Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Biochemistry

ISSN 1815-9923
DOI: 10.3923/ajb.2017.79.84

Research Article
Pomegranate (*Punica granatum* L.) Juice Improves Liver Damage in Carbon Tetrachloride-induced Rats

[1]Prasetyastuti, [1]Adhi Tanjung Laksono, [1]Ricky Taufiqurrohman, [1]Tantra Dewi Rahardyanti, [1]Gandhi Anandika Febryanto, [1]Yan Mahmud Fau, [2]Sri Herwiyanti, [1]Ngadikun and [1]Sunarti

[1]Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Histology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

Background and Objective: Carbon tetrachloride (CCl_4), a carcinogenic compound commonly used in household cleaning agents and spot removers can cause free radical-mediated liver damage. Recent research shows polyphenols in pomegranate (*Punica granatum* L.), rich in exogenous antioxidants, may protect the liver from hepatotoxic damage. This study aimed to investigate the effect of pomegranate juice on CCl_4-induced liver damage in a rat model. **Materials and Methods:** A total of 25 rats, aged 8 weeks and weighing an average of 200 g were used. They were divided into 5 groups as follows: (1) Normal, (2) Hepatotoxic rats and (3), Hepatotoxic rats with pomegranate juice 0.4 mL/200 g body weight (BW) (4) Hepatotoxic rats with pomegranate juice 0.8 mL/200 g BW and (5) Hepatotoxic rats with pomegranate juice 1.2 mL/200 g BW, respectively. Hepatotoxicity was done by intraperitoneal injection of 0.5 mL^{-1} CCl_4 kg^{-1} BW for 5 days. Before and after 2 weeks administration of treatment, blood samples were collected for assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT) and malondialdehyde (MDA). At the end of the study, the animals were euthanized and the livers were used for histopathological assessment. Data were analyzed by paired t-test and one-way ANOVA followed by the Games-Howell test. **Results:** Supplementation with three different doses of pomegranate juices in CCl_4-induced rats significantly reduced the serum levels of ALT, AST, ALP, γ-GT and MDA ($p<0.05$) and pomegranate juice as much as 0.8 and 1.2 mL/200 g BW reduced liver damage in CCl_4-induced rats. **Conclusion:** According to findings of this study supplementation with pomegranate juice can improve liver damage induced by CCl_4 in rats.

Key words: Pomegranate, carbon tetrachloride, hepatotoxicity, malondialdehyde, hepatocyte enzyme

Citation: Prasetyastuti, Adhi Tanjung Laksono, Ricky Taufiqurrohman, Tantra Dewi Rahardyanti, Gandhi Anandika Febryanto, Yan Mahmud Fau, Sri Herwiyanti, Ngadikun and Sunarti, 2017. Pomegranate (*Punica granatum* L.) juice improves liver damage in carbon tetrachloride-induced rats. Asian J. Biochem., 12: 79-84.

Corresponding Author: Prasetyastuti, Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
Tel: (+62)81227882379

Copyright: © 2017 Prasetyastuti *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Most chemicals induce free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues[1]. Carbon tetrachloride (CCl_4), a common household compound is a carcinogenic agent that causes hepatic injury. Biotransformation of CCl_4 by hepatic microsomal cytochrome P-450 produces toxic metabolites[2]. According to Adewale *et al.*[2] the toxic effect of various chemicals can be prevented by antioxidants. Some studies showed that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation[3], suppressing alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities[4] and increasing antioxidant enzyme activity[5].

Phenolic compounds in vegetables and fruits have antioxidant properties and are important to investigate because these compounds have strong antioxidant activity but low toxicity compared to the synthetic phenolic antioxidants, such as butylated hydroxytoluene[6]. One of the most powerful antioxidant-containing fruits is pomegranate which contains flavonoids, anthocyanins and ellagitanin[7]. These substances have been known to protect pancreatic β-cells from damage due to free radicals[8]. Flavonoids are powerful antioxidants with free radical scavenging properties and can reduce free radicals formation[9]. In comparison, antioxidant activity of pomegranate juices is 3 times higher than red wine and green tea infusion[10]. According to Anoosh *et al.*[11] pomegranate juice has anti-atherogenic action in hypercholesterolemia patients. Considering these benefits, this study evaluated the effect of pomegranate juice on carbon tetrachloride-induced liver damage in rat.

