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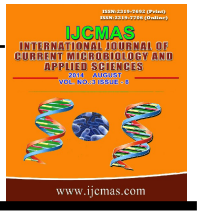

Original Research Article

Role of the Functional Food (Pomegranate-Yoghourt) as Hepatoprotective Effect on Liver Injured Rats

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Keywords

Pomegranate juice, Yoghourt, antioxidant activity, phenolic compounds,

A B S T R A C T

Pomegranate juice (PJ) is commonly used in traditional medicine due to its therapeutic properties according to its high content of total phenolic content (TPC) and antioxidant activity (AA) which related to the management of many disorders. So, supplementing fresh buffalo skim milk with (PJ) with different ratios (2, 4, 6, 8, and 10%) and the textural properties, TPC and AA in the resultant fresh yoghurt during the cold storage for 12 days were evaluated. As the percentage of PJ was increased, the TPC and AA were increased, while the addition of PJ up to 6% had an adverse effect on the textural properties. The effect of pomegranate-yoghurt against hepatic injury (CC14 treatment) in rats was investigated through determination of some biochemical parameters in serum and liver in addition to the histophathological examination of liver under different treatments. Forty adult albino rats were equally divided into five groups; a negative control (C^-), CC14-treated rats (C^+) and groups that received a dietary yoghurt-PJ in 0, 4, 8% ratios. Results revealed that pomegranate-yoghurt improved the level of biomarkers scores of CC14-treated rats, and reduced the histopathological changes in liver with the elevating of PJ ratio. Consequently, administration of yoghurt supplemented with PJ is acceptable as "functional food" alleviates the harmful effect of CC14-induced liver injury and offers a pleasant and effective route in increasing the TPC and antioxidant intake in our daily diet.

Introduction

The challenge is, however, choosing the right functional nutrients and formulating a message that works. Yoghurt is a fermented dairy product, having several health benefits, so is healthier for consumption (lourens-Hattingh and Viljoen 2001). Yoghurt is beneficial to our digestive system, especially stomach and colon; it enhances the immune response which will be in turn increase resistance to immune-related diseases (Meydani *et al* 2000). Yoghurt can be developed with fruit juice which can be part of a healthy diet (Aswal *et al* 2012), which increases the aesthetic value of the new product as a functional pigment (Ghadge *et al* 2008; Coïsson *et al* 2005).

Pomegranate (*Punica granatum Linn*) is a potential medicinal plant of family punicaceae (Heber 2011). Pomegranate juice was used as a healthful beverage (Basu and Penugonda 2009), since it is a natural rich source of polyphenols, flavonoid and other antioxidant. It could be considered as functional ingredients for its anti radical activities. It is good supplement for food and dietetics (Tomás-Barberán *et al* 2006; Basu and Penugonda 2009). Pharmacological properties of the juice were anti-inflammatory (Adams *et al* 2006), anticancer (Adams *et al* 2010) and protection of hepatoxicity (Kaneria *et al* 2012) by carbon tetrachloride (Pirinccioglu *et al* 2012).

Therefore the aim of the study was to evaluate the textural parameters; total phenolic content and DPP-radical scavenging activity of yoghurt supplemented with pomegranate juice, also, the protective roles of pomegranate juice against the hepatoxicity induced by carbontetrachlorid for rats feeding on yoghurt supplemented with pomegranate juice.

Materials and Methods

Preparation of pomegranate juice

Fresh mature pomegranate fruits were purchased from local market at Giza. They were cleaned and cut; arils were manually separated from the peals and piths. The fruit juice was extracted using an electric juicer and then filtered through cotton mesh. Fresh juice (12.2% total solids) was stored in freezer.

Preparation of yoghurt

Fresh skim buffalo milk was obtained from dairy department of Agriculture College, Cairo University. Milk was heated to 90^{O} C for 10 min. and cooled to 40^{O} C. Pomegranate juice (PJ) (pH 4.5) was added at the rate of 2, 4, 6, 8, 10% immediately after incubation with 3% starter culture (local market) to avoid the quick reducing of pH and transferred into 100 ml plastic containers, lightly sealed and incubated at 45^{O} C until the complete curd formation, then stored at refrigerator (6 ± 2^{O} C) for 3, 6, 9, 12 days.

