Food Technology Research Journal, Vol. 1, issue 2, 77– 90, 2023

FTRJ

Health Benefits of Pomegranate (peel & juice) and preparation of functional Pomegranate drink using probiotic *Lactobacillus plantarum*

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Original Article

Article information
Received 22/1/2023
Revised 10/3/2023
Accepted 15/3/2023
Published 17/3/2023
Available online 19/3/2023

Keywords
bio waste, antidiabetic, hyperlipidemic

ABSTRACT

Pomegranate (*Punica granatum L.*) is rich in bioactive compounds, antioxidant, and volatile substances; under this investigates the impact of pomegranate peel (wastes), juice and preparation of probiotic juice by *Lactobacillus plantarum* on antimicrobial activity, antidiabetic *in vitro* and hypocholesterolemic agents by rats. Pomegranate peel extract has the highest total phenol, flavonoid and antioxidant more than juice .but no significance with probiotic juice. Antimicrobial compounds from pomegranate peel and probiotic juice are effective against a range of pathogenic microorganisms. The anti-amylase activity was significantly influenced by pomegranate tissues. The biological experiment used (n=36 rats up to 6 weeks) which were divided into six groups G1 (- ve control). G2 (positive control) by high-fat diet + 1% cholesterol, G3 (positive control+ 300 mg/kg/day/rat pomegranate peel extract), G4 (positive control+ 1 ml juice / day/rat), G5 (positive control+1 ml probiotic juice / day/rat), and G6 (positive control + reference drug 0.18 mg/kg/day/rat (ATOR 20 mg). Results, on the other hand, demonstrate the impact of peel extract, juice, and probiotic juice on lipid content (LDL, HDL, total cholesterol and triglycerides) and liver-related enzymes (ALT, ALP, AST). The pomegranate peel extracts and probiotic juice is more effective than juice due to the rich phenolic content and fermentation of juice by the probiotic strain *Lactobacillus plantarum*.

1. Introduction

The pomegranate, or Punica granatum L., is a very old fruit that has been consumed extensively throughout history by many different cultures. It is regarded as a super fruit with numerous health-improving qualities, including antimicrobial, antiallergic, antitumor, and antioxidant (Alkhatib et al., 2022). Pomegranate which contains several phytochemicals, each of which has more than one bioactivity, has various medicinal uses, such as being antibacterial, antiviral, and antifungal. It has been used since ancient times until now, according to Stefanou et al. (2020). Pomegranate is a fruit with a biowaste (peel) that has the ability to be transformed into added value for a wide range to products. Which use pomegranate rind has antioxidant and antibacterial effects which able to inhibit the main pathogenic microorganism (Ko et al., 2021). Elzoghbiy et al. (2022) and Xiang Luo et al. (2020) reported that Punica granatum L. cultivation dates back to the dawn of civilization, and since the turn of the 20th century, as the fruit's health advantages have been confirmed by science, production and consumption have expanded. Pomegranate fruit peels are one of the main byproducts that are highly concentrated in broad-spectrum antioxidants and antibacterial agents; they can even stop food from going bad. Pomegranate is useful for food preservation and possesses antibacterial properties, making it a complete resource for farmers, the food processing and storage industries. Probiotic bacteria have been used as food additives that improve the balance of intestinal microflora, however dairy products are now the best Frequent food carriers to reliable source

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of probiotics, an raising variety of non-dairy food substrates display the ability for transmission of probiotics,"according to studies on the functional food industry (Min et al., 2019).

Probiotic bacteria appear to be used in the fermentation of juice to produce beverages with high nutritional benefits and regarded organoleptic quality (Stavros et al., 2020). Due to their improved nutritional value, sensory qualities, improved storage life, and addition of derived components that promote health, fermented fruit beverages serve functional manufacturing needs (Hua et al ., 2021).

Amri et al. (2020) shows that leaves, rind, or juice extracts of pomegranate promote decrease insulin sensitivity and promote glycemic control, and limit carbohydrate and lipid absorption by lowering the activities of amylase and lipase. Andi Alfira et al. 2020) studied In Vitro that the peel powder of fruit Pomegranate had great effect on amylase inhibitor. Seba et al. (2021) assayed that the pomegranate, seeds ,peel, meso-carp and juice displayed higher inhibiting behavior on α-glucosidase and α-amylase, this is definitely connected to a lower IC_{50} value. The enzyme that causes the postprandial glucose level in diabetics to increase is due to the release of sugar molecules (oligosaccharides and monomers) from eating complex carbohydrates is alpha-amylase, which plays a role in regulating blood plasma glucose levels. The plan was implemented to study the chemical, microbiological, and biological activity of pomegranate (peel, juice, and probiotic juice).