MATERIALS AND METHODS

This study was conducted at Universitas Gadjah Mada from June, 2016 until January, 2017. Pomegranate (*Punica granatum* L.) fruit was purchased from the local market in Jakarta, Indonesia. Twenty-five male test rats (*Rattus norvegicus*), 8 weeks old, weighing an average of 200 g were obtained from the Food and Nutrition Department/PAU (*Pangan dan Gizi*), Universitas Gadjah Mada, Yogyakarta, Indonesia.

Reagents: Assay kits for measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) were purchased from Dyasis® (Holzheim, Germany) and diagnostics for gamma-glutamyl transferase (γ-GT) from Human Gesellschaft for Biochemica Und Diagnostica mbH (Wiesbaden, Germany), while malondialdehyde (MDA) level was measured using TBAR methods. All other chemicals were of analytical grade.

Experimental procedure: The rats were housed in individual cages and acclimatized to the laboratory condition (22-25°C room temperature and 12 h daylight cycle) for 7 days with free access to food and water during the experimental period. The standard diet was AIN 93 M consisting of (g kg^{-1} mix): Cornstarch (465.692), casein (140), dextrinized cornstarch (155), sucrose (100), soybean oil (40), alphacel (50), AIN-93-M-MX (35), L-cysteine 1.8, AIN-93-VM (10), choline bitartrate (2.5) and tert-butylhydroquinone (0.008). The present study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada. Twenty-five rats were divided into 5 Groups: (1) Normal, (2) Hepatotoxic rats, (3) Hepatotoxic rats with pomegranate juice 0.4 mL/200 g BW, (4) Hepatotoxic rats with pomegranate juice 0.8 mL/200 g BW and (5) Hepatotoxic rats with pomegranate juice 1.2 mL/200 g BW. Hepatotoxicity of rats was done by intraperitoneal injection of CCl_4 at dose of 0.5 mL kg^{-1} BW for 5 days according to the method described by Hewawasam *et al.*[12]. The pomegranate juice was given orally to groups 3, 4 and 5 for 2 weeks. Before and after treatment, fasting blood samples were collected from the retro orbitalis plexus for analysis of ALT, AST, ALP, γ-GT and MDA. Histopathological assessment of liver damage was done by haematoxylin and eosin (HE) staining.

Data analysis: All results were expressed as Mean±SE. Differences in blood ALT, AST, ALP, γ-GT and MDA were compared by one-way ANOVA followed by the Games-Howell test. Paired t-test was used to analyze the level of ALT, AST, ALP, γ-GT and MDA before and after treatment. The statistical analysis was performed using the SPSS program (Version 18; SPSS Inc., USA) with significance considered at $p<0.05$.

RESULTS

Administration of pomegranate juice significantly decreased serum MDA and ALT levels. The greatest decline in serum MDA and ALT levels were seen in the group with 1.2 mL/200 g BW of pomegranate juice. Compared to the hepatotoxic and normal groups, the effects of the administration of 0.4, 0.8 and 1.2 mL/200 g BW pomegranate juice were significantly different (Table 1 and 2). After 2 weeks administration of pomegranate juice serum ALP levels were significantly $p<0.05$ decreased. The greatest decline was seen

Table 1: Serum malondialdehyde (MDA) levels in rats

Groups	Before	After	p-value	Mean difference
I	1.53±0.13[a]	1.63±0.48[a]	0.003	-0.1(-0.15;-0.05)
II	14.48±0.25[b]	14.85±0.29[b]	0.023	-0.36 (-0.65;0.07)
III	15.05±0.48[b]	12.65±0.44[c]	<0.001	2.4 (1,9;2,9)
IV	14.77±0.49[b]	8.54±0.44[d]	<0.001	6.23 (5.64;6.82)
V	14.29±0.25[b]	6.06±0.26[e]	<0.001	8.24 (7.89;8.58)
p-value	<0.001	<0.001		

Values are presented as Mean±SE (n = 5). p-value in column indicates p<0.05 according to one-way ANOVA test. [a,b,c,d,e]indicated significant difference between groups according to one-way ANOVA test followed by the Games-Howell test. p-value in bottom row indicates p<0.05 according to paired t-test