Textural evaluation

Texture profile analysis (TPA) performed on the yoghurt samples using the double corporation, Slinfold, W. Sussex, UK. From the force time curve the following parameters were evaluated by TPA according to the definitions by International Dairy Federation (1991).

Hardness*:* force necessary to attain a given deformation

Cohesiveness: the extant to which a material can be deformed before it rupture.

Springiness: in the rate which the sample returns to its original shape when the deforming force is removed.

Gumminess: Force needed to disintegrate the sample to a state ready for swallowing

Chewiness: work needed to masticate the sample to state ready for swallowing

Total phenolic content (TPC)

The total phenolic content determined by Folin-Ciocalteu Reagent according to **Zheng et al (2001),** and calculated as Gallic acid.

Radical scavenging activity (RSA)

The stable free radical DPPH is a rapid test

can provide information on the ability of a food compound contains antioxidants to act as free radical scavenger's ability or hydrogen donor's atom, according to (Xuewu et al., 2007).

Design of animal experiment

Animals

Forty adult (male and female) albino Speaque-Dawley rats with body weights 80-90g were obtained from the Animal Home in National Research Center Egypt. Animals were kept individually in stainless steal cages, water was allowed adlib tam, and the room temp. was adjusted at 25^{O} C.

Experimental Design

Rates divided into 5 groups, 8 for each group as follow:

Group (1): C^{-} fed on standard died only (according to AIN 1993)
Group (2): C^{+} treated with CC_{14} , fed on the standard diet only.

Group (3): y_0 treated with CC_{14} , fed on yoghurt only.

Group (3): y_4 treated with CC_{14} , fed on yoghurt with 4% PJ.

Group (3): y_8 treated with CC_{14} , fed on yoghurt with 8% PJ.

Rats were administered (carbon tetrachloride, Sigma) by back subcutaneous injection (0.5 ml of 1:1 mixture of CC_{14} and olive oil) based on a calculated 3 ml/kg dosage for inducing liver injuries according to lee *et al* 2005. The yoghurt diet was 180 cc^3 per kg body weight per day. The feeding period continued for 6 weeks. During the experimental period, the food consumption and body weight of the rats were followed.

At the end the experimental period, rats were fasted overnight and in the morning blood samples were taken from each rat by open heart puncture under slight ether anesthesia, and serum was separated. The abdomen was opened and the liver was excised then washed with saline plotted between sheets of filter paper and weighed. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin, and albumin were estimated according to Reirtman and Frankel (1957); Dreper and Hadley (1990); Bartholomev and Delang (1966) respectively. Reduced glutathione (GSH) and glutathione peroxidase (GPX) according Ellman (1959), and Weiss *et al* (1980) respectively.

Histopathology study

After blood collection, all rats were rapidly sacrificed and the liver of each animal was dissected, weighted and portion of it was preserved in 10% buffered neutral formalin and paraffin embedded. Four sections (5mictons in thickness) were taken from each liver tissue, each section being at a distance at least 500 µ from the proceeding one sections were stained with haematoxylin and eosin for the histological examination according Ross *et al* (1989).

Statistical analysis

Data of the textural analysis were statistically analyzed using GLM procedures of the SAS (SAS 1996). Means were separated by using Duncan's multiple rage test (Duncan 1955).

The values of the biochemical analysis were expressed as mean ± SD and ± SEM and statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance. Differences were considered significant at $p<0.05$ according to Artimage and Berry (1987).

Results and Discussion

Textural properties

Table (1) presents the results of texture analysis performed on yoghurt supplemented with pomegranate juice (PJ) and the control sample. Data revealed that hardness of yoghurt samples was not significantly affected with increasing the concentration of (PJ) and storage periods until 6% then decreased significantly with increasing the (PJ) added (8%, 10%) and at the end of storage (12 days). This may be attributed to the moisture content of (PJ) at higher concentration which weakens the protein network resulting in a less firmness (Ahmed *et al* 2005). In yoghurt, the protein matrix consists of short interconnected chains; consequently the liquid phase of juice is immobilized in the interstitial spaces in the protein matrix (Shiby and Mishra 2012). Values of cohesiveness were relatively lower for yoghurt samples supplemented with (PJ) than in plain yoghurt samples, this may due to the strength of protein-protein interaction bonds in control yoghurt rather that in the mixture of milk and (PJ) which weakened this phenomena. Also, noticed the same results in springiness data. Moreover, chewiness and gumminess of the yoghurt supplemented with (PJ) were reduced significantly rather than control yoghurt (without PJ).

