (0.557  $\mu\text{g/g}$ ) and vitamin B9 (0.806  $\mu\text{g/g}$ ). The beverages content of tocopherols can be attributed to the almond as the main component in these beverages since Keser et al. (2014) reported that almond extract contained 3.05 mg/kg gamma-tocopherol and 104.40 mg/kg alpha-tocopherol. Beverages I and II were reported to contain beta-carotene may be due to the presence of carrot since Sharma et al. (2012) mentioned that carrot pomace contained 50% of beta-carotene.

#### 3.3. Sensory properties of the prepared beverages

Sensory properties are a very important parameter for evaluating the consumer attitude towards any food product. Hence, in the present research, the sensory properties of the prepared beverages were assessed. The results of the sensory properties of the beverages' samples are presented in Table 4. With regard to color, odor, taste, mouthfeel, and appearance, the three beverages recorded accepted scores (above 5). The

Table 2. . Proximate analysis, physicochemical and antioxidant properties of the prepared beverages.

Parameters	Beverage I	Beverage II	Beverage III	LSD
Proximate analysis (%)				
Moisture	84.92 <sup>b</sup> ± 0.49	80.53 <sup>a</sup> ± 0.47	88.52 <sup>c</sup> ± 0.51	1.70
Protein	2.93 <sup>a</sup> ± 0.17	5.22 <sup>b</sup> ± 0.30	2.55 <sup>a</sup> ± 0.15	0.75
Ash	0.46 <sup>c</sup> ± 0.03	0.35 <sup>b</sup> ± 0.02	0.27 <sup>a</sup> ± 0.02	0.08
Fiber	0.91 <sup>b</sup> ± 0.05	0.84 <sup>b</sup> ± 0.05	0.61 <sup>a</sup> ± 0.03	0.15
Fat	0.38 <sup>a</sup> ± 0.02	0.50 <sup>b</sup> ± 0.03	0.42 <sup>ab</sup> ± 0.02	0.09
Carbohydrates	10.40 <sup>b</sup> ± 0.76	12.56 <sup>b</sup> ± 0.87	7.63 <sup>a</sup> ± 0.73	2.73
Physicochemical parameters				
Soluble solids (°Brix)	8.60 <sup>b</sup> ± 0.35	15.30 <sup>c</sup> ± 0.63	5.90 <sup>a</sup> ± 0.24	1.52
pH	4.82 <sup>a</sup> ± 0.14	4.65 <sup>a</sup> ± 0.13	4.55 <sup>a</sup> ± 0.13	NS
Acidity (%)	3.60 <sup>a</sup> ± 0.21	3.45 <sup>a</sup> ± 0.20	3.22 <sup>a</sup> ± 0.18	NS
Brix/Acidity	2.39 <sup>b</sup> ± 0.04	4.44 <sup>c</sup> ± 0.08	1.82 <sup>a</sup> ± 0.03	0.18
Total solids (%)	12.50 <sup>b</sup> ± 0.72	18.35 <sup>c</sup> ± 1.06	9.15 <sup>a</sup> ± 0.53	2.77
Viscosity (cP)	40.50 <sup>a</sup> ± 0.23	59.00 <sup>c</sup> ± 0.34	50.00 <sup>b</sup> ± 0.29	1.01
Color attributes				
Lightness ( $L^*$ )	78.08 <sup>c</sup> ± 0.45	65.42 <sup>a</sup> ± 0.46	67.13 <sup>b</sup> ± 0.47	1.58
Redness ( $a^*$ )	9.54 <sup>c</sup> ± 0.06	8.79 <sup>b</sup> ± 0.06	4.34 <sup>a</sup> ± 0.03	0.19
Yellowness ( $b^*$ )	36.84 <sup>c</sup> ± 0.25	29.93 <sup>b</sup> ± 0.21	15.07 <sup>a</sup> ± 0.10	0.69
Antioxidants and antioxidant activity				
Total phenolic compounds ( $\mu\text{g GAE/g}$ )	91.48 <sup>b</sup> ± 0.53	147.43 <sup>c</sup> ± 0.85	81.60 <sup>a</sup> ± 0.47	2.21
DPPH ( $\mu\text{g TE/g}$ )	85.08 <sup>a</sup> ± 0.59	122.19 <sup>c</sup> ± 0.85	89.70 <sup>b</sup> ± 0.62	2.41
ABTS ( $\mu\text{g TE/g}$ )	275.07 <sup>b</sup> ± 1.91	326.70 <sup>c</sup> ± 2.26	198.39 <sup>a</sup> ± 1.37	6.52

The same letters in the same row mean non-significant difference, the different letters in the same row mean significant difference, ND = Not detected, The confidence level is 95%. The data are expressed as mean values ± standard deviation, NS = Not Significant, GAE = gallic acid equivalent, TE = Trolox equivalent.