Table 2: Serum alanine aminotransferase (ALT) levels in rats

Groups	Before	After	p-value	Mean difference
I	22.30±0.31[a]	22.41±0.60[a]	0.635	-0.11 (-0.7;0.47)
II	40.38±0.24[b]	40.49±0.21[b]	0.002	-0.11(-0.16;-0.06)
III	41.54±0.19[b]	34.13±0.82[c]	<0.001	7.4 (6.71;8.11)
IV	41.13±0.13[b]	29.00±0.20[d]	0.380	12.12 (11.46;12.79)
V	41.75±1.32[b]	25.60±0.88[e]	<0.001	16.31 (15.01;17.61)
p-value	<0.001	<0.001		

Values are presented as Mean±SE (n = 5). p-value in column indicates p<0.05 according to one-way ANOVA test. [a,b,c,d,e]indicated significant difference between groups according to one-way ANOVA test followed by the Games-Howell test. p-value in bottom row indicates p<0.05 according to paired t-test

Table 3: Serum ALP levels in rats

Groups	Before	After	p-value	Mean difference
I	96.81±3.82[a]	97.78±3.94[a]	0.003	-0.97 (-1.45;-0.49)
II	115.41±1.59[b]	117.29±1.63[b]	0.008	-1.89 (-3.02;-0.75)
III	118.61±1.00[b]	99.27±0.58[a]	<0.001	46.91(44.01;49.82
IV	117.92±0.38[b]	71.01±0.89[c]	<0.001	46.91 (44.01;49.82)
V	118.78±1.25[b]	59.56±2.04[d]	<0.001	59.22 (55.09;63.35)
p-value	<0.001	<0.001		

Values are presented as Mean±SE (n = 5). p-value in column indicates p<0.05 according to one-way ANOVA test. [a,b,c,d]indicated significant difference between groups according to one-way ANOVA test followed by the Games-Howell test. p-value in bottom row indicates p<0.05 according to paired t-test

Table 4: Serum AST levels in rats

Groups	Before	After	p-value	Mean difference
I	20.55±0.20[a]	21.02±0.13[a]	0.026	-0.48 (-0.87;-0.09)
II	34.86±0.19[b]	35.04±0.23[b]	0.015	-0.17 (-0.29;-0.05)
III	34.42±0.27[b]	31.02±0.11[c]	<0.001	3.4 (2.7;4.09)
IV	32.12±0.17[b]	27.63±0.25[d]	<0.001	4.5 (3.86;5.14)
V	32.19±0.35[b]	20.96±0.11[a]	<0.001	11.23 (10.17;12.29)
p-value	<0.001	<0.001		

Values are presented as Mean±SE (n = 5). p-value in column indicates p<0.05 according to one-way ANOVA test. [a,b,c,d]indicated significant difference between groups according to one-way ANOVA test followed by the Games-Howell test. p-value in bottom row indicates p<0.05 according to paired t-test

in the hepatotoxic group with 1.2 mL/200 g BW. Compared to the hepatotoxic group, the hepatotoxic with pomegranate juice groups were significantly p<0.05 different. However, administration of 0.4 mL/200 g BW pomegranate juice gave similar effects with the normal group (Table 3). Decreased serum AST levels in all of the hepatotoxic groups with pomegranate juice were observed, with the greatest decline in the hepatotoxic group with 1.2 mL/200 g BW pomegranate juice. It is found that administration 1.2 mL/200 g BW had similar effects with the normal group (Table 4). Reduced serum gamma-GT levels were also observed in all hepatotoxic groups with pomegranate juice (Table 5). The greatest decline was found in the hepatotoxic group with 1.2 mL/200 g BW. Compared with the normal group, administration of 0.8 mL/200 g BW pomegranate juice had similar effects.