Total phenolic content (TPC):

Results illustrated in Fig. (1) revealed that TPC of yoghurt fortified with (PJ) increased with increasing the concentration from 2% to 10% this ascribed to the TPC of (PJ) (3425 gallic acid mg/l). Many researchers reported that (PJ) is a very rich source of polyphenols including ellagitannins (Uzuner et al 2011; Com and Hisil 2010; Negi et al 2003; Gil et al 2000).

Moreover Turfan et al (2012) reported that the antioxidative properties of pomegranate products are correlated with their polyphenolic content including tannins and anthocyannins. The rate of increase in TPC in fresh yoghurt supplemented with 4% and 8% (PJ), Table (2) was 27.7% and 103.6% respectively, while it was 19.9% and 92.8% in the same order after 3 days of storage. During cold storage the TPC gradually decreased for all samples, this may be attributed to the transformation of phenolic compounds which unstable and undergo numerous enzymatic and chemical reactions during storage as stated by (Cheynier (2005); Poncet-Legrand *et al* (2005); ES-Safi *et al* (2007). Uzuner *at al* (2011) and Turfan *et al* (2012) found that anthocyanins in pomegranate lost its stability during storage and some of polyphenols formed di-esters with α-D-glucose.

Antioxidant activity (AA)

DPPH radical scavenging activity (RSA) of yoghurt supplemented with (PJ) had higher antioxidant activity than control yoghurt (without juice) and this was directly proportional to the percentage of added juice Fig (2). This ascribed to the phenolic compounds found in pomegranate which are responsible for 92% of its AA (Seeram *et al* 2006 and Kulkarni *et al* 2004). The rate of increase in RSA by adding 4% and 8% pomegranate was 198% and 398 % respectively for fresh yoghurt samples, Table (3), while the corresponding rates after 3 days of storage was 106.5% and 321.7%. After 12 days of storage, the rate of increase in RSA for 4% and 8% concentration of pomegranate were 28.6% and 125.7% respectively.

Table.1 Texture analysis performed for yoghurt supplemental with pomegranate and the control sample

Analysis	Days	0%	2%	4%	6%	8%	10%
hardness	fresh	1.0	1.0	1.0	1.1	0.7	0.6
	3 days	1.0	1.0	1.0	1.0	0.7	0.6
	6 days	1.0	1.0	1.0	1.0	0.6	0.5
	12 days	0.8	0.8	1.0	1.0	0.6	0.5
Cohesivness	fresh	0.82	0.416	0.488	0.404	0.339	0.513
	3 days	0.846	0.424	0.496	0.399	0.343	0.516
	6 days	0.813	0.419	0.437	0.438	0.429	0.506
	12 days	0.641	0.435	0.492	0.589	0.468	0.431
Spinginess	fresh	0.649	0.469	0.495	0.515	0.499	0.583
	3 days	0.647	0.469	0.492	0.582	0.514	0.524
	6 days	0.67	0.448	0.655	0.561	0.504	0.534
	12 days	0.682	0.601	0.642	0.669	0.552	0.532
Gumminess	fresh	0.82	0.416	0.586	0.515	0.237	0.308
	3 days	0.846	0.424	0.595	0.399	0.24	0.309
	6 days	0.813	0.419	0.568	0.482	0.258	0.253
	12 days	0.852	0.479	0.541	0.589	0.281	0.216
Chewiness	fresh	0.532	0.195	0.29	0.265	0.118	0.179
	3 days	0.544	0.199	0.293	0.232	0.123	0.162
	6 days	0.544	0.188	0.372	0.27	0.13	0.135
	12 days	0.581	0.288	0.347	0.512	0.155	0.115

Fig.1 Total phenolic content equivalent gallic acid (mg/l) of yoghurt samples supplemented with pomegranate and storage in refrigerator at 6±2°C