2. Materials and Methods

Materials

Plant materials

One variety of pomegranates (*Punica granatum L.*) Manfaloty was received from the Agricultural Research Center, Giza, Egypt's Horticulture Research Institute, at their full maturity. The pomegranates were rinsed thoroughly with distilled water after being thoroughly cleaned under running tap water, and then left to dry at room temperature in the air for a few minutes.

Pomegranate peel powder preparation

Peels were chopped into bits. At a temperature of 45°C for 72 hours, the peel was dried in the tray dryer. The grinder was used to process dried pomegranate peels into smaller particles. Thereafter, a sieve with a mesh size of 50 is used to catch the ground particles. Then stored at ambient temperature (4±1°C) until use. (Devatkal and Naveena, 2010).

Pomegranate juice preparation

The pomegranate fruits and peeled off to create pure juice, the seeds were manually separated and crushed in a blender. The extraction step was put through the juicer once again to extract more juice then filtered through muslin cloth and kept at 4°C until use. 25 ml of a 50% (v/v) solution of water and ethanol were added to 5 g of juice. The peel powder was processed using the same method; the extracts were filtered and concentrated using a powerful evaporator (Eyela rotary vacuum evaporator N-11; Tokyo ridadidai Co., Ltd., Japan). (Gözlekçi et al., 2011).

Preparation of starter culture

Lactobacillus plantarum was starter culture which was cultivated 24h at 37°C in the MRS broth. 10 mL of the individually grown MRS broths at 4000 rpm for 10 min were centrifuged to get the biomass. To remove the remaining MRS culture, the resulting biomass was twice cleaned with a sterile saline solution. Initially, the inoculum starter were ready had around 4 $x10^7$ cfu/ml of cells. From Chr. Hansen, (Copenhagen, Denmark) probiotic strain of *L. plantarum* strain was obtained.

Preparation of probiotic pomegranate juice

Pomegranate juice extract 100 mL was pasteurized at 80 °C for 5 minutes, after cooling to 30 °C inoculated with 1 % *L. plantarum* under aseptic conditions and 24 hour incubation at 37 °C. Later kept at 4 °C in refrigerator until use after fermentation, no sugar or preservatives were used (Mamta et al., 2018).

Methods

1-Assessment of total phenolic concentration for pomegranate peel, pomegranate juice, and probiotic pomegranate juice extracts.

by Folin-Ciocalteu Colorimeter method, spectrophotometrically at 765nm. Gallic Acid Equivalents (mg GAE/g) were used to measure the total phenolic content (Mamta and Sharma, 2017).

2- Assessment of total Flavonoids content of pomegranate peel, pomegranate juice and probiotic pomegranate juice extracts. Total flavonoids content was measured spectrophotometrically at 510nm by the method of (Zhishen et al., 1999). Expressed as mg Quercetin Equivalent /g of dry weight sample on a calibration plot.

3- Assessment of antioxidant properties of pomegranate peel, pomegranate juice and probiotic pomegranate juice extracts. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to make the determination Brand (Williams et al., 1995).

4- Phenolic and flavonoid fractionation and identification using high-performance liquid chromatography (HPLC) the polyphenolic compounds determined by (Pinto et al., 2008) but the flavonoids described by (Pirjo Mattila et al., 2000).

5- Determination of vitamins A, E, D and K detected by Plozza et al., (2012). Ascrobic acid (Vitamin C) studied with (Romeu-Nadal et al., 2006).

6- Antimicrobial activity evaluation of pomegranate peel extracts, pomegranate juice and probiotic pomegranate juice. It was done using the well-agar diffusion method by (Perez et al., 1990).

The microorganisms used in this study included bacteria gram positive *Bacillus cereus* ATCC 33018 and *Staphylococcus aureus* ATCC 25923 and, bacteria gram negative *Pseudomonas aerugonisa* (ATCC 27853) and *Salmonella typhimurium* (ATCC 20231) and *Escherichia coli* (ATC C 25922). foodborne fungi such as *Aspergillus niger* (Rizk et al., 2009), *Fusarium verticillioides* (Sacc.) Nirenberg, and *Candida albicans* (CAIM-22) was acquired at Ain-Shams University in Cairo, Egypt, from MIRCEN (Microbiology Research Center). The bacterial and fungus cultures were diluted with sterile saline to nearly 1.0 x 10^5 CFU/mL to make the spore suspension.

7- Antidiabetic *in Vitro by* inhibition of α-Amylase Used the 3,5-dinitrosalicylic acid (DNSA) technique to conduct the experiment by (Wickramaratne *et al*., (2016).