Histological studies also provided supporting evidence for the results of the biochemical analysis. The HE staining revealed normal rat's hepatocyte (Fig.1a) with level of damage less than pathological control (Fig.1b). Hemorrhage, widening sinusoid, hepatocyte necrosis and hepatocyte karyolysis were observed in the livers of the rats treated with CCl_4 (Fig. 1b). A similar pattern was observed in rat liver within 2 weeks after treatment of 0.4 mL/200 g BW pomegranate juice compared to the pathologic control. Interestingly, the necrotic area was less observed in the group 32 weeks after treatment of 0.4 mL/200 g BW (Fig. 1c). In the rat liver

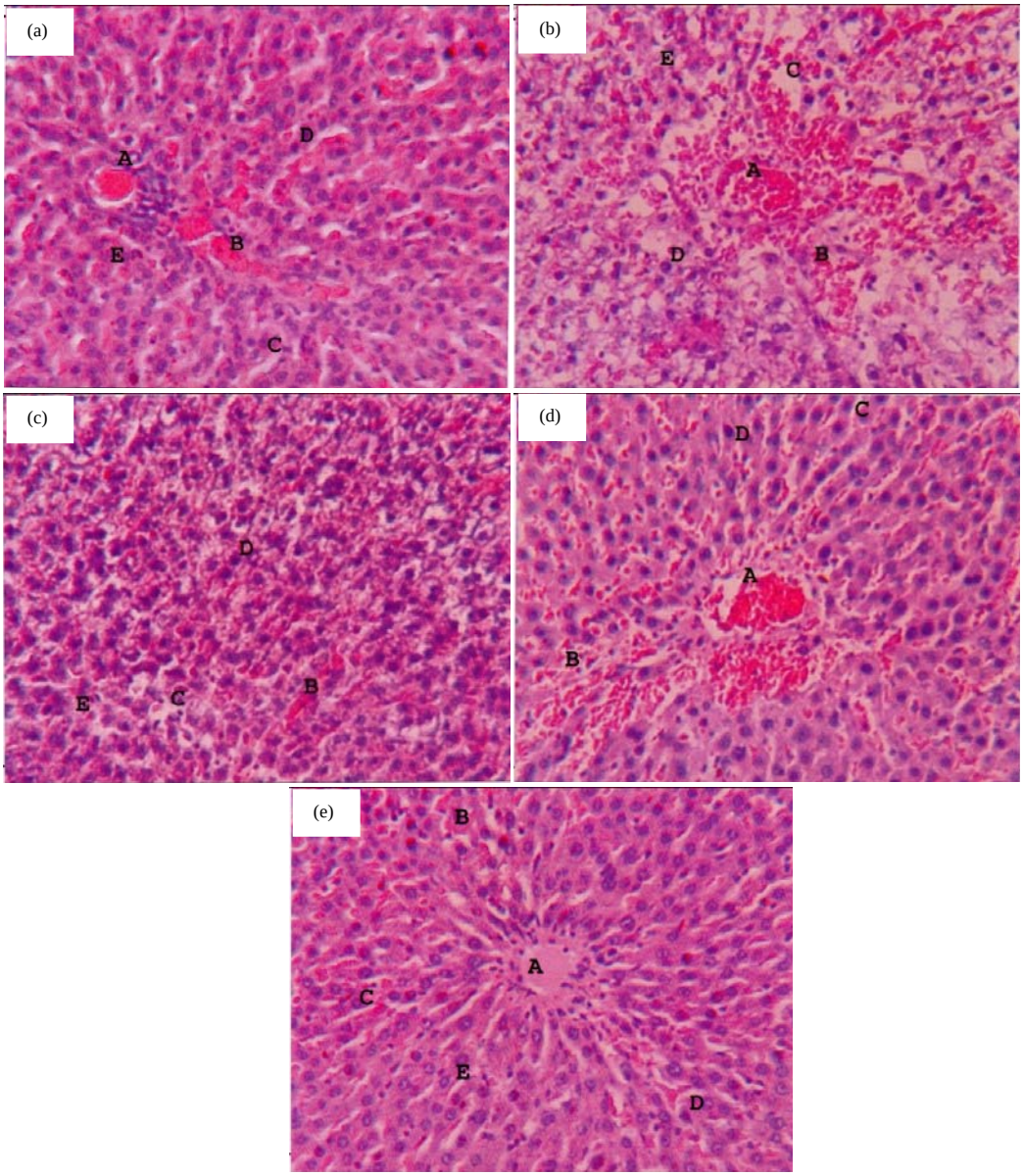

Fig. 1(a-e): Light microphotographs of HE-stained sections of the formalin-fixed livers. The effect of pomegranate juice on histopathological damages induced by CCL4 in rat liver, (a) Normal control group (b) Carbon tetrachloride group (c) Carbon tetrachloride received 0.4 mL/200 g BW pomegranate juice group (d) Received 0.8 mL/200 g BW pomegranate juice group and (e) Received 1.2 mL/200 g BW pomegranate juice group. A: Vena centralis, B: Hemorrhage, C: Sinusoid, D: Hepatocytes, E: Karyolysis and F: Cloudy cytoplasma