**Table 3.** Vitamins content and bioactive components ( $\mu\text{g/g}$ ) of the prepared beverages.

Vitamin	Beverage I	Beverage II	Beverage III
Thiamine (B1)	8.80 <sup>a</sup> $\pm$ 0.52	54.37 <sup>c</sup> $\pm$ 0.37	20.05 <sup>b</sup> $\pm$ 0.05
Riboflavin (B2)	1.30 <sup>b</sup> $\pm$ 0.21	1.25 <sup>b</sup> $\pm$ 0.15	0.43 <sup>a</sup> $\pm$ 0.03
Niacin (B3)	22.29 <sup>b</sup> $\pm$ 0.95	16.13 <sup>a</sup> $\pm$ 0.94	21.87 <sup>b</sup> $\pm$ 0.57
Pyridoxine (B6)	1.11 <sup>a</sup> $\pm$ 0.10	1.56 <sup>a</sup> $\pm$ 0.41	1.19 <sup>a</sup> $\pm$ 0.01
Folic (B9)	0.42 <sup>a</sup> $\pm$ 0.01	0.81 <sup>c</sup> $\pm$ 0.01	0.48 <sup>b</sup> $\pm$ 0.01
Gamma-tocopherol	0.49 <sup>b</sup> $\pm$ 0.06	0.56 <sup>b</sup> $\pm$ 0.03	0.15 <sup>a</sup> $\pm$ 0.01
Alpha-tocopherol	14.99 <sup>c</sup> $\pm$ 0.47	7.46 <sup>b</sup> $\pm$ 0.23	0.93 <sup>a</sup> $\pm$ 0.02
Beta-carotene	104.54 <sup>b</sup> $\pm$ 0.32	81.48 <sup>a</sup> $\pm$ 0.95	ND

The same letters in the same row mean non-significant difference, the different letters in the same row mean significant difference, ND = Not detected, The confidence level is 95%. The data are expressed as mean values  $\pm$  standard deviation.

beverage I showed the highest scores in all the studied parameters. The high viscosity of the beverage II in addition to the starchy taste of quinoa seeds powder may reduce its scores. The beverage III recorded the lowest scores may be due to the presence of banana juice that is characterized by browning and high turbidity caused by polyphenol oxidases (PPO) enzyme as confirmed by Koffi et al. (1991).

### 3.4. Evaluation of the prepared beverages in a metabolic syndrome rat model

#### 3.4.1. Effect of the prepared beverages on the body and organs' weights

Metabolic syndrome was induced in the current study throughout the feeding of rats on high-fat high-fructose diet. The nutritional parameters are presented in Table 5. Non-significant ( $p > 0.05$ ) differences in the body weight gain were observed in the metabolic syndrome control rats in comparison to normal control rats. Mohamed et al. (2019) also found that feeding rats on diet rich in fructose and contained saturated fat did not produce a significant change in the body weight gain. Administration of all the prepared beverages reduced the final body weight and body

weight gain compared with the metabolic syndrome control and normal control rats. It was observed in a previous study (Mohamed et al., 2020a) that foods rich in dietary fiber reduced the body weight gain in rats. Hence, the richness of almond, carrot, quinoa seeds, oat, and banana (the components of the prepared beverages) with dietary fiber as confirmed by Bolling et al. (2011), Sharma et al. (2012), Mohamed et al. (2019), Decker et al. (2014), and Kumar and Kumar (2011), respectively contributed in the reduction of the rats' weight in the present study. Relative liver weight was higher in rats of metabolic syndrome control rats in comparison to normal control rats, while relative heart weight in rats of metabolic syndrome control rats showed a non-significant change in comparison to normal control rats. Oral administration of all the prepared beverages reduced liver weight and relative liver weight compared with the metabolic syndrome control rats. The beverage II showed the maximum reduction in body weight, body weight gain, liver weight, and relative liver weight.

#### 3.4.2. Effect of the prepared beverages on the plasma lipid profile

Poudyal et al. (2013) reported that feeding rats on a diet rich in fat and simple sugars developed all the metabolic syndrome signs (dyslipidemia, impaired glucose, and diminished cardiac function). Fructose also contributes to the development of the metabolic syndrome and the appearance of all its symptoms, as well as the occurrence of oxidative stress. A high level of fructose stimulates the liver to increase lipogenesis and accumulate triglycerides. The results of triglycerides accumulation are insulin resistance and impaired glucose intolerance (Basciano et al., 2005).

Feeding rats on HFHF diet mediated dyslipidemia, as one of the metabolic syndrome signs, which reflected by the significant elevation of plasmatic levels of lipid profile markers (T-Ch, TGs, and LDL-Ch) and the significant reduction in HDL-Ch levels. Also, the atherosclerosis indicators, the ratio of T-Ch to HDL-Ch and TGs to HDL-Ch, elevated significantly in the rats of the metabolic syndrome model (Table 6). The elevation in atherosclerosis indicators and dyslipidemia are risk factors for cardiovascular disease as confirmed by Mohamed et al. (2010). Administration of all the prepared beverages improved plasma lipid profile with different degrees. The beverage II was superior in the

**Table 4.** Sensory properties of the prepared beverages.

Samples	Color (10)	Odor (10)	Taste (10)	Mouthfeel (10)	Appearance (10)	Overall acceptability (10)
Beverage I	8.19 <sup>c</sup> $\pm$ 0.15	8.90 <sup>c</sup> $\pm$ 0.12	9.40 <sup>c</sup> $\pm$ 0.14	8.55 <sup>c</sup> $\pm$ 0.14	8.39 <sup>c</sup> $\pm$ 0.12	8.68 <sup>c</sup> $\pm$ 0.07
Beverage II	7.15 <sup>b</sup> $\pm$ 0.13	8.15 <sup>b</sup> $\pm$ 0.08	7.65 <sup>b</sup> $\pm$ 0.15	7.90 <sup>b</sup> $\pm$ 0.12	7.60 <sup>b</sup> $\pm$ 0.12	7.69 <sup>b</sup> $\pm$ 0.05
Beverage III	6.35 <sup>a</sup> $\pm$ 0.11	7.25 <sup>a</sup> $\pm$ 0.08	6.50 <sup>a</sup> $\pm$ 0.13	6.18 <sup>a</sup> $\pm$ 0.11	6.35 <sup>a</sup> $\pm$ 0.15	6.52 <sup>a</sup> $\pm$ 0.04

In each column same letter means non-significant difference while different letter means significant difference. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error.