8- Experimental Animals and diet

In these investigation 36 male albino rats of Wister strain weighing at 150 .00 ± 20 g was used. The rats were supplied from the National Research Center (NRC), Giza, Egypt. All experiment rats were done under the animal research ethics applied in the NRC up to 6 weeks (Abo-Taleb et al ., 2017). Hypocholesterolemic ATOR (Atorvastatin) 20mg was obtained from the (EIPICO) Egyptian International Pharmaceutical Industries Company (10^{th} of Ramadan city, Egypt) at a dose of 0.18 mg/Kg body weight /day (Guerin et al., 2000). Rats were given a standard diet and water (Reeves et al., 1993). Six groups of six rats each were created from the 36 rats. Group (1): Control rats (normal stander diet). Group (2): (positive control) High Fat Diet +1 % cholesterol.

Group (3): positive control treated with extract of pomegranate peel 300 mg /kg BW per day. Group (4): positive control treated with 1ml/day/rat pomegranate juice. Group (5): positive control treated with 1ml /day/rat from probiotic pomegranate juice. Group (6): positive control treated with ATOR standard drug. Daily checks on the rats' health revealed no detrimental developments during the period of the study. The dose for groups (3,4,5 and 6) take orally by stomach tube.

Biochemical Examination

The blood samples from overnight-fasted rats were centrifuged for 10 minutes at 4 °C at 3000 rpm to get clear serum, which was then frozen at -18°C. Assay kits obtain from (Bio diagnostic, 29 Tahreer st., Dokki, Giza, Egypt).

Serum analysis: detection of serum cholesterol by Richmond (1973), triglycerides by Fossati and Prencipe (1982), and LDL and HDL by Castelli and Levitar (1977). (Reitman and Frankel's, 1957) method is used for liver enzymes tests of (AST) Aspartate amino transferase and (ALT) Alanine amino transferase, Alkaline phosphatase (ALP) by Belfield and Goldberg, (1971).

Histopathological examination

The experiment's effects on the liver and heart were assessed. At Cairo University's The Pathology Department at the University of Veterinary Medicine in Giza, Egypt prepared livers for histological analysis. Sections of liver samples that were placed in paraffin and were 3- to 5-mm thick were used. Hematoxylin-eosin (H&E, 400X) was stained the sections for light microscopy analysis (Hirsch et al., 1997).

Statistical analysis

Using Minitab Statistical Software version 15, one analysis of Variance **(**ANOVA) was performed on the data (Minitab Inc, State College, USA). The information presented in each table as mean ± SD. (AboAllam, 2003).

Table 1. Formulation composition of stander basal and High Fat diets (g/100g diet)

Components	Stander basal diet (negative control)	Positive control (High fat diet + 1 % cholesterol)
Casein(90% protein)	18	18
Starch	62	47.62
Corn oil	10	8
Vitamin mixture(AIN-93-VX)	1	1
Mineral mixturex (AIN-93-MX)	4	4
Cellulous	5	5
Beef fat	____	15
Cholesterol	____	1
Choline bitartrate	____	0.20
Cholic acid	____	0.18

According to Terpstra et al., (2002)

3. Results and Discussion

Pomegranate peel, seeds, and arils are three different anatomical origins that can be recognized. Pomegranate juice, which can be made from the fruit's whole pomegranate or its arils (seed + juice). Results in Table 2. showed that about 42.63±3.61% of the fruit weight in regard to peel 57.37±3.61 % arils, 16.71 ± 0.78 % seeds and 40.66 ± 3.58 % Juice. (Karimi et al., 2017) reported that when compared to the edible portion of the fruit, which was made up of 10% seeds and 40% arils, the peel, which form 50% of the fruit's non-edible portion, is distinguished by its antioxidant ability. The total phenolic concentration (mg Gallic acid/gm), total flavonoid contents (mg Quercetin /gm) and Antioxidants activity.

Table 2. Percentage parts content of pomegranate fruit

Parts of pomegranate fruit	Percentage content (%)
Peel	42.63±3.61
Arils (seed +juice)	57.37±3.62
Seeds	16.71±0.78
Juice	40.66±3.58