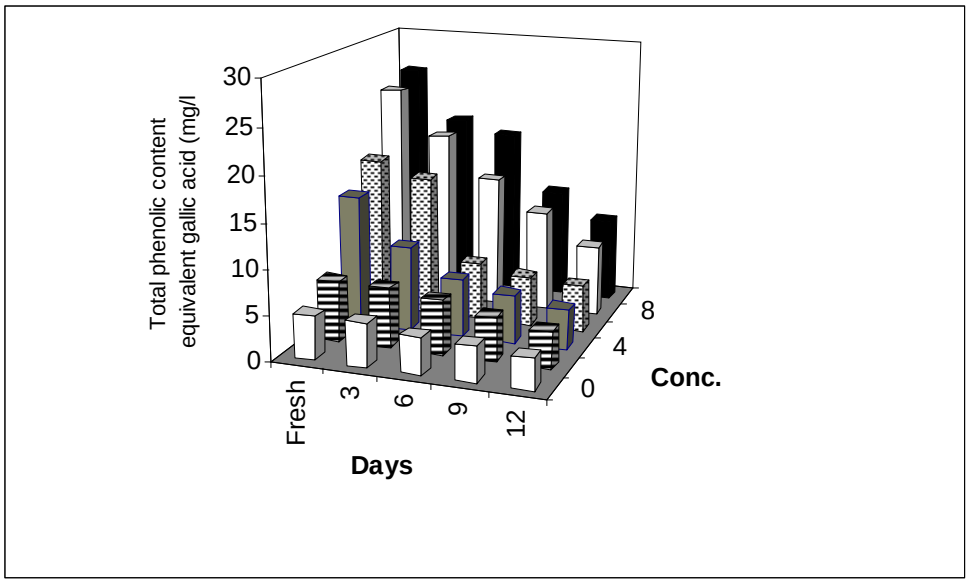


Table.2 Percentage rate of increase in total phenolic content equivalent gallic acid (mg/l) of yoghurt samples supplemented with pomegranate and storage in refrigerator at 6±2°C

Conc. of pomegranate	Storage periods (days)				
	Fresh	**3**	**6**	**9**	**12**
2	14.458	10.843	6.580	7.576	2.941
4	24.711	14.880	19.079	27.273	8.823
6	95.783	87.952	20.395	36.364	9.804
8	103.614	92.771	46.053	50.758	46.078

Table.3 Rate of increase in DPPH-radical scavenging activity (RSA) for yoghurt supplemented with pomegranate and storage in refrigerator at 6±2°C

Conc. of pomegranate	Storage periods (days)				
	Fresh	**3**	**6**	**9**	**12**
2	38.776	45.652	48.780	25.641	17.143
4	194.959	106.522	58.536	38.462	28.571
6	255.102	243.478	60.976	43.590	48.571
8	394.959	321.739	260.976	192.308	125.714

Table.4 The weight gain, food intake, liver weight and antioxidant enzyme (Glutathione & Glutathione peroxidase)

variables / Groups	Weight gain (g)	Food intake (g/day)	Liver weight (g)	GSH (mmol/g)	GPx (µ /mg)
C^-					
Range	(25.00 - 49.00)	(12.00- 14.00)	(5.00 - 7.60)	(25.00 – 31.00)	(250.00 -280.00)
Mean ±SD	32.80±9.39	12.80 ±0.84	6.18 ±0.93	28.33 ±3.05	265.00 ± 15.00
Mean ± SE	32.80 ± 4.20^{a}	12.80 ±0.37^{a}	6.18 ±0.41^{a}	28.33 ±1.76^{a}	265.00 ± 8.66^{a}
C^+					
Range	(25.00 – 28.00)	(7.00 – 9.00)	(5.40 - 6.70)	(19.00 -27.00)	(215.00 –240.00)
Mean ±SD	26.20 ±1.30	8.20 ±0.84	5.80 ±0.52	22.00 ±4.36	228.33 ±12.58
Mean ±SE	26.20 ±0.58^{b}	8.20 ±0.37^{c}	5.80 ±0.23^{a}	22.00 ±2.52^{a}	228.33 ±7.26^{b}
Y_0					
Range	(26.00 – 32.00)	(8.00 – 10.00)	(5.80 - 6.60)	(23.00 – 34.00)	(210.00 -260.00)
Mean ±SD	28.60 ±2.40	9.00 ± 0.71	6.14 ±0.31	28.00 ±5.59	236.66 ± 25.16
Mean ± SE	28.6 ±1.07^{ab}	9.00 ± 0.32^{bc}	6.14 ±0.14^{a}	28.00 ±2.21^{a}	236.33 ±14.53^{ab}
Y_4					
Range	(27.00 – 32.00)	(9.00 - 11.00)	(6.00 - 6.80)	(18.00 – 26.00)	(220.00 -270.00)
Mean ± SD	29.80 ±1.92	10.00 ±1.00	6.32 ±0.31	22.33 ±4.04	245.00 ±25.00
Mean ± SE	29.80 ±0.86^{ab}	10.00 ±0.45^{b}	6.32 ±0.14^{a}	22.33 ±2.33^{a}	245.00 ±14.43^{ab}
Y_8					
Range	(28.00 – 36.00)	(9.00 – 11.00)	(6.10 - 6.80)	(22.00- 29.00)	(235.00 -260.00)
Mean ±SD	31.80 ±3.03	10.00 ±1.00	6.50 ±0.29	25.66 ± 3.51	250.00 ±13.23
Mean ± SE	31.80 ±1.36^{ab}	10.00 ±0.13^{a}	6.50 ±0.13^{a}	25.66 ±2.02^{a}	250.00 ±7.64^{ab}