**Table 5.** Effect of the prepared beverages on the nutritional parameters.

Parameters	Normal control	Metabolic syndrome model control	Beverage I	Beverage II	Beverage III
Initial body weight (g)	144.67 <sup>a</sup> $\pm$ 2.98	144.67 <sup>a</sup> $\pm$ 4.32	144.83 <sup>a</sup> $\pm$ 5.00	144.67 <sup>a</sup> $\pm$ 6.31	144.67 <sup>a</sup> $\pm$ 7.91
Final body weight (g)	266.83 <sup>b</sup> $\pm$ 4.53	274.17 <sup>b</sup> $\pm$ 6.07	246.33 <sup>a</sup> $\pm$ 7.37	234.33 <sup>a</sup> $\pm$ 4.34	240.17 <sup>a</sup> $\pm$ 7.91
Body weight gain (g)	122.17 <sup>b</sup> $\pm$ 3.51	129.50 <sup>b</sup> $\pm$ 6.55	101.50 <sup>a</sup> $\pm$ 3.39	89.67 <sup>a</sup> $\pm$ 2.96	95.50 <sup>a</sup> $\pm$ 6.80
Total Food Intake (g)	1020.50 <sup>b</sup> $\pm$ 7.73	1025.00 <sup>b</sup> $\pm$ 22.39	998.33 <sup>b</sup> $\pm$ 13.27	901.83 <sup>a</sup> $\pm$ 15.21	898.50 <sup>a</sup> $\pm$ 13.77
Total calories intake (Kcal)	4204.46 <sup>a</sup> $\pm$ 31.85	5196.75 <sup>c</sup> $\pm$ 113.53	5061.55 <sup>c</sup> $\pm$ 67.27	4572.29 <sup>b</sup> $\pm$ 77.10	4555.39 <sup>b</sup> $\pm$ 69.79
Food Efficiency Ratio	0.12 <sup>b</sup> $\pm$ 0.004	0.13 <sup>b</sup> $\pm$ 0.005	0.10 <sup>a</sup> $\pm$ 0.002	0.10 <sup>a</sup> $\pm$ 0.002	0.11 <sup>a</sup> $\pm$ 0.006
Liver weight (g)	6.89 <sup>a</sup> $\pm$ 0.35	8.29 <sup>b</sup> $\pm$ 0.25	6.94 <sup>a</sup> $\pm$ 0.20	6.47 <sup>a</sup> $\pm$ 0.23	6.88 <sup>a</sup> $\pm$ 0.26
Relative liver weight	2.58 <sup>a</sup> $\pm$ 0.10	3.03 <sup>b</sup> $\pm$ 0.07	2.83 <sup>a</sup> $\pm$ 0.12	2.77 <sup>a</sup> $\pm$ 0.15	2.87 <sup>a</sup> $\pm$ 0.13
Heart weight (g)	0.77 <sup>a</sup> $\pm$ 0.03	0.80 <sup>a</sup> $\pm$ 0.03	0.78 <sup>a</sup> $\pm$ 0.04	0.76 <sup>a</sup> $\pm$ 0.03	0.83 <sup>a</sup> $\pm$ 0.025
Relative Heart weight	0.29 <sup>a</sup> $\pm$ 0.01	0.29 <sup>a</sup> $\pm$ 0.02	0.31 <sup>a</sup> $\pm$ 0.01	0.32 <sup>a</sup> $\pm$ 0.02	0.35 <sup>a</sup> $\pm$ 0.018

In each row same letter means non-significant difference while different letter means significant difference. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error.

**Table 6.** Effect of the prepared beverages on the plasma lipid profile.

Parameters	Normal control	Metabolic syndrome model control	Beverage I	Beverage II	Beverage III
T-Ch (mg/dl)	81.04 <sup>a</sup> ± 2.41	158.27 <sup>d</sup> ± 3.94	98.97 <sup>bc</sup> ± 4.00	91.30 <sup>b</sup> ± 1.97	100.94 <sup>c</sup> ± 1.42
HDL-Ch (mg/dl)	42.97 <sup>c</sup> ± 1.06	24.14 <sup>a</sup> ± 1.53	33.25 <sup>b</sup> ± 1.76	39.43 <sup>c</sup> ± 1.54	33.32 <sup>b</sup> ± 1.34
LDL-Ch (mg/dl)	19.54 <sup>a</sup> ± 0.63	131.60 <sup>d</sup> ± 2.11	59.81 <sup>c</sup> ± 4.48	39.64 <sup>b</sup> ± 2.39	54.77 <sup>c</sup> ± 2.79
TGs (mg/dl)	90.94 <sup>a</sup> ± 2.39	184.69 <sup>c</sup> ± 5.34	105.68 <sup>b</sup> ± 2.52	98.22 <sup>ab</sup> ± 1.94	101.20 <sup>b</sup> ± 3.41
T-Ch/HDL-Ch	1.90 <sup>a</sup> ± 0.09	6.68 <sup>c</sup> ± 0.42	3.01 <sup>b</sup> ± 0.16	2.33 <sup>a</sup> ± 0.10	3.06 <sup>b</sup> ± 0.15
TGs/HDL-Ch	2.10 <sup>a</sup> ± 0.06	7.80 <sup>c</sup> ± 0.50	3.20 <sup>b</sup> ± 0.22	2.50 <sup>a</sup> ± 0.12	3.11 <sup>b</sup> ± 0.20