In Table 3. the total phenolic content of pomegranate peel was great than pomegranate juice and probiotic pomegranate juice, according to the data. The most significant results obtained in the extracts of pomegranate peel, probiotic juice and juice which contained 665.64±1.02, 345.30±0.414 and 219.42±1.13 mg GAE/g respectively. The pomegranate peel has a high phenolic content because it is full of ellagitannins, which are phenolic chemicals. Ellagitannins' polar nature prompted their extraction in ethanol, methanol, and water, two polar solvents (Hasan et al., 2018). As evidenced in Table 3. that the concentration of total flavonoid content the best amount of flavonoids were present in extracts of pomegranate peel, probiotic juice and juice. the results agree with Kennas,(2019) which showed The greatest amounts of flavonoids 53.85±1.95, 52.68±1.97 µg QE/mg respectively, in ethanolic and combination water/methanol (50:50) extracts, were little different from one another (P>0.05). Also detected that the total phenolic content in pomegranate peel extract ranged from 242.05±7.99 to 638.17±10.59 mg GAE/g but flavonoid content of pomegranate peel extracts varied 63.85±1.9 (µg QE/mg of dry extract).In comparison to other extracts, the pomegranate peel extracts demonstrated stronger antioxidant effects. As a result of fermentation, the radical scavenging rate increased (up to 40%), the highest antioxidant rate of probiotic pomegranate juice more than and unfermented pomegranate juice (Pontonio et al., 2019). Because of the effect of sunlight and variances in local production areas on the formation of phenolic compounds and antioxidant properties. Elfalleh et al., (2012). The results are in coincide with the Alyaa et al., (2020) who reported that the order of Phenolic compound , flavonoid and antioxidants contents were as follows pomegranate peel extract > probiotic pomegranate juice > more than its juice . Gözlekçi et al., (2011) reported that the greatest amount of total phenolic concentration were detected in extract of pomegranate peel more than its juice .

Abdel-salam et al., (2018) studied that probiotic juice of pomegranate was great in total phenolic concentration, antioxidant rate more than control juice. Also in Table (3) Pomegranate peel had the highest vitamin C content (37.025± 0.02mg/100g), while the pomegranate juice (11.044± 0.001mg/100g) but the probiotic pomegranate juice 14.021± 0.005mg/100g. Vahid Akbarpour et al., (2009) found that the pomegranate juices vitamin C content also ranged between 9.68-17.45 mg/100 ml. the Vitamin A of Manfaloty peel powder is 0.593 mg/100g. Pomegranate juice had the highest vitamin K (18.568 mg/100g). (Pranav et al., 2017) who detected that peel of pomegranate contained (14.06 ± 0.08 µ g/gm) vitamin A.

Table 3. The total phenolic, total flavonoid, Antioxidants activity and Vitamines content for pomegranate peel, pomegranate juice and probiotic pomegranate juice.

Samples	pomegranate peel	pomegranate juice	probiotic pomegranate juice
Total phenol (mgGE/g)	665.64±1.02	219.42±0.133	345.30±0.414
Total flavonoid (mg QE /g)	66.20±0.451	31.2±0.442	48.2±0.432
Antioxidant activity %	93.82±0.202	51.16±0.161	60.44±0.102
	Vitamines		
Vit. C (L-scarobic acid) mg/100g	37.025± 0.062	11.044± 0.001	14.021± 0.005
Vit. A (Retinol) mg/100g	0.593± 0.001	0.377± 0.003	0.533± 0.004
Vit. E (α-Tochoferol) mg/100g	0.012± 0.001	0.017± 0.007	0.076± 0.00
Vit.D (Ergocalciferol) mg/100g	0.180± 0.002	0.259± 0.035	0.399± 0.003
Vit.K (Phylloquinone) mg/100g	3.032± 0.002	18.568± 0.007	20.068± 0.004

Data represent mean ± SD, are not significant difference at $P < 0.05$.

Table 4. presents that composition of polyphenolic compounds of pomegranate peel powder, probiotic pomegranate juice and juice which were fractionated into 19 different components, respectively by HPLC.

Table 4. Composition of polyphenolic and flavonoid concentration of pomegranate peel powder, pomegranate juice and probiotic pomegranate juice by HPLC

Phenolic compounds	Pomegranate peel (µg/g)	pomegranate juice (µg/g)	probiotic pomegranate juice (µg/g)
Gallic acid	22.2	3.5	4.8
Pyrogallol	1609.2	144.2	166.1
4-Amino-benzoic acid	18.4	2.2	4.2
Protocatchuic acid	282.3	76.2	91
Catechein	1766.1	530.1	607
Chlorogenic acid	413.3	43.5	56.4
Catechol	9.3	1.4	2.2
Caffeine	113.1	23.3	38.3
P-OH- benzoic acid	165.02	56.1	71
Caffeic acid	1.3	0.002	0.01
Vanillic acid	97	2.6	4.7
Ferulic acid	38	8.1	10.04
Iso-Ferulic acid	2.1	0.42	0.58
Ellagic acid	2039	43.3	60.2
Benzoic acid	223	20.1	33.6
Alpha-Coumaric acid	4.2	0.3	0.51
3,4,5-methoxy-cinnamic acid	30.1	3.6	4.9
Coumarin acid	24.6	nd	nd
Salicylic acid	28.5	0.008	0.02
Flavonoid compounds mg/100g			
Apig.6-rhamnose8-glucose	20.412	2.063	3.145
Naringin	26.259	2.074	3.011
Rutin	37.654	2.659	4.234
Quercetin-3-o-glucoside	4.738	0.385	0.512
Apig.7-o-neohespiroside	69.995	3.362	5.427
Quercetin	60.674	1.456	2.321
Rhamentin	4.150	0.580	0.644
Apigenin	1.874	0.085	0.011
Kampferol	1.213	0.136	0.266
Naringenin	2.025	0.116	0.209