^{abc} Means in each column having the same letter were not significantly different.
Values with different superscript letters are significantly different at $p<0.05$.

Fig.2 DPPH- radical scavenging activity (RSA) for yoghurt supplemented with pomegranate and storage in refrigerator at 6±2°C

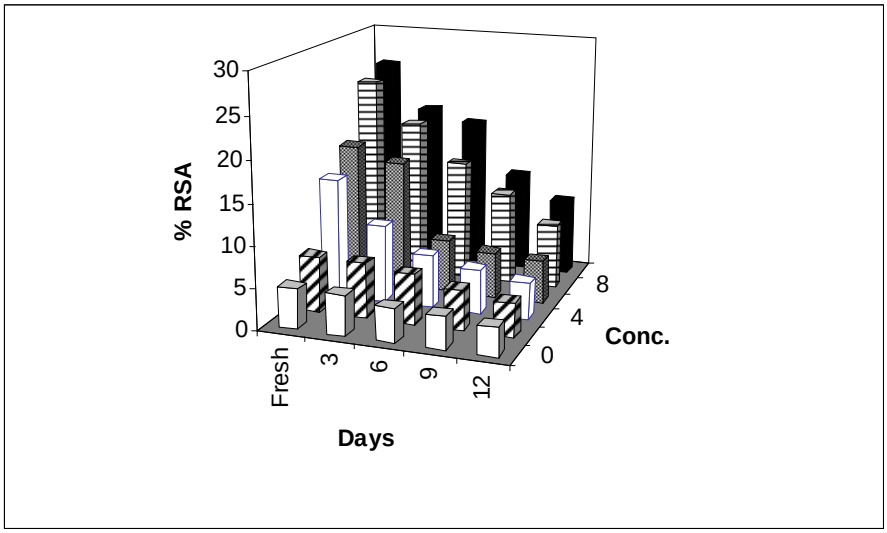
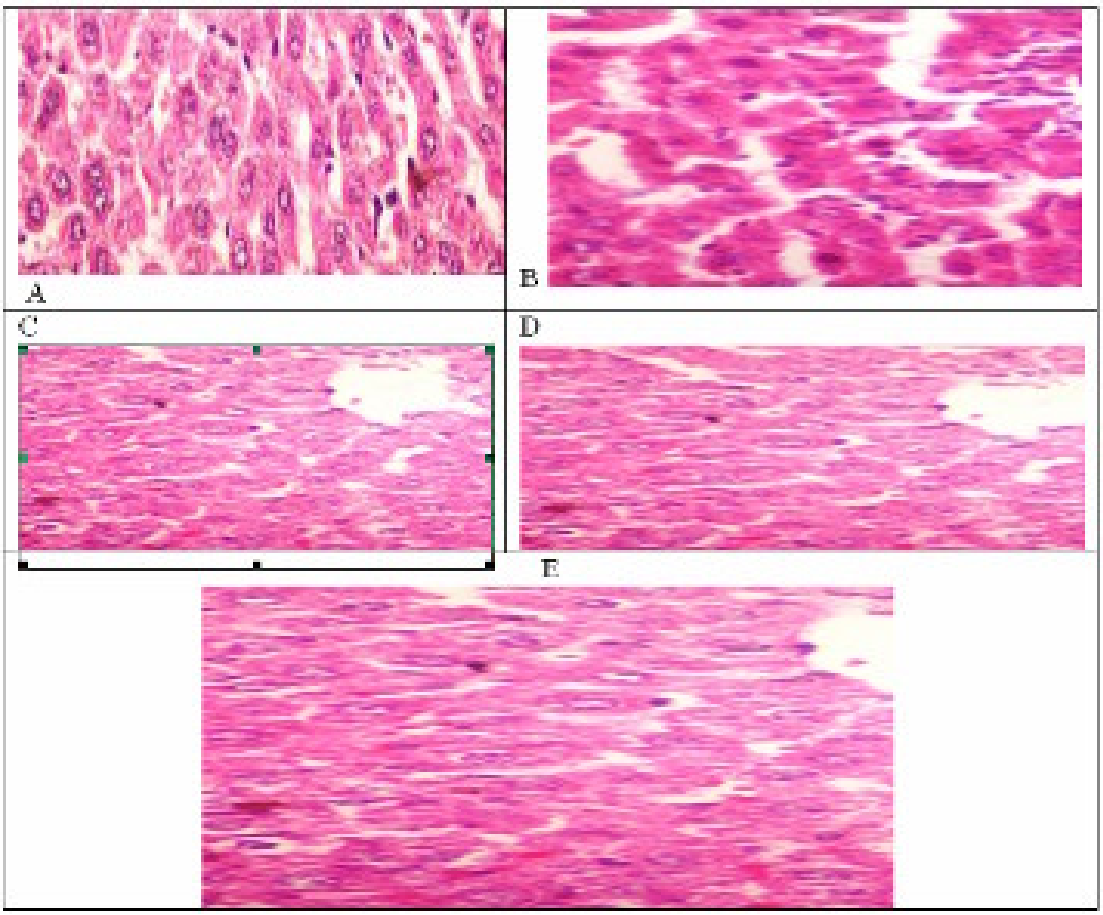