In each row same letter means non-significant difference while different letter means significant difference. The confidence level is 95%. The data are expressed as mean values ± standard error.

improvement of dyslipidemia. Also, the ratio of T-Ch to HDL-Ch and TGs to HDL-Ch reduced significantly in rats given oral administration of the prepared beverages in comparison to metabolic syndrome control rats but still significantly different than normal control. The hypolipidemic and anti-atherosclerotic effects of the prepared beverage may be attributed to their content of not only the polyunsaturated fatty acids in almond (Keser et al., 2014) and quinoa seeds (Mohamed et al., 2019) but also to the bioactive therapeutic peptides (Siddiqui et al., 2020) and the phenolic compounds which possess lipid-lowering effect in the liver and can regulate genes expression that involved in lipogenesis and fatty acid oxidation (Pisonero-Vaquero et al., 2015). Reduction of cholesterol and lipid absorption, adjusting postprandial insulin response, promoting cholesterol conversion to bile acids, and improving gut microbiota can be mediated by the dietary fiber (De Carvalho et al., 2014). Also, beta-carotene has the potential to inhibit atherogenesis in humans (Harari et al., 2008) through improving lipid metabolism, cell migration, apoptosis and inflammation as confirmed by Chinetti et al. (2001); Day et al. (2006); Ji et al. (2001), and Marx et al. (1999), respectively.

### 3.4.3. Effect of the prepared beverages on the carbohydrate metabolism

Insulin resistance and fatty liver can be progressed by lipid accumulation and loss of insulin action caused by the high-fat diet (Satapati et al., 2012). Feeding rats on HFHF diet mediated impaired glucose and insulin resistance which reflected by the significant elevation in fasting plasma glucose, fasting insulin and HOMA-IR in metabolic syndrome control rats in comparison to normal rats (Figure 1A–C, respectively). Oral administration of the prepared beverages significantly suppressed the elevation in plasma insulin, fasting glucose, and insulin resistance with different degrees. The beverage II was the most promising in this concern. It was reported that almonds reduces postprandial glucose (Tan and Mattes, 2013), decreases insulin resistance (Wien et al., 2010), and elevates insulin secretion (Jenkins et al., 2008) due to the presence of unsaturated fatty acids, phytosterol, fiber, and other bioactive components (Berryman et al., 2011). Additionally, the cereals (quinoa and oat) content of folate, magnesium, potassium, and fiber may be contributed to the hypoglycemic effect of the prepared beverages (Hosseinpour-Niazi et al., 2011).

### 3.4.4. Effect of the prepared beverages on oxidative stress status and inflammatory marker

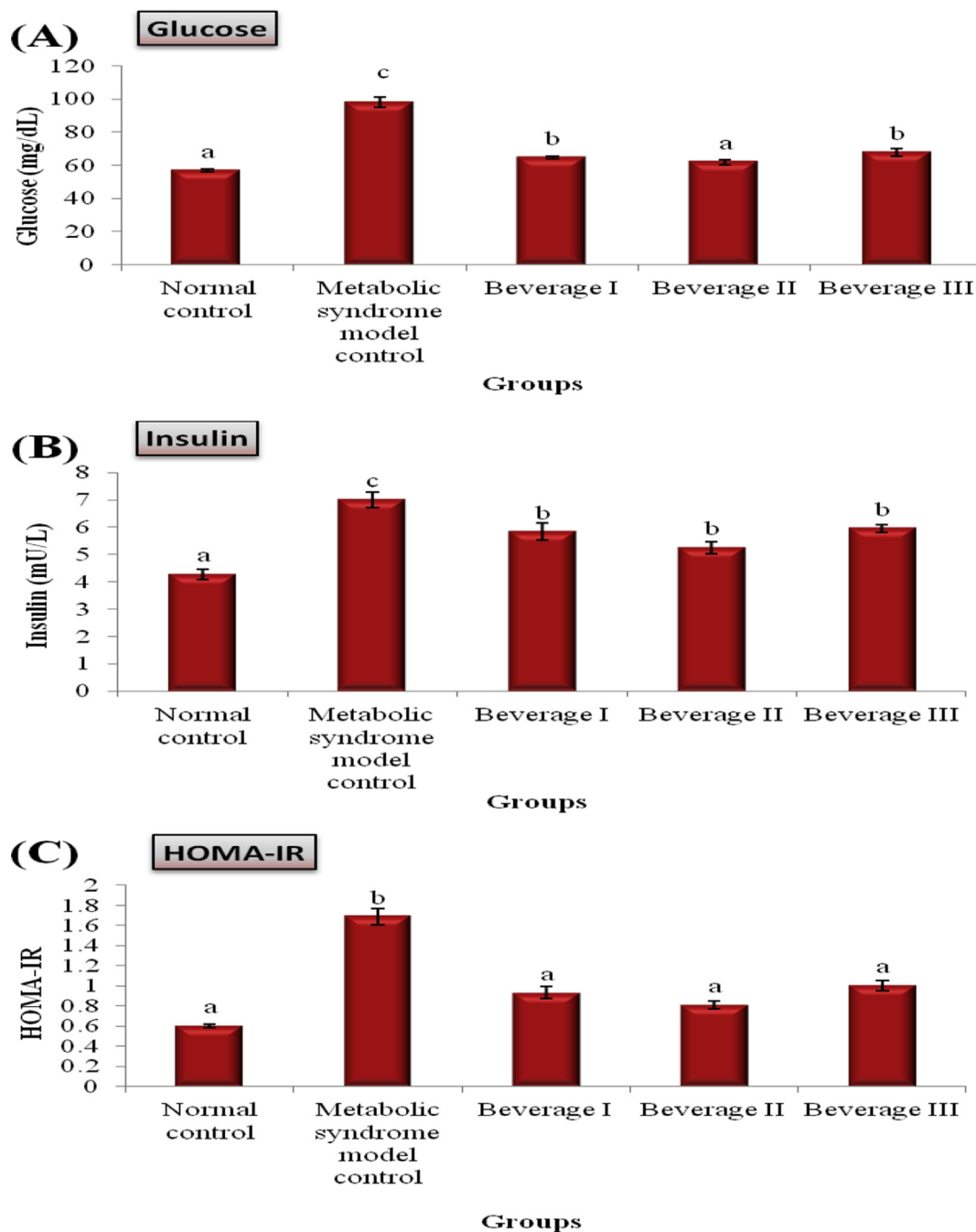
It was reported by O'Neill and O'Driscoll (2015) that the existence of inflammation and oxidative stress is an implicit cause of the metabolic syndrome, which leads to increase broaden of cardiovascular disease, non-alcoholic fatty liver, and type 2 diabetes. Plasma MDA was determined as a lipid peroxidation marker and TNF-alpha was determined as an inflammatory marker, while the activity of plasma catalase was determined as an antioxidant status enzyme. Plasma MDA and TNF-alpha elevated significantly in rats of metabolic syndrome control rats in comparison to normal control rats, while the activity of plasma catalase

