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Production of a Yogurt Drink Enriched with Golden Berry (*Physalis pubescens* L.) Juice and Its Therapeutic Effect on Hepatitis in Rats

Magdy Ramadan Shahein ¹, El Sayed Hassan Atwaa ², Hanan A. Radwan ³, Abdelmoneim Ahmed Elmeligy ⁴, Amin A. Hafiz ⁵, Ashraf Albrakati ⁶ and Ehab Kotb Elmahallawy ^{7,*}

- ¹ Department Food Science and Technology, Faculty of Agriculture, Tanta University, Tanta 31527, Egypt; magdrsh10@gmail.com
- ² Food Science Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; elsayedhassanattwa@gmail.com
- ³ Department of Home Economics, Faculty of Specific Education, Zagazig University, Zagazig 44511, Egypt; drhananalsadeq81@gmail.com
- ⁴ Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt; elmeligy52@yahoo.com
- ⁵ Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm AlQura University, Makka Al-Mukarama 21961, Saudi Arabia; aahafiz@uqu.edu.sa
- ⁶ Department of Human Anatomy, College of Medicine, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia; a.albrakati@tu.edu.sa
- ⁷ Department of Zoonoses, Faculty of Veterinary Medicine, Sohag University, Madinet Nasser 82524, Egypt
- * Correspondence: eehaa@unileon.es

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Abstract: Fermented dairy products have been associated with multiple health benefits. The present study aimed to produce a functional yogurt drink fortified with golden berry juice and assess its therapeutic effect on hepatitis rats. Thirty male albino rats were randomly divided into two major groups. The first group included the control (-) animals (six rats) and was fed a standard diet, whereas the second group included 24 rats that were fed a standard diet and injected with carbon tetrachloride (CCl₄) for 2 weeks to trigger chronic damage of the liver (hepatitis); they were then divided into four groups (six rats/group): Group 2: hepatitis, fed on a standard diet as a positive control group; Group 3: received a basal diet with 5 mL of the yogurt drink; Group 4: received a basal diet with 5 mL of the yogurt drink fortified with 10% golden berry juice. Group 5: received a basal diet with 5 mL of the yogurt drink fortified with 20% golden berry juice. Various biological parameters were determined. Yogurt drink treatments were evaluated for their chemical, phytochemical, and sensory properties, as well as for their effects on hepatoprotective activity by determining various biochemical parameters. We found that the yogurt drinks containing golden berry juice exhibited no significant differences in fat, protein, and ash content compared with the control samples. Moreover, the yogurt drinks containing golden berry juice exhibited the highest content of total phenolic compounds, antioxidant activity, and organoleptic scores among all treatments. In addition, rats fed on a diet fortified with yogurt drinks containing golden berry juice for 8 weeks exhibited higher potential hepatoprotective effects compared with the liver injury control group. This improvement was partly observed in the group that received the yogurt drink containing golden berry juice. Therefore, we concluded that golden berry juice can be recommended as a natural additive in the manufacture of functional yogurt drinks, as it showed a potential hepatoprotective effect in rats with hepatitis.

Keywords: *Physalis pubescens* L.; yogurt drink; phytochemicals; liver function; hepatoprotective

1. Introduction

Liver diseases remain among the most serious health problems worldwide; however, prevention and treatment options are limited in this context [1]. It is well established that

pathogenesis, oxidative stress, and inflammation are the causes of liver disease, and that blocking and retarding the chain reactions of the oxidation and inflammation processes is a promising therapeutic strategy for the prevention and treatment of liver diseases [2,3]. Among others, carbon tetrachloride (CCl₄) poisoning has been associated with the production of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, which play an important role in the development of liver disease [4,5]. Trichloromethyl, which is a product of the primary metabolism of CCl₄, is believed to initiate the biochemical processes that cause oxidative stress, which is the direct cause of many pathological conditions, such as cancer, hypertension, diabetes mellitus, kidney and liver damages, and even death [5,6]. Clinical and experimental evidence has largely demonstrated that oxidative stress is a major inducer of apoptosis in various types of acute and chronic liver injuries and in hepatic fibrosis [7]. Hepatic fibrosis induced by CCl₄ is associated with the depletion of the antioxidant status and the exacerbation of lipid peroxidation [5,8]. Accordingly, the use of antioxidants and their interactions in the diet, which has attracted the attention of many researchers, presents a potential and effective therapeutic strategy for preventing or delaying the occurrence of hepatic fibrosis [9,10].