The percentage composition of polyphenolic compounds for pomegranate peel powder, that Ellagic acid had the highest composition for peel powder (2039 µg/g), while caffeic acid for Manfaluty peel powder showed the lowest value (1.3 µg/g). (Seba et al., 2021) showed that ellagic acid content and catechin are the greatest amount in phenolic compounds, ellagic acid was major phenolic in Pomegranate peel (1034.7 µg/g), and Pomegranate juice (26.5 µg/g). Also in Table 4., quantitative

and qualitative analysis of flavonoids by HPLC were fractionated into 10 different components, Hespirtin, Luteo.7-glucose and Hesperidine were not found.

Antimicrobial activity of pomegranate peel, probiotic pomegranate juice and juice

Pomegranate has an antimicrobial effects on different foodborne pathogens, which has an inhibit effect towards bacteria gram negative, gram-positive, fungi and yeast. The data of antimicrobial testing are showed in the Table 5. pomegranate showed the good results against different microbes ,the extracts of pomegranate peel powder has maximum inhibition zone than pomegranate juice, the phenolic compounds were more stable in solvent extracts than water extract. Similarly to Parimala et al., (2018) shown that the extracts obtained from the solvent extracts ethanol, methanol, acetone higher than water extract. The extract of pomegranate peel produced greatest inhibition zones against *Staphylococcus aureus* 34 mm but the inhibition zone formed with, *Fusarium verticillioides , Escherichia coli, , Candida albicans, Bacillus cereus* , *Salmonella typhimurium*, *Pseudomonas aerugonisa* and *Aspergillus niger* are 31, 30, 29, 28, 27, 25 and 24mm, respectively.

As shown in Table 5. the probiotic pomegranate juice was higher than juice in the diameter of inhibition zone. The result agrees with Abdel-Aziz *et al .,* (2021) studied that peel extracts of pomegranate exhibit antibacterial inhibition against the tested *E. coli* isolate, with the aqueous extract of pomegranate peel having a greater impact more than methanol extract on the tested *E. coli* strain.

Dimitra et al., (2015) demonstrated that the ellagitannins and flavonoids found in pomegranate juices and fruit peels contributed to the substantial antibacterial activity of these matrices. Pomegranate juices showed considerable antibacterial inhibition and antifungal activity, which could increase their shelf time and stop deterioration brought on by the enzymatic activity of the microorganisms. Stefanou et al ., (2020) demonstrated that the juice, extracts, and isolated chemicals from pomegranates are effective against a variety of infections, with results that are frequently better than those of conventional antibiotics.

The data Similarly to Saad et al., (2010) and Priyanka Kesur et al., (2016) reported that comparing peel extract to other juice and seed extracts, peel extract has the perfect antibacterial activity. The strongest antibacterial activity toward *Staphylococcus aureus* was observed among the chosen bacterial and fungal cultures, and high antifungal activity toword *Aspergillus niger*.

Akhtar et al., (2015) Based on their ability to precipitate cell membrane proteins with bacterial which causes lysis of bacterial, it has been hypothesised that polyphenolic compounds play an important part in the inhibition of microorganisms. These compounds may also stop microbial enzymes by acting with proteins by nonspecific interactions or by precipitating with sulfhydryl groups.

It has been demonstrated that pomegranate juice and peel have an unusually wide range of powerful antimicrobial and antiparasitic action (Celiksoy et al., 2021). The reduced pH and higher amount of organic acids in the fermented fruit juices were primarily responsible for the increased antimicrobial activity (Hao et al., 2020).

In Vitro Antidiabetic Activity

By releasing oligosaccharides and monomeric molecules from dietary complex carbs, the alpha-amylase enzyme increases postprandial glucose levels in diabetics. Preventing the first enzyme needed for starch breakdown, α -amylase and can slow down the rate at which blood glucose is released when a person consumes carbohydrates (Ponnusamy, et al ., 2011).

In Table 6. Antidiabetic activity of pomegranate peel, juice and probiotic fermented juice was described by 3,5- dinitrosalicylic acid assay. From the results, the pomegranate peel have the highest of α-amylase inhibition (97.8±0.002%) compared to all the others, which is associated with lower IC_{50} value. (Seba et al., 2021) reported that the *Punica granatum L.* peel has great carbohydras inhibitory activity more than juice.

Anthocyanin pigment, phenolic concentration found in the food chain from different fruits and vegetables and fermentation have been stopped and block digestive enzymes (Mojica et al ., 2015).