enzyme reduced significantly in rats of metabolic syndrome control rats in comparison to normal rats as shown in Figure 2 (A, B and C, respectively). It was explored in a previous study that feeding rats on high-fat diet induced oxidative stress (elevation of MDA and reduction of catalase activity) and inflammation (elevation of TNF- alpha) especially that TNF-alpha is secreted by the white adipose tissue in addition to that both reactive oxygen species (ROS) and inflammatory cytokines stimuli the secretion of each other (Mohamed et al., 2020b). Oral administration of the prepared beverages reduced the elevation in MDA and TNF-alpha in the association of significant elevation in catalase activity which may be interpreted by the antioxidant activity of the prepared beverages as elucidated in the present results.

### 3.4.5. Effect of the prepared beverages on the liver and kidney functions

Kidney functions (creatinine and urea) as well as liver functions (ALT, AST, total protein, and albumin) are shown in Table 7. Plasma creatinine and urea were found to be significantly elevated in the metabolic syndrome control rats in comparison to normal rats. Ishimoto et al. (2012) reported that renal damage is associated with metabolic syndrome. Fructose induces glomerular hypertension and decreased the blood flow of renal which related to preglomerular vascular disease (Sánchez-Lozada et al., 2008). Also, Fan et al. (2014) and Al-Okbi et al. (2014) explored that feeding rats on high-fructose diet induced renal injury in association with inflammation and increment of lipid accumulation. Transaminases activity (AST and ALT) elevated significantly in metabolic syndrome control rats in comparison to normal rats. Plasma total protein and albumin reduced significantly in the metabolic syndrome control rats in comparison to normal rats. The present results coincide with those of Emamat et al. (2018) and Mohammed et al. (2018) who found that high-fat high-sugar diet produced elevation of AST and ALT.

The administration of all prepared beverages showed significant reduction in the liver and kidney functions in association with significant elevation of plasma total protein and albumin. The ameliorative effect of the prepared beverages on kidney and liver functions may be due to their hypolipidemic and antioxidant activities in addition to the hepatoprotective effect of phenolic compounds. Almond content of unsaturated fatty acid, phytosterol, fiber, and other bioactive components (Berryman et al., 2011) may be contributed to the beneficial effect of the prepared beverage on kidney and liver functions. Mohamed et al. (2019) confirmed that quinoa seeds suppressed the elevation in kidney and liver functions which mediated by feeding rats on diet rich in fructose and contained saturated fat. Additionally, there are several bioactive compounds present in oat (phenolic compounds, tocols, and beta-glucan) as confirmed by Emmons et al. (1999), banana (phenolics, biogenic amines, and phytosterols) as confirmed by Singh et al. (2016a) and in carrots (carotenoids, especially beta-carotene, anthocyanins, dietary fiber, and vitamins) as confirmed by Que et al. (2019) which possessed antioxidant