The last few decades have witnessed a growing interest in the development of enriched dairy products for health-enhancing foods such as products fortified with fiber, low and non-fat products and functional foods [11,12]. It is noteworthy to state that dairy products are among the most important consumer preferences among functional foods since they are ideal products for fortification with functional ingredients, and therefore, these fortified dairy products have been extensively examined [13–16]. However, it should be taken into account that there is a growing trend to avoid dairy products due to some associated health problems such as lactose intolerance, cow's milk allergy and hypercholesterolemia [17,18]. Among others, yogurt is one of the most widely consumed dairy products due to its numerous health benefits [13–15,19]. The acceptance of consumers for functional fermented yogurt remains very high, particularly among older people and females of all ages, and they have expressed their willingness to incorporate such functional foods into their diet [12]. Numerous studies have indicated that natural substances from edible and medicinal plants have a strong antioxidant activity and can, thus, act against hepatotoxicity [20,21]. One of those candidate plants is *Physalis peruviana* L. or *P. pubescens* L., which is known locally in Egypt as harankash and in English-speaking countries as the golden berry. This fruit has many medicinal and edible uses [22], as it contains many active components, such as vitamins A, B, C, E and K, minerals, polyunsaturated fatty acids, carbohydrates, hydroxyester disaccharides, withanolides (steroidal lactones), carotenoids, phenolic acids (such as gallic, chlorogenic, ferulic, caffeic, and p-coumaric acids), flavonoids (such as myricetin, kaempferol, catechin, quercetin, and rutin), and epicatechin biologically active components that reduce the risk of certain diseases and provide health benefits (such as antiproliferative effects on hepatoma cells, anti-hepatotoxic effects, and anti-inflammatory activity) [23]. In addition, it has excellent potential as a food-based strategy to manage hypertension and diabetes [23–27].

The demand for yogurt drinks by consumers has increased because of their unique properties and their many health benefits. Yogurt drinks are rich in protein, B vitamins, calcium and potassium, which help to stabilize the immune system [28]. Several researchers studied the effect of some different additives on the properties of the resulting yogurt. The addition of stabilizers, such as xanthan and guar gum, and skim milk powder to non-fat yogurt showed a significant effect on sensory attributes [29]. In another study [30], the addition of 0.06 mg/mL of nanopowdered eggshell powder could be applicable to manufacture probiotic yogurt with an acceptable composition and quality as compared to the control. Furthermore, nanopowdered eggshell increased the shelf-life of probiotic yogurt, as compared to the control, with a range of 5 to 7 log cfu/g of probiotic yogurt. Likewise, the incorporation of zeaxanthin nanoparticles in yogurt allowed the dispersion of a hydrophobic compound in a hydrophilic matrix which provides more stability [31]. Another previous study [32] revealed that the addition of inulin in the manufacturing of

probiotic yogurt as a prebiotic enhanced the growth of Bb and increased the shelf-life of the resultant yogurt. It is, therefore, not surprising to mention that the supplementation of yogurt with phenolic-rich products such as golden berry juice could be an ideal method for the optimization of the benefits of fermented dairy products with a high phenolic compound intake. Revising the available literature, several researchers demonstrated the potential benefits of the addition of fruit or vegetable juices in the preparation of functional fruit yogurt that included high acceptability, besides its role in the enhancement of the flavor, phenolic content and free radical scavenging activity of yogurt [11,33]. However, no previous research was performed on the addition of golden berry juice to yogurt, and therefore, it seems that the supplementation of yogurt with phenolic-rich products such as golden berry juice might optimize the benefits of fermented dairy products with a high phenolic compound intake. There is a need to clarify whether the addition of golden berry juice might change some specific aspects of fermented dairy products, such as fermentation time, growth of starter bacteria, acidification rate and the main physicochemical characteristics [11]. Given the above information, the present study aimed to investigate the impact of incorporating golden berry fruit juice in yogurt drink manufacturing and to fortify different proportions of yogurt drinks with their functional properties. Furthermore, the fortified yogurt drink was tested for its therapeutic effect as a functional food to treat artificially induced hepatitis in albino rats.