Table 5: Serum gamma-GT level in rats

Groups	Before	After	p-value	Mean difference
I	19.96±1.10[a]	20.27±1.16[a]	0.006	-0.31 (-0.48;-0.13)
II	35.08±0.56[b]	35.55±0.58[b]	0.003	-0.47 (-0.70;-0.24)
III	33.29±1.11[b]	26.64±0.72[c]	<0.001	6.66 (5.37;7.94)
IV	33.13±0.41[b]	19.07±0.44[a]	<0.001	14.07 (13.63;14.51)
V	31.22±0.69[b]	15.93±0.30[d]	<0.001	15.23 (14.52;15.93)
p-value	<0.001	<0.001		

Values are presented as Mean±SE (n = 5). p-value in column indicates p<0.05 according to one-way ANOVA test. [a,b,c,d]indicated significant difference between groups according to one-way ANOVA test followed by the Games-Howell test. p-value in bottom row indicates p<0.05 according to paired t-test

treated with 0.8 mL/200 g BW pomegranate juice (Group 4), hemorrhage with multinucleated normochromatic hepatocytes were observed (Fig. 1d) and compared to CCl_4-induced rats the extent of damage was less. The group treated with 1.2 mL/200 g BW pomegranate juice, compared to the pathologic control group (Group 2), showed induced recovery faster as seen by the absence of hemorrhage and necrosis (Fig. 1e).

DISCUSSION

The present study revealed that CCl_4 induction in rats increased ALT, AST, ALP, γ-GT and MDA levels, signaling hepatotoxic liver damage. CCl_4 is one of the xenobiotics reported to induce acute and chronic tissue injuries[13,14]. CCl_4 causes acute hepatocyte injuries and membrane integrity alteration, which leads to hepatocyte enzymes leak out[15]. However, after treatment with pomegranate juice the increase of ALT, AST, ALP and γ-GT were significantly reduced. These results indicated that pomegranate juice has the ability to protect the liver from CCl_4-induced hepatocyte injury. This finding is in agreement with one study[16] that showed polyphenolic compounds protect liver from CCl_4-induced liver cirrhosis. According to other research[4] the liver protective and antioxidative effects of phenolic containing plant extracts in CCl_4-induced liver injury were related to free radical scavenging effects.

The decrease of these enzymes after treatment with pomegranate juice correlates with the antioxidant and anti-inflammatory potential in pomegranate juice. Pomegranate juice contains flavonoid compounds which have antioxidant and anti-inflammation properties[7,10]. Flavonoid compounds are known to decrease hepatotoxicity caused by xenobiotic chemicals and are protective against damage caused by oxidative stress[17]. The antioxidant effect in pomegranate juice stops radical trichloromethyl formation from CCl_4 metabolism. The protective factor in pomegranate can be due to its action as an electron donor to free radicals thus stopping the radical reaction damage. As seen in our results, damage that was caused by the radical reaction stopped[18]. Balancing of enzymes against free radicals such as superoxide dismutase, catalase and peroxidase was maintained by antioxidants from pomegranate. This result is because the antioxidant potential of pomegranate minimized hydrogen peroxide and lipid peroxidation thus preventing membrane damage. Similar research results have shown that the antioxidant effect of pomegranate decreased lipid peroxidation and increased plasma antioxidants[19]. The results in this study showed that the antioxidant effect of pomegranate also reduced free radicals and decreased oxidative stress in macrophage activity. There are three indicators for decreased oxidative stress. They are decreased cell and DNA damage, decreased glutathione and oxidized glutathione and decreased liver antioxidant enzyme levels.

CONCLUSION

The present study concluded that administration of pomegranate juice with three different doses decreased serum ALT, AST, ALP, gamma-GT and MDA. Administration of 0.8 and 1.2 mL/200 g BW of pomegranate juice effectively reduced liver damage in carbon tetrachloride-induced rats.