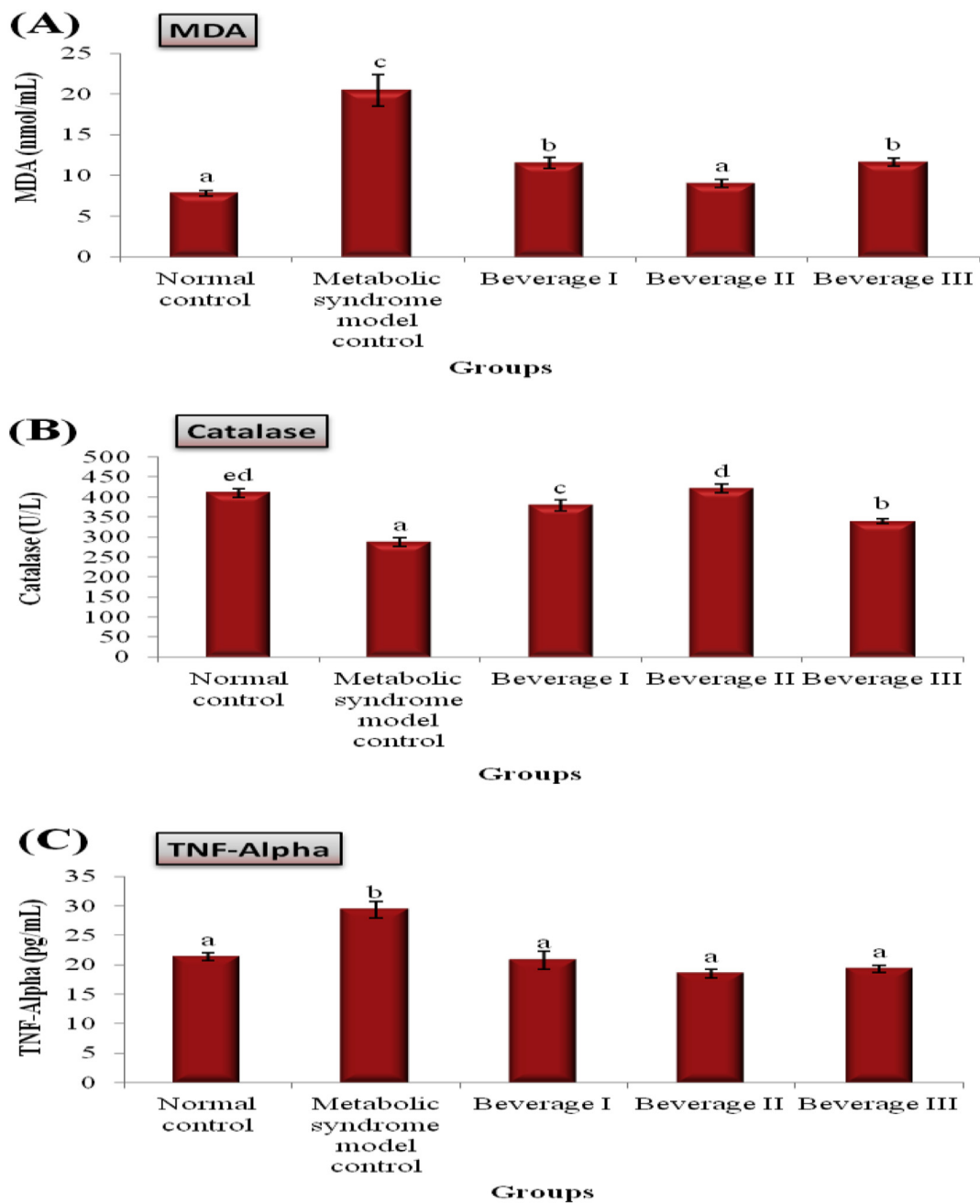
**Figure 1.** Fasting plasma glucose (A), fasting insulin (B) and insulin resistance index, HOMA-IR (homeostasis model assessment) (C) of the different experimental groups. On pairs of each figure, same letters mean non-significant difference; different letters mean the significance among the tested groups. The confidence level is 95%. The data are expressed as mean values  $\pm$  standard error.

activity and may be involved in the beneficial effect of the prepared beverages on kidney and liver functions.

#### 3.4.6. Effect of the prepared beverages on the histopathological findings of liver and heart tissues

Photomicrographs of livers from different groups (H&E X 400) are shown in Figure 3. The liver from the normal control group (Figure 3a) showed normal hepatic parenchyma, normal hepatocytes, and normal blood sinusoids. Staining of liver sections showed increased lipid deposition and inflammatory cell infiltration in metabolic syndrome control rats (Figure 3b) compared to normal rats (Figure 3a). Also, the liver from metabolic syndrome control rats (Figure 3b) showed diffuse fatty

degeneration and circumscribed vacuolated hepatocytes with peripheral nucleus (arrows). Accumulation of fat in the liver tissues possessed fatty liver in the rats, as one metabolic syndrome complications, and these results are supported by the elevation transaminases (AST and ALT) activities in plasma. The liver from rats given oral administration of beverage I (Figure 3c) showed improvement in the hepatic parenchyma than control metabolic syndrome rats; note normal hepatocytes and normal blood sinusoids. The liver from rats given oral administration of beverage (II) (Figure 3d) showed improvement in the hepatic parenchyma than control metabolic syndrome rats; note normal hepatocytes and normal blood sinusoids. The liver from rats given oral administration of beverage (III) (Figure 3e) showed slight regression in the fatty