2. Materials and Methods

2.1. Materials and Reagents

Golden berry fruits were obtained from a local market (Zagazig, Egypt). Fresh cow standardized milk (3% fat) was obtained from the Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Zagazig University. Yogurt cultures containing *Streptococcus salivarius* subsp. *thermophilus* EMCC104 and *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC1102 were obtained from the Microbiological Resources Center, Faculty of Agriculture, Ain Shams University, Egypt. Moreover, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, CCl₄, and Tris-HCl buffer were purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade. Double-distilled water was used as the solvent. The basal pellet diets were obtained from the central animal house of the National Research Center, Dokki, Giza, Egypt. Water was provided ad libitum. The basal diet was prepared based on AIN 1993 [34]. Thirty-six male albino rats weighing between 150 and 200 g (± 10 g) were obtained from the Agricultural Research Center, Giza, Egypt.

2.2. Preparation of Golden Berry Fruit Juice

The ripe golden berry fruits were de-husked manually, then washed. Subsequently, the fruits were pulped using a high-speed electric mixer, for juice extraction. The juice was filtered using cheesecloth to separate the seeds and skins and the fresh fruit juice was analyzed directly after production.

2.3. Determination of Phytochemical Properties

The total phenolic content (TPC) (mg GAE/100 g) and antioxidant activity (AO; %) of the juice were assessed as described by Odabasoglu et al. [35] and Illupapalayam et al. [36], respectively, whereas the TPC and AO of the prepared yogurt drink were assessed as described by Maksimovic et al. [37] and Apostolidis et al. [38], respectively. Ascorbic acid (mg 100 mL⁻¹) was assessed as described by Bajaj and Kaur [39]. The total carotenoid content ($\mu\text{g mL}^{-1}$) was determined as described elsewhere [40].

2.4. Yogurt Drink Manufacture

Different treatments of yogurt drink were manufactured according to the procedure of [41], with some alterations as follows: Fresh Egyptian bulk cow's milk was separated to skim-milk and the resulting standardized cow's milk (3% fat) was heated at 85 °C for

10 min, then cooled to $42\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ before adding the yogurt starter culture containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) at a rate of 3%, followed by incubation at $42\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and pH reached 4.65 until uniform coagulation was obtained. The developed yogurts were cooled overnight at $5\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. The yogurt drink treatments were carried out as follows:

- Control yogurt drink (C): plain yogurt was mixed with 50% sterilized distilled water sweetened with 10% sugar (to achieve 5% sugar in the yogurt drink) at $25\text{ }^{\circ}\text{C}$.
- Yogurt drink fortified with 10% golden berry juice (T1): yogurt was mixed with 30% sterilized distilled water sweetened with 10% sugar and 20% pasteurized golden berry juice sweetened with 10% sugar (to achieve 5% sugar and 10% golden berry juice in the yogurt drink).
- Yogurt drink fortified with 20% golden berry juice (T2): yogurt was mixed with 10% sterilized distilled water sweetened with 10% sugar and 40% pasteurized golden berry juice sweetened with 10% sugar (to achieve 5% sugar and 20% golden berry juice in the yogurt drink). The drinking yogurt mixes were placed in 250-g plastic cups and then refrigerated until use in rat feeding. The samples were analyzed at day one.

2.5. Methods of Analysis

The total solids, fat, total protein, crude fiber, ash content, and titratable acidity of the yogurt samples were determined according to [40]. The changes in pH in the yogurt samples during storage were measured using a laboratory pH meter with a glass electrode (HANNA, Instrument, 4495-129 Amorim, Portugal).