Probiotic pomegranate juice is more effective and inhibition more than juice in comparison to the fresh juice, fermentation with various LAB strains of mango juice results in considerably higher inhibitory activities of α amylase ($p \leq .05$), (Florence et al., 2021). Fermentation have been stopped and block digestive enzymes (Mojica et al ., 2015).

Table 5. shows the antimicrobial activity of extracts of pomegranate peel extract, pomegranate juice and probiotic pomegranate juice.

Type of Extract	Antibacterial activity (diameter of inhibition zone mm)					Antifungal activity		
	Gram positive		Gram negative			fungi		yeast
	Staphylo-coccus aure-us	*Bacillus cereus*	*Pseudomo-nas aeru-gonisa*	*Salmonella typhimurium*	*Escherichia coli*	*Aspergillus niger*	*Fusarium verticil-lioides*	*Candida albicans*
Peel extract	34	28	25	27	30	24	31	29
Pomegranate juice	26	18	20	19	20	17	22	18
Probiotic pomegranate Juice	28	20	21	21	22	18	23	20

Table 6. Inhibition of α-amylase and IC_{50} values in pomegranate peel, pomegranate juice and probiotic pomegranate juice.

Samples (Conc. µg/ml)	Amylase Inhibition %		
	Pomegranate peel	pomegranate juice	Probiotic pomegranate juice
1000	97.8^{a}±0.002	64.3^{c}±0.003	77.9^{b}±0.009
800	90.4^{a}±0.002	55.4^{c}±0.002	68.3^{b}±0.008
600	80.6^{a}±.0017	47.2^{c}±0.003	59.9^{b}±0.003
400	71.3^{a}±0.006	39.6^{c}±0.003	53.2^{b}±0.004
200	58.5^{a}±0.011	31.6^{c}±0.004	48.8^{b}±0.007
100	44.2^{a}±0.011	22.0^{c}±0.009	39.8^{b}±0.003
80	35.4^{a}±0.005	13.7^{c}±0.005	31.7^{b}±0.008
40	27.9^{a}±0.009	5.4^{c}±0.004	22.4^{b}±0.017
20	18.7^{a}±0.006	3.0^{c}±0.002	12.5^{b}±0.008
10	10.6^{a}±0.002	1.1^{c}±0.008	4.5^{b}±0.001
Control 100%	0.0±0.037	0.0±0.037	0.0±0.037
IC_{50}	34.43	84.8	40.04

Results represent mean ± SD, the Identical letters in each Colum are not significant at $P < 0.05$.

Effect of pomegranate peel extract, pomegranate juice and probiotic juice on lipid profile and liver enzymes in hypercholesterolemic rats induced by HFD

Table 7. showed that the comparison effect between the pomegranate peel extract, pomegranate juice and probiotic pomegranate juice on high fat diet rats and negative group (basal diet), significant difference for all results. The group of rats takes 300mg of pomegranate peel extract was better

than other and its data near the reference drug (ATOR) which increases total cholesterol, LDL and triglyceride but raising the rate of HDL. The data agree with (Abo-Taleb, *et al*., 2017) which study the improvement action of juice and peel pomegranate extract on the total cholesterol, LDL, HDL and triglycerides in as hypercholesterolemic. Also (Lotfy and Alamri, 2019) reported that consumption of pomegranate peel which considered a high fiber source effect on anti-oxidative activities. It is used for weight loss and to improve the lipids profile. Also natural agent against obesity and high lipid levels. In Table 7. significant increase of ALT, AST and ALP values in diet high with cholesterol and fat in comparison to negative control rats while significant reduction was detected in the several parameters after 6 weeks of treatment with pomegranate peel extract, probiotic juice, pomegranate juice and reference drug post induction with high fat diet in comparison to high fat diet (positive control group). These outcomes are consistent with earlier research that discovered rats fed pomegranate peel extract up to six weeks had significantly lower value of the ALT and AST enzymes. Mansour et al., (2020) showed that significantly improves the liver markers after receiving gradient doses of. Pomegranate peel and juice also showed a substantial reduction in AST and ALT in rats. Alireza et al ., (2014) obtained that rats fed a high-lipid diet, peel extract of the pomegranate dramatically lower serum levels of lipid composition profile and hepatic enzyme, while raising serum HDL-C in comparison to stander control.

Table 7. Activity of peel extract, juice and probiotic juice of pomegranate on lipid composition profile and liver enzymes of hypercholesterolemic rats.