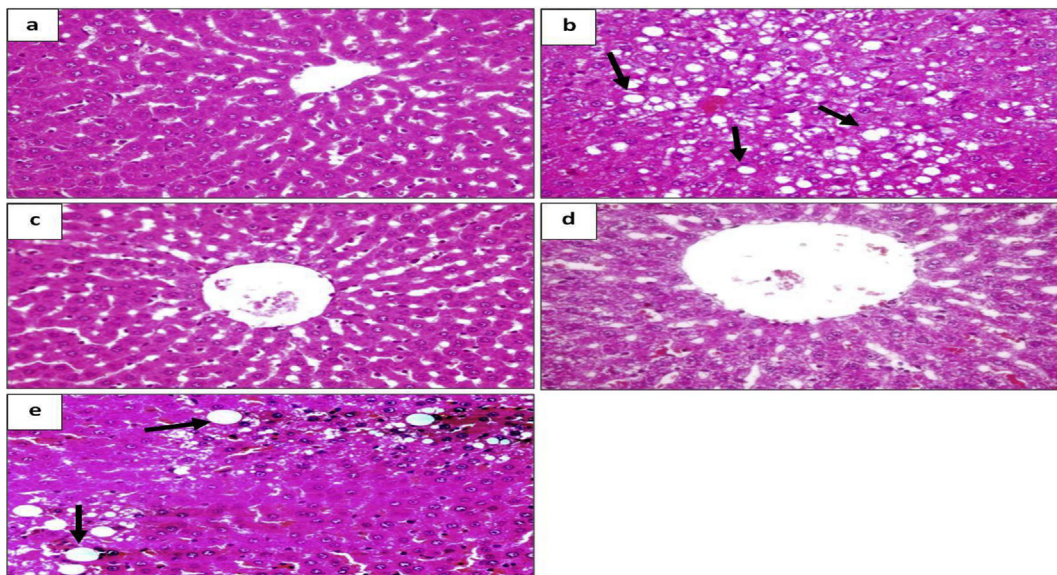
**Figure 2.** Plasma malondialdehyde (A), catalase (B) and tumor necrosis factor alpha (C) of the different experimental groups. On pars of each figure, same letters mean non-significant difference; different letters mean the significance among the tested groups. The confidence level is 95%. The data are expressed as mean values ± standard error.

**Table 7.** Effect of the prepared beverages on the liver and kidney functions.

Parameters	Normal control	Metabolic syndrome model control	Beverage I	Beverage II	Beverage III
Creatinine (mg/dl)	0.54 <sup>a</sup> ± 0.01	0.81 <sup>b</sup> ± 0.04	0.56 <sup>a</sup> ± 0.01	0.53 <sup>a</sup> ± 0.02	0.56 <sup>a</sup> ± 0.02
Urea (mg/dl)	26.00 <sup>a</sup> ± 0.58	30.67 <sup>b</sup> ± 0.99	29.47 <sup>b</sup> ± 0.26	29.41 <sup>b</sup> ± 0.61	29.52 <sup>b</sup> ± 0.47
ALT (U/l)	18.83 <sup>a</sup> ± 0.87	26.00 <sup>b</sup> ± 1.15	20.83 <sup>a</sup> ± 2.21	20.00 <sup>a</sup> ± 0.58	19.33 <sup>a</sup> ± 1.82
AST (U/l)	39.00 <sup>a</sup> ± 1.90	53.33 <sup>b</sup> ± 1.54	42.67 <sup>a</sup> ± 191	41.17 <sup>a</sup> ± 1.74	42.50 <sup>a</sup> ± 0.76
Total protein (g/dl)	6.87 <sup>b</sup> ± 0.14	5.85 <sup>a</sup> ± 0.24	6.48 <sup>ab</sup> ± 0.24	6.65 <sup>b</sup> ± 0.15	6.43 <sup>ab</sup> ± 0.32
Albumin (g/dl)	3.19 <sup>b</sup> ± 0.33	1.80 <sup>a</sup> ± 0.15	3.10 <sup>b</sup> ± 0.20	3.13 <sup>b</sup> ± 0.21	2.96 <sup>b</sup> ± 0.05

In each row same letter means non-significant difference while different letter means significant difference. The confidence level is 95%. The data are expressed as mean values ± standard error.