2.6. Sensory Evaluation

A sensory evaluation of the yogurt drinks was carried out by a team of 10 professional panelists from the Faculty of Agriculture, Zagazig University, Egypt using the 5-point Hedonic Scale, according to Heymann and Lawless [42]. The panelists were instructed to wash their mouths with low sodium spring water during the sensory evaluation session, and they were encouraged to write down any criticisms on the tested products. Plain and treated yogurt samples were presented in plastic cups coded with three-digit random codes. Each cup contained 100 mL of yogurt samples freshly removed from the refrigerator. The sensory evaluation was conducted using a comparative test with fresh yogurt as a reference sample. The data were collected in specially designed ballots.

2.7. Experimental Design

After acclimation on a basal diet for 7 days, 30 male albino rats weighing about $150 \pm 5\text{ g}$ were classified into two groups. The first ($n = 6$) was used as the normal control group and fed a standard diet. The second group of rats ($n = 24$) was injected twice per week with CCl_4 in paraffin oil (50% v/v 2 mL/kg) via subcutaneous injection (for 2 weeks), to induce chronic damage in the liver, as described elsewhere [43]. The rats with hepatitis were classified into four groups as follows. Group 2: hepatitis positive control group, fed a basal diet and water ad libitum; Group 3: received a basal diet of 5 mL of a yogurt drink via an epigastric tube for 8 weeks; Group 4: received a basal diet and 5 mL of a yogurt drink fortified with 10% golden berry juice via an epigastric tube for 8 weeks; and Group 5: received a basal diet and 5 mL of a yogurt drink fortified with 20% golden berry juice via an epigastric tube for 8 weeks. Dosage of yogurt drink treated was 5 mL/mouse.

2.8. Blood Sampling and Biochemical Analyses

At the end of the trial term (8 weeks), all rats were fasted overnight before sacrificing. Blood samples were collected from the hepatic portal vein; one part was used for various analyses, and the remaining blood was kept in a centrifuge tube at room temperature for 15 min and then centrifuged at 4000 rpm for 10 min, to obtain serum, which was placed in plastic vials and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Subsequently, the livers were dissected, washed, and homogenized immediately to yield a 50% (w/v) homogenate in

ice-cold medium containing 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant (10%) was used in the various biochemical determinations.

2.9. Biochemical Analysis

Serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was assayed according to Reitman and Frankel [44]. Alkaline phosphatase (ALP) was assayed according to Belfield and Goldberg [45]. γ -Glutamyltransferase (γ -GT) was assessed according to Szasz and Persijn [46]. Moreover, total bilirubin (TB) in serum was assayed according to Garber [47]. The total antioxidant capacity (TAC) was determined according to Koracevic et al. [48]. Liver catalase (CAT) and Superoxide dismutase (SOD) activities were determined according to [49] and Nishikimi et al. [50], respectively. Malondialdehyde (MDA) was assayed in serum and homogenates of the liver according to Ohkawa et al. [51], whereas glutathione (GSH) was measured as described elsewhere [52].

2.10. Statistical Analysis

Data were statistically analyzed using the Statistic software, version 9 [53], and the differences between the means of the treatments were considered significant when they were greater than the least significant differences at the 5% level.

3. Results and Discussion

3.1. Approximate Composition of the Golden Berry Juice

Table 1 presents the approximate chemical composition of the golden berry juice. The moisture, protein, fat, and ash contents of the golden berry juice were 88.40, 1.06, 0.16, and 0.80 g/100 g, respectively. The total phenolic, ascorbic acid, and carotenoid contents, and the % DPPH inhibition of the golden berry juice were 112.40 mg GAE/100 mL, 52.68 mg/100 mL, 86.54 μ g/mL, and 78.34%, respectively. These results of chemical composition were in line with those reported by El Sheikha et al. [54,55], who found that the moisture, protein, fat, and ash contents of golden berry juice were 89.34, 1.02, 0.13, and 0.75 g/100 g, respectively, but in the same study, the results of the total phenolic, ascorbic acid and carotenoids contents of golden berry juice were slightly different from the results we reached, where the total phenolic, ascorbic acid, and carotenoid contents of golden berry juice were 76.60 mg GAE/100 mL, 38.77 mg/100 mL, and 70.0 μ g mL⁻¹, respectively.