Parameter	Negative control	Positive (HFD) control	(HFD) + 300 mg Peel extract	(HFD) +1ml juice	(HFD) + 1ml Probiotic juice	HFD + ATOR
Total cholesterol (mg/dl)	$92.10^{e} \pm 0.33$	$157.20^{a} \pm 1.04$	$95.70^{d} \pm 0.32$	$100.80^{b} \pm 0.45$	$97.83^{c} \pm 0.05$	$96.07^{d} \pm 1.08$
LDL(mg/dl)	$29.83^{f} \pm 0.65$	$107.10^{a} \pm 0.09$	$36.07^{d} \pm 0.55$	$50.07^{b} \pm 0.26$	$41.03^{c} \pm 0.35$	$34.60^{e} \pm 1.10$
HDL(mg/dl)	$53.33^{a} \pm 0.23$	$31.05^{f} \pm 1.00$	$50.71^{c} \pm 0.65$	$43.77^{e} \pm 0.02$	$48.74^{d} \pm 0.22$	$51.5^{b} \pm 0.51$
Triglycerides (mg/dl)	$46.06^{f} \pm 0.24$	$93.11^{a} \pm 0.76$	$48.06^{d} \pm 0.50$	$55.07^{b} \pm 0.97$	$49.05^{c} \pm 0.51$	$47.05^{e} \pm 0.01$
liver function enzymes						
ALT (U/l)	$50.06^{f} \pm 0.82$	$90.23^{a} \pm 0.34$	$51.21^{e} \pm 0.65$	$59.06^{b} \pm 0.82$	$55.06^{c} \pm 0.13$	$52.36^{d} \pm 0.12$
AST (U/l)	$43.30^{f} \pm 0.57$	$70.01^{a} \pm 0.20$	$55.65^{e} \pm 0.22$	$60.65^{b} \pm 0.22$	$57.65^{c} \pm 0.22$	$56.65^{d} \pm 0.65$
ALP (U/l)	$70.90^{f} \pm 4.00$	$136.84^{a} \pm 1.10$	$85.08^{e} \pm 0.42$	$95.33^{b} \pm 1.06$	$90.20^{c} \pm 1.29$	$88.14^{d} \pm 0.15$

Data represent mean ± SD, the identify letters in each column are not significant at $P < 0.05$.

Histological Assessment

In Figure 1. no histopathological modification in the negative control rats due to the parenchyma's major vein and bordering hepatocytes had their usual histological structure, whereas the blood vessels of revealed . But in positive control high-fat diet-fed rats showed in Figure 2. addition to substantial dilatation and congestion in the portal vein, the histology of blood vessels in showed many intracytoplasmic micro fat vacuoles in the hepatocytes as a fatty change. After being treated with pomegranate peel extract, high-fat diet rats revealed normal liver lobule histology with low inflammatory cell infiltration in the blood vessels in Figure 3.

In both Figures 4 and 5 treated of the portal region which had fibrosis and dilatation in the portal vein by pomegranate juice 1ml/day/rat and 1ml /day/rat from probiotic pomegranate juice .

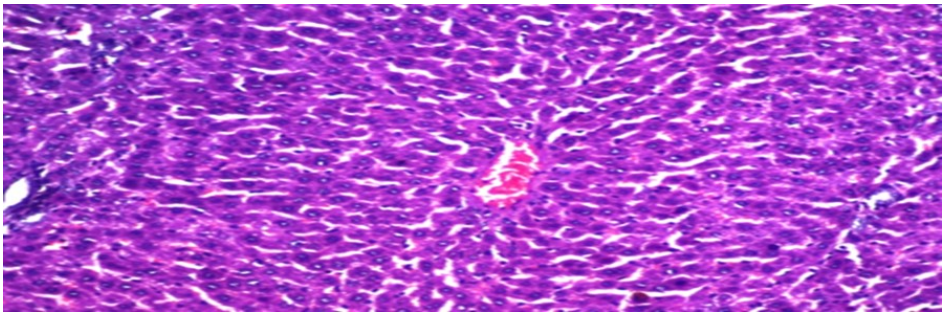

Figure 1. The negative control rats revealed no histopathological modification (H & E X 400) .

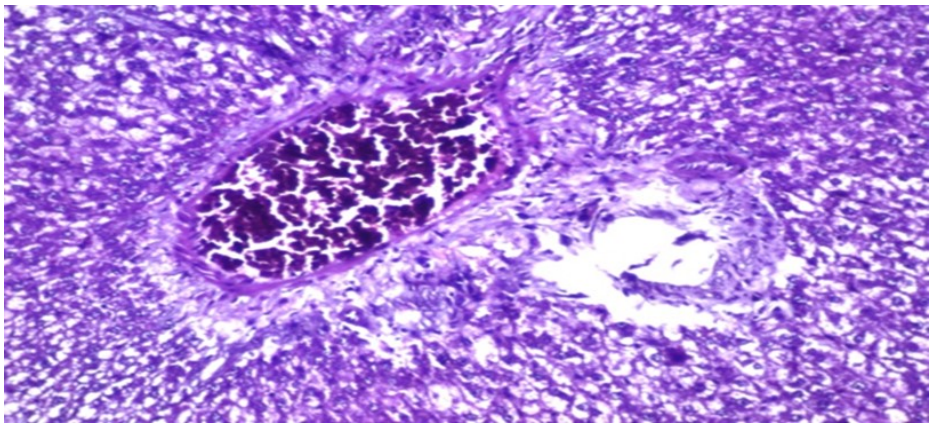

Figure 2. Positive control (high-fat diet-fed rats) hepatic lobule (H & E X 400)