Table.5 Liver biomarkers (GOT, GPT, BILE total, BILE direct and Albumin)

Variables / Groups	AST (μ /ml)	ALT (μ /ml)	T.Bilirubin. (μ /ml)	D.Bilirubin (μ /ml)	Albumin (g/dl)
C^-					
Range	(16.00 – 21.00)	(14.00 – 21.00)	(0.25 – 0.54)	(0.01 – 0.10)	(3.50 - 3.80)
Mean ± SD	18.60 ± 2.52	17.33 ± 3.51	0.37 ± 0.11	0.05 ± 0.05	3.63 ± 0.15
Mean ± SE	18.60 ± 1.45[b]	17.33 ± 2.03[a]	0.37 ± 0.06[a]	0.05 ± 0.03[a]	3.63 ± 0.09[ab]
C^+					
Range	(21.00 – 29.00)	(19.00 – 23.00)	(0.42 – 0.53)	(0.01 – 0.18)	(3.40 – 3.50)
Mean ± SD	25.60 ± 4.16	21.00 ± 2.00	0.48 ± 0.05	0.09 ± 0.09	3.43 ± 0.05
Mean ± SE	25.60 ± 2.40[a]	21.000 ± 1.15[a]	0.48 ± 0.03[a]	0.09 ± 0.05[a]	3.43 ± 0.03[b]
Y_0					
Range	(23.00 – 27.00)	(17.00 – 23.00)	(0.30 – 0.60)	(0.01 – 0.15)	(3.60 – 3.90)
Mean ± SD	25.00 ± 2.00	20.33 ± 3.05	0.44 ± 0.15	0.06 ± 0.08	3.76 ± 0.15
Mean ± SE	25.00 ± 1.15[a]	20.33 ± 1.76[a]	0.44 ± 0.08[a]	0.06 ± 0.05[a]	3.76 ± 0.09[a]
Y_4					
Range	(19.00 -26.00)	(16.00 – 21.00)	(0.24 – 0.70)	(0.01 – 0.09)	(3.50 - 3.80)
Mean ± SD	23.00 ± 3.61	18.33 ± 2.52	0.43 ± 0.24	0.06 ± 0.04	3.63 ± 0.15
Mean ± SE	23.00 ± 2.08[ab]	18.33 ± 1.45[a]	0.43 ± 0.14[a]	0.06 ± 0.03[a]	3.63 ± 0.09[ab]
Y_8					
Range	(18.00 – 23.00)	(15.00 – 19.00)	(0.26 – 0.59)	(0.01 - 0.15)	(3.60 – 3.80)
Mean ± SD	20.60 ± 2.52	16.66 ± 2.08	0.39 ± 0.18	0.06 ± 0.08	3.70 ± 0.10
Mean ± SE	20.60 ± 1.45[ab]	16.66 ± 1.20[a]	0.39 ± 0.10[a]	0.06 ± 0.05[a]	3.70 ± 0.06[a]

[abc] Means in each column having the same letter were not significantly different.
Values with different superscript letters are significantly different at $p<0.05$.