Table 1. Approximate chemical composition (total phenol, ascorbic acid, and carotenoid content) and % DPPH inhibition of golden berry juice.

Components (%)	Golden Berry Juice
Moisture	88.40
Protein	1.06
Fat	0.16
Ash	0.80
Total phenol (mg GAE/100 mL)	112.40
Ascorbic acid (mg/100 mL)	52.68
Carotenoids (μ g mL ⁻¹)	86.54
% DPPH Inhibition	78.34

3.2. Chemical Composition of a Yogurt Drink Fortified with Golden Berry Juice

Table 2 shows that fortification of a yogurt drink with golden berry juice increased the TS content in the fortified yogurt drink, whereas the protein, fat, and ash contents of the yogurt drink were not affected by fortification with golden berry juice at two ratios, which may be due to the low protein, fat, and ash contents of golden berry juice [55]. This result

agrees with that of Ismail et al. (2020) [33], who found that the fortification of a yogurt drink with fruit juices (red grape or apricot) increased the TS but did not affect the protein, fat, and ash contents of the resultant yogurt drink.

Table 2. Chemical composition of a yogurt drink fortified with golden berry juice on the first day of storage.

Components (%)	Treatments			
	C	T ₁	T ₂	LSD
Total solids	11.70 ^C	12.78 ^B	13.84 ^A	0.1681
Protein	2.12 ^A	2.24 ^A	2.46 ^A	0.6162
Fat	1.25 ^A	1.28 ^A	1.30 ^A	0.1269
Ash	0.50 ^A	0.56 ^A	0.64 ^A	0.1513

Means followed by different uppercase letters in the same column are significantly different ($p \leq 0.05$). LSD, least significant difference; C, yogurt drink made from cow milk, as a control (C); T₁, yogurt drink fortified with 10% golden berry juice; T₂, yogurt drink fortified with 20% golden berry juice.

3.3. Titratable Acidity, pH Values, TPC, and % DPPH Inhibition of a Yogurt Drink Fortified with Golden Berry Juice

Table 3 indicates that the control yogurt drink had the lowest titratable acidity (TA) value. The acidity of the yogurt drink fortified with golden berry juice increased as the fortification ratio increased, which may be due to the high TA of golden berry juice [56]. Accordingly, the pH values of all treatments exhibited a reverse trend compared with that observed for TA because they expressed the level of alkalinity. Similar results were obtained by Dimitrellou et al. (2020) [11], who found that the fortification of yogurt with juices from grapes and berries increased the TA values of the resultant yogurt.

Table 3. Titratable acidity, pH values, total phenolic content, and % DPPH inhibition of a yogurt drink fortified with golden berry juice on the first day of storage.

Parameters	Treatments			
	C	T ₁	T ₂	LSD
Acidity%	0.45 ^C	0.62 ^B	0.75 ^A	0.1177
pH values	6.02 ^A	5.37 ^{AB}	4.72 ^B	1.0176
Ascorbic acid (mg/100 mL)	0.78 ^C	4.34 ^B	6.92 ^A	1.798
Carotenoids (µg/mL)	0.84 ^C	7.40 ^B	11.78 ^A	1.748
TPC (mg/100 g)	15.33 ^C	24.72 ^B	36.84 ^A	1.680
% DPPH Inhibition	11.46 ^C	19.54 ^B	28.60 ^A	6.723

Means followed by different uppercase letters in the same column are significantly different ($p \leq 0.05$). LSD, least significant difference.