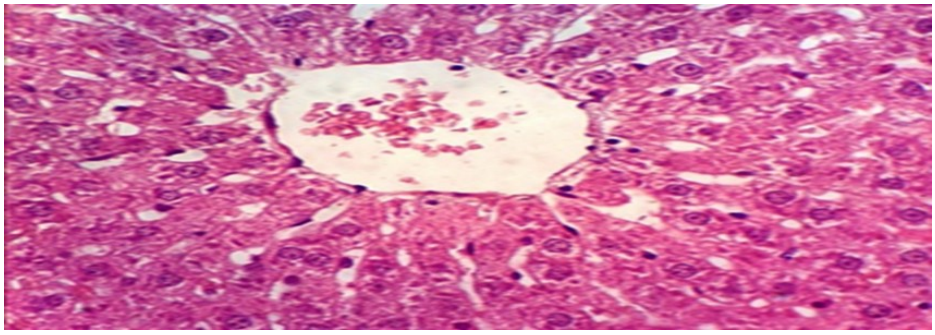

Figure 3. After being treated with pomegranate peel extract300 mg Peel extract

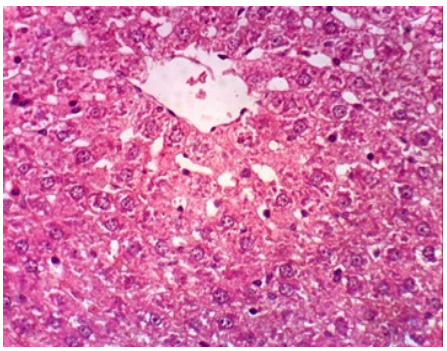

Figure 4. Liver of rats from positive control+1ml /day /rat pomegranate juice

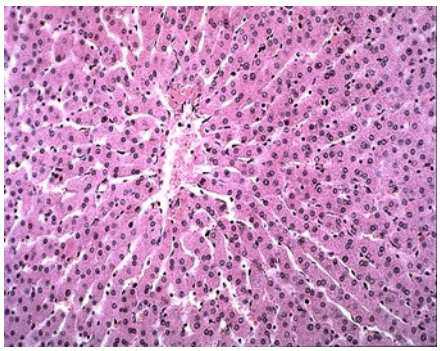

Figure 5. Liver of rats from positive control+1ml /day /rat probiotic pomegranate juice.

In the Figure 6. showed normal histological structure of the hepatic lobule and normal histological structure of the central vein when treated with the reference medication ATOR in high lipid diet. The results are in agreement with Alireza et al. (2014) which reported that the pomegranate peel extract formed treatment and reduced hepatic damage in rats fed a high-lipid diet in comparison to a negative control. Our results are in agreement also with the results of Abo-Taleb et al. (2017) and Binmowyna et al. (2021).

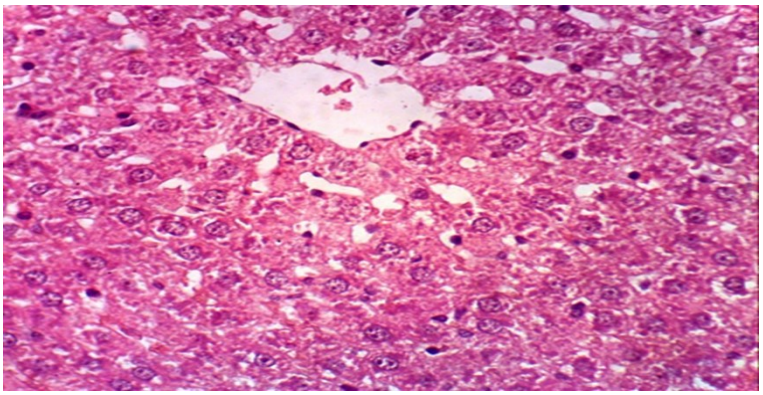

Figure 6. showed normal histological structure when treated with the reference medication ATOR in high lipid diet.

4. Conclusion

Pomegranate bio waste, pomegranate juice and probiotic juice are a good source of bioactive compound and antioxidant activity, also have strong antimicrobial activity. The benefits of drinking non-dairy fermented beverages for health are confirmed by probiotic juice, which vegetarians and those with high cholesterol can also use., the Pomegranate is a potential treatment alternative as an anti-hyperlipidemic drug, but more clinical research is required to validate this.

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