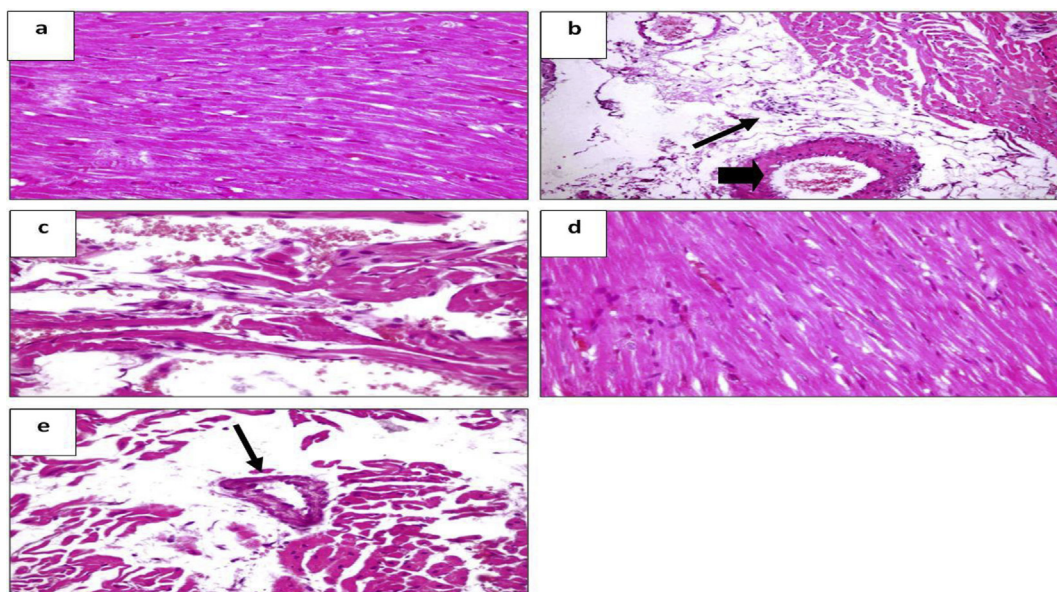


**Figure 3.** Photomicrographs of Livers from different groups (H&E X 400). Liver from normal control group (a). Liver from metabolic syndrome model control group (b), balloon score = 1, NAFLD score = 5, note the circumscribed vacuolated hepatocytes with prepheral nucleous (arrows). Liver from rats given oral administration of beverage I (c), balloon score = 0, NAFLD score = 0. Liver from rats given oral administration of beverage (II) (d), balloon score = 0, NAFLD score = 0. Liver from rats given oral administration of beverage (III) (e), balloon score = 0, NAFLD score = 0, note the circumscribed vacuolated hepatocytes with prepheral nucleous (arrows).

degenerated hepatocytes; note the few numbers of the in the circumscribed vacuolated hepatocytes with prepheral nucleous (arrows). The liver histology findings can be graded as minimal (for normal rats, rats given beverage I and rats given beverage II), mild (for rats given beverage III), and moderate (for metabolic syndrome control rats) according to Brunt et al. (1999) who suggested a three grade system (mild, moderate and marked) for the histological livers' lesions. Liver tissues of normal rats, rats given beverage I and rats given beverage II did not show ballooned hepatocytes (balloon score = 0, NAFLD score = 0). Liver tissue of metabolic syndrome control rats showed few ballooned hepatocytes (balloon score = 1, NAFLD score = 5) according to Kleiner et al. (2005) who explored these scores for non-alcoholic fatty liver disease and

Caldwell et al. (2010) who confirmed that the hepatocellular ballooning is used for histological grading and staging of NAFLD. Although liver tissue of rats given beverage III did not show ballooned hepatocytes, the hepatic lobule contained macro vacuoles which indicate to the presence of fat in the liver.

The heart from the normal rats (Figure 4a) showed normal myocardial muscles; note normal striations and normal nucleations (X 400). The heart from the metabolic syndrome control rats (Figure 4b) showed intramuscular edema with leucocytic cells infiltrations (arrows) together with dilatation in the blood vessels (arrowhead) (X200). These changes possessed cardiovascular diseases which are metabolic syndrome complications. Although metabolic syndrome control rats recorded



**Figure 4.** Photomicrographs of hearts from different groups (H&E). Heart from normal control group (a) (X 400). Heart from metabolic syndrome model control group (b) showed intramuscular edema with leucocytic cells infiltrations (arrows) together with dilatation in the blood vessels (arrow head) (X200). Heart from rats given oral administration of beverage I (c) (X400). Heart from rats given oral administration of beverage (II) (d) (X 400). Heart from rats given oral administration of beverage (III) (e) (X200).

significant elevation (Table 6) in the plasma lipid profile levels, the heart tissues showed moderate changes and did not reach to the changes which observed in type I atherosclerosis (including the presence of foamy cells) as reported by Stary (2000) who stated that atherosclerosis types start from I to VIII (the presence of fibrous tissues). The heart from rats given oral administration of beverage (I) (Figure 4c) showed improvement in the myocardial muscles than metabolic syndrome control rats; note the slight regression in the edema with the absence of neither inflammatory cells nor blood vessel dilatation (X400). The heart from rats given oral administration of beverage (II) (Figure 4d) showed improvement in the myocardial muscles than metabolic syndrome control rats; note normal striations and normal nucleations (X 400). The heart from rats given oral administration of beverage (III) (Figure 4e) showed slight regression in the intramuscular edema with slight regression in the blood vessel dilatation (arrow) (X200).

#### 4. Conclusion

It was manifested that the HFHF diet prompted the induction of Mets and its subsequent hepatic and vascular complications in rats. The prepared fortified almond milk reduced plasma total cholesterol, triglycerides, LDL-Ch, and the atherosclerosis indicators (the ratio of T-Ch to HDL-Ch and TGs to HDL-Ch) and elevated plasma level of HDL-Ch. Also, the prepared fortified almond milk suppressed the elevation of insulin, lipid peroxidation, TNF-alpha, AST, and ALT which indicates the beneficial effect of these fortified almond milk on cardiovascular diseases and non-alcoholic fatty liver as disorders of MetS. The beverage II was the most promising in this concern. Further studies are required to investigate the effect of the prepared beverages on MetS patients, also further studies are required to assess the efficacy of almond milk fortified with other vegetables and fruits.

#### Declarations

##### Author contribution statement

Ahmed M. S. Hussein, Doha A. Mohamed, Rasha S. Mohamed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Karem Fouda, Fathy M. Mehaya, Ayman A. Mohammad, Sherein S. Abdelgayed: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Competing interest statement

The authors declare no conflict of interest.

##### Additional information

No additional information is available for this paper.

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