The ascorbic acid, carotenoid, and TPCs and the % DPPH inhibition of the yogurt drink supplemented with golden berry juice increased as the supplementation ratio increased, compared with the control yogurt drink (Table 3). This may be due to the high ascorbic acid, carotenoid, and TPC of the golden berry fruit and juice [23,55]. These results agreed with those reported by Dimitrellou et al. [11], who found that the TPC and radical scavenging activity of yogurt increased after the fortification of yogurt milk with juices from grapes and berries. Moreover, Naeem et al. [56] found that fortification of ice cream with concentrated golden berry juice increased its total phenolic content and radical scavenging activity.

3.4. Sensory Properties of a Yogurt Drink Fortified with Golden Berry Juice

The data presented in Table 4 showed that the fortification of a yogurt drink with golden berry juice increased greatly the sensory attributes of the resultant yogurt drink, especially

its taste, odor, and overall acceptance compared with the control yogurt drink; moreover, this increment was proportional to the fortification ratios. All treatments yielded acceptable sensory properties. These results agree with those reported by Dimitrellou et al. [11], who observed a great improvement in the sensory properties of yogurt after fortification with juices from grapes and berries. Moreover, Naeem et al. [56] found that the fortification of ice cream with concentrated golden berry juice improved greatly its sensory properties.

Table 4. Sensory properties of a yogurt drink fortified with golden berry juice on the first day of storage.

Items	Treatments			
	C	T ₁	T ₂	LSD
Taste	3.90 ^C	4.70 ^B	5.0 ^A	0.1681
Odor	3.70 ^B	4.30 ^{AB}	4.80 ^A	0.6724
Appearance	4.80 ^B	4.50 ^{AB}	4.30 ^A	0.3362
Overall acceptance	3.90 ^C	4.40 ^B	4.90 ^A	0.1681

Means followed by different uppercase letters in the same column are significantly different ($p \leq 0.05$). LSD, Least significant difference.

3.5. Effect of a Yogurt Drink Fortified with Golden Berry Juice on Serum AST, ALT, γ -GT, ALP, and TB Levels in Rats with Hepatitis

The effects of the treatments described above on the levels of AST, ALT, γ -GT, ALP, and TB are illustrated in Table 5. The negative control group showed significantly low serum AST, ALT, γ -GT, ALP, and TB levels compared with the hepatitis rat group. The lowest mean values of AST, ALT, γ -GT, ALP, and TB in the negative control (Group 1) were 74.14 U/mL, 60.22 U/mL, 138.40 U/mL, 40.02 U/mL, and 3.04 mg/dL, respectively. In contrast, the two rat groups that were fed a yogurt drink containing golden berry juice (Groups 4 and 5) exhibited a significant reduction in AST, ALT, γ -GT, ALP, and TB levels compared with the hepatitis positive control group (Group 2). Furthermore, the rats with hepatitis had significantly higher levels of AST, ALT, γ -GT, ALP, and TB than did the control rats. In turn, rats with hepatitis that were fed a yogurt drink containing 20% golden berry juice exhibited non-significant differences in AST, ALT, γ -GT, ALP, and TB levels compared with the control rats. The best improvement after treatment was observed in the group that consumed the yogurt drink containing 20% golden berry juice, which may be due to the general chemical composition of golden berry juice, which has antiproliferative effects on hepatoma cells [57], antihepatotoxic effects [58], antioxidant activity [25], and anti-inflammatory activity [59]. The hepatoprotective effect may be attributed to the presence of ascorbic acid and phenolic components, such as chlorogenic, ferulic, caffeic, gallic, and p-coumaric acids, as well as flavonoids, such as kaempferol, catechin, epicatechin rutin, myricetin, and quercetin [27,60], which are well-known hepatoprotective agents [60]. The first indication of the liver damage caused by CCl₄ can be obtained by assessing ALT, AST, ALP, and γ -GT levels, as these enzymes are upregulated in cytotoxic liver injury and cholestasis. Golden berry juice is a clear indication for the improvement of the functional status of liver cells while preserving cellular architecture [61], which confirms the protective activity of golden berries on the liver. The data were in line with those of Al-Olayan et al. [2], who reported that golden berry juice showed a potential protective effect against CCl₄-induced hepatotoxicity in rats. Furthermore, Taj et al. [62] found that animals treated/fed with various preparations of golden berries exhibited a significant decrease in the elevated levels of serum markers, such as ALAT, ASAT, ALP, LDH, creatinine, urea, and bilirubin, indicating protection against hepatic cell damage.