However, the percentage of RSA gradually decreased during cold storage for all samples, this presumably due to the decrease stability of phenolic compounds and anthocyannin pigments during storage, this finding was agreement with Sagdic *et al* (2012); Mirsaeedghazi *et al* (2011).

Biochemical assessments

As expected, the subcutaneous injection of CC14 into rats is known to cause liver necrosis, alter various biomarkers such as increasing serum amino transferase activity and lipid peroxidation and depress the antioxidant system in the liver (Lee *et al* 2007). A similar action was noticed in group C^+. Table (4) showed that weight gain value in negative control (C^-) had a higher mean value (32.80± 4.2g) than other four groups, followed by Y_8 group (31.80± 1.36g). Food intake values as comparable with weight gain values, showed significant elevation in groups Y_4 and Y_8 (10±0.45 and 10.00 ± 0.13 g/day respectively) rather than Y_0 (9.00± 0.32 g/day). Also, it was noticed that liver weight was increased for groups Y4 and Y8 with mean ± SR 6.32± 0.14g and 6.5± 0.13g respectively, while it was 5.8± 0.23 for group C^+.

The antioxidant enzyme glutathione in C^+ group reduced to 22.33±2.33 mmol/g than C^- group (28.33± 1.76 mmol/g). Moreover GPX value was significantly increased in groups feed on yoghurt supplemented with pomegranate in comparison to C^+.

The effect of pomegranate on GSH level is due to its polyphenols, where, it is know to be able to modulate the transcription and expression of proteins related to the endogenous antioxidant defense by interacting with antioxidant response elements in genre promoter regions of genes encoding proteins related to oxidative injury management (Moskaug *et al* (2005); Myhrstad *et al* (2002).

Changes in liver biomarkers values in Table (5) demonstrated that injection with CC14, group C^+ increased blood AST, ALT, T. and D. Bilirubin and deceased Albumin, whereas groups Y_4 and Y_8 exhibited an ability to protect the hepatotoxicity by deceasing the level of AST, ALT and blood bilirubin as well as deceasing the blood albumin and this was parallel with the increase of pomegranate added to yoghurt in Y_4 and Y_8 group. Similar results were reported by Luangpirom *et al* (2013).

Histopathological studies

The microscopical examination on liver section of control rat (C^- group) showed a normal histological structure of hepatic lobule (Fig. 3, Pic. A), while, liver section of CC14-treated rats (C^+ group) revealed to the presence of focal hepatic necrosis associated with inflammatory cells infiltration (Fig. 3, Pic B). Administration with yoghurt only (Y0 group) for CC14-treated rates, the liver cells still had vacuolation of hepatocytes, congestion of hepatic sinusoids and degeneration of hepatocytes (Fig. 3, Pic. C). supplementing yoghurt with 4% pomegranate (Y4 group), live section (Fig. 3, Pic. D) Showed cytoplasmic vacuolization of hepatocytes, while rats feed on yoghurt supplemented with 8% pomegranate (Y8 group) showed no histopathological changes (Fig. 3, Pic. E).

This ascribed to presence of different bioactive compounds in pomegranate mainly polyphenols, falvonoids and anthocyannins which had antioxidant properties. Similar results were reported by Basu and Penugonda (2009); Amal *et al* (2012); Pirinccioglu *et al* (2012); Fatma *et al* (2013); Luangpiram *et al* (2013).

Consequently, supplementing yoghurt with 8% pomegranate juice had a slight effect on its textural properties, but had a high positive effects on its total phenolic content and antioxidant properties. Moreover, the biochemical and histopathological studies evidenced a remarkable improvement of the hepatic cells. This confirm the ability of pomegranate to protect afainis hepatotoxicity (liver damage) induced by chemicals.

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