Table 5. Effect of a yogurt drink fortified with golden berry juice on serum ALT, AST, ALP, and γ -GT activities in rats with hepatitis.

Parameters	Groups					LSD
	Group 1	Group 2	Group 3	Group 4	Group 5	
AST (U/mL)	74.14 ^C	156.70 ^A	86.24 ^B	80.46 ^B	76.84 ^{BC}	11.192
ALT (U/mL)	60.22 ^D	128.52 ^A	96.60 ^B	84.32 ^C	66.50 ^D	9.036
ALP (IU/L)	138.40 ^D	240.84 ^A	190.62 ^B	170.24 ^{BC}	146.30 ^{CD}	29.848
γ -GT (U/L)	40.02 ^C	54.86 ^A	48.24 ^B	43.58 ^{BC}	40.60 ^C	4.887
TB (mg/dL)	3.04 ^D	6.22 ^A	4.20 ^B	3.78 ^C	3.22 ^C	0.2768

Means followed by different uppercase letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference. Group 1: Negative control rats fed a basal diet. ALT: Serum Alaninealanine aminotransferase; AST: aspartate aminotransferase; ALP: Alkaline phosphatase; γ -GT: γ -Glutamyltransferase; TB: total bilirubin. Group 2: Hepatitis rats (as a positive control group) fed a basal diet. Group 3: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink. Group 4: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink fortified with 10% golden berry juice. Group 5: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink fortified with 20% golden berry juice.

It is noteworthy to mention that understanding the sex's similarities, differences, and/or complex seems crucial for correctly applying research-derived knowledge. The present study pointed out the use of male rats were used in the experiment since there was a great concern among scientists about including female animals in preclinical biomedical research for fear of increasing costs and diversity in the resulting data [63]. In this concern, a previous study [64] revealed that biomedical research might be affected by the impact of the animal's sex on the cellular, molecular and biochemical levels. Importantly, there is substantial bias in biomedical research to not study female rats/mice [65,66]. The exclusion of female rats/mice in biological research was justified due to the variable physiological nature of female data caused by hormonal fluctuations associated with the female's reproductive cycle. However, it should be stressed also that some recent studies documented that female mice were not inherently more variable than male mice across diverse physiological traits [67]. Regardless of which sex was used in the present work, including both sexes in the same experiment might influence the findings and the resulting data. Furthermore, several previous studies used male rats/mice in hepatitis in rats [68–71].

3.6. Effect of a Yogurt Drink Fortified with Golden Berry Juice on Serum MDA, GSH, and TAC and Liver MDA, GSH, CAT, and SOD Levels in Rats with Hepatitis

The data illustrated in Table 6 showed that the rats with hepatitis had significantly lower serum GSH and CAT levels and lower serum MDA values compared with the control rats. The groups of rats with hepatitis that were fed the yogurt drink fortified with golden berry juice showed a significant increase in serum GSH and CAT levels and a decrease in serum MDA levels compared with the hepatitis positive control rats. This represented evidence of the protective effect of the yogurt drink fortified with golden berry juice in the liver. Moreover, the rats with hepatitis had significantly lower liver SOD, CAT, and GSH levels and a higher liver MDA level compared with the control rats. The groups of rats with hepatitis that were fed a yogurt drink fortified with golden berry juice exhibited a significant increase in liver SOD, GSH, and CAT levels and a decrease in the liver MDA level compared with the hepatitis positive control rats. These improvements indicated that the yogurt drink fortified with golden berry juice exerted antioxidative and beneficial effects regarding liver recovery from CCl_4 injury.

Table 6. Effect of a yogurt drink fortified with golden berry juice on serum MDA, GSH, and TCA and Liver MDA, GSH, CAT, and SOD levels in rats with hepatitis.

Parameters	Groups					LSD
	Group 1	Group 2	Group 3	Group 4	Group 5	
Serum MDA (nmol/mL)	35.22 ^C	52.48 ^A	42.84 ^B	38.60 ^{BC}	36.54 ^C	5.043
Serum GSH (mmol/mL)	65.40 ^B	36.72 ^E	58.26 ^D	62.86 ^C	68.38 ^A	1.259
Serum TAC (mmol/L)	0.94 ^A	0.58 ^D	0.66 ^{CD}	0.74 ^{BC}	0.82 ^B	0.1168
Liver MDA (nmol/g tissue)	412.86 ^D	574.50 ^A	486.24 ^B	442.66 ^C	404.80 ^E	7.342
Liver GSH (mmol/g tissue)	38.30 ^B	24.52 ^D	32.62 ^C	40.06 ^B	46.74 ^A	3.084
Liver CAT (U/g)	92.46 ^B	65.30 ^E	73.84 ^D	86.22 ^C	96.20 ^A	1.542
Liver SOD (U/g)	132.82 ^A	66.14 ^E	80.76 ^D	94.60 ^C	104.54 ^B	6.169

Means followed by different uppercase letters in the same column are significantly different ($p \leq 0.05$). LSD: Least significant difference. TAC: total antioxidant capacity; CAT: Liver catalase (CAT); SOD: Superoxide dismutase; MDA Malondialdehyde; GSH: glutathione (GSH). Group 1: Negative control rats fed a basal diet. Group 2: Hepatitis rats (as a positive control group) fed a basal diet. Group 3: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink. Group 4: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink fortified with 10% golden berry juice. Group 5: Hepatitis rats fed a basal diet with 5 mL of a yogurt drink fortified with 20% golden berry juice.

The toxicity of CCl_4 is due to oxidative stress caused by free radicals, which contributes to both the onset and development of fibrosis. This appears during lipid peroxidation, which is associated with a decrease in the levels of GSH and the antioxidant enzymes SOD, CAT, and GSH [5,72]. These enzymes constitute a strong defense against ROS [73].

The yogurt drink fortified with golden berry juice attenuated the intoxication effects triggered by CCl_4 and significantly improved the activity of these enzymes, which indicates the antihepatotoxic and antioxidant effects of golden berry juice, similar to the results obtained elsewhere [2]. Ascorbic acid and the phenolic components present in golden berries, such as chlorogenic, ferulic, caffeic, gallic, and p-coumaric acids, as well as flavonoids, such as kaempferol, catechin, epicatechin rutin, myricetin, and quercetin, may explain the antioxidant activity recorded in golden berries, which are considered to be a strong antioxidant because of their ability to scavenge free radicals and active oxygen species [74]. These properties of phenolic and flavonoid compounds allow them to inhibit lipid peroxidation and exert anti-inflammatory effects [5,75].

4. Conclusions

Collectively, the golden berry fruit juice showed high contents from total phenol (112.40 mg GAE/100 mL), ascorbic acid (52.86 mg/100 mL⁻¹) and carotenoids (86.54 µg/mL⁻¹). Based on the results presented above, the golden berry fruit juice can be used to fortify several vital food products, such as yogurt drinks. The addition of golden berry fruit juice, up to a concentration of 20%, during the manufacture of yogurt drinks did not affect the contents of protein, fat and ash, while it greatly affected the total sensorial preference, acidity values, total phenolic content and antioxidant activity, and added health benefits to them because of its highly bioactive components. Yogurt drinks fortified with golden berry juice proved effective in protecting against hepatitis in albino rats. The bioactive components of golden berry juice were able to prevent hepatitis in rats by lowering the levels of several relevant parameters, i.e., markers of liver function, and upregulating the antioxidant enzymes SOD, CAT, and GSH. Therefore, golden berry juice can be recommended as a natural additive in the manufacture of functional yogurt drinks.

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