SIGNIFICANCE STATEMENTS

This study examines the benefits of pomegranate juice in improving liver damage induced by oxidative stress. This study will help researchers to uncover critical areas of liver disease that may have previously not been available. Thus a more comprehensive understanding about antioxidants in pomegranate juice, may be applied in health sciences and nutrition.

ACKNOWLEDGMENT

This research was supported by the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

REFERENCES

1. Ayala, A., M.F. Munoz and S. Arguelles, 2014. Lipid peroxidation: Production, metabolism and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-nonenal. Oxidat. Med. Cell. Longevity. 10.1155/2014/360438.
2. Adewale, O.B., A.O. Adekeye, C.O. Akintayo, A. Onikanni and S. Sabiu, 2014. Carbon tetrachloride (CCl_4)-induced hepatic damage in experimental Sprague Dawley rats: Antioxidant potential of *Xylopia aethiopica*. J. Phytopharmacol., 3: 118-123.
3. Kalyani, G.A., C.K. Ramesh and V. Krishna, 2011. Hepatoprotective and antioxidant activities of *Desmodium triquetrum* DC. Indian J. Pharmaceut. Sci., 73: 463-466.
4. Li, R., W. Guo, Z. Fu, G. Ding, Y. Zou and Z. Wang, 2011. Hepatoprotective action of *Radix Paeoniae Rubra* aqueous extract against CCl_4-induced hepatic damage. Molecules, 16: 8684-8693.
5. Richhariya, A., A.K. Singh, N. Singh and S.K. Singh, 2012. Hepatoprotective and antioxidants activity of ethanolic extract of *Cuscutta reflexa* Roxb. IOSR J. Pharm., 2: 142-147.
6. Elfalleh, W., N. Tlili, N. Nasri, Y. Yahia and H. Hannachi *et al*., 2011. Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits. J. Food Sci., 76: C707-C713.

7. Zarfeshany, A., S. Asgary and S.H. Javanmard, 2014. Potent health effects of pomegranate. Adv. Biomed. Res., Vol. 3. 10.4103/2277-9175.129371.
8. Wang, J.Y., C. Zhu, T.W. Qian, H., D.D. Wang, F. Zhang and X. Yin, 2015. Extracts of black bean peel and pomegranate peel ameliorate oxidative stress-induced hyperglycemia in mice. Exp. Therapeut. Med., 9: 43-48.
9. Kumar, S. and A.K. Pandey, 2013. Chemistry and biological activities of flavonoids: An overview. Scient. World J. 10.1155/2013/162750.
10. Gil, M.I., F.A. Tomas-Barberan, B. Hess-Pierce, D.M. Holcroft and A.A. Kader, 2000. Antioxidant activity of pomegranate Juice and its relationship with phenolic composition and processing. J. Agric. Food Chem., 48: 4581-4589.
11. Anoosh, E., E. Mojtaba and S. Fatemeh, 2010. Study the effect of juice of two variety of pomegranate on decreasing plasma LDL cholesterol. Procedia-Social Behav. Sci., 2: 620-623.
12. Hewawasam, R.P., K.A.P.W. Jayatilaka, C. Pathirana and L.K.B. Mudduwa, 2004. Hepatoprotective effect of *Epaltes divaricata* extract on carbon tetrachloride induced hepatotoxicity in mice. Indian J. Med. Res., 120: 30-34.
13. Ferreira, E.A., E.F. Gris, K.B. Felipe, J.F.G. Correia, E. Cargnin-Ferreira, D.W. Filho and R.C. Pedrosa, 2010. Potent hepatoprotective effect in CCl_4-induced hepatic injury in mice of phloroacetophenone from *Myrcia multiflora*. Libyan J. Med., Vol. 5. 10.3402/ljm.v5i0.4891.
14. Hermenean, A., T. Mariasiu, I. Navarro-Gonzalez, J. Vegara-Meseguer, E. Miutescu, S. Chakraborty and H. Perez-Sanchez, 2017. Hepatoprotective activity of chrysin is mediated through TNF-α in chemically-induced acute liver damage: An *in vivo* study and molecular modeling. Exp. Therapeut. Med., 13: 1671-1680.
15. Khan, R.A., M.R. Khan and S. Sahreen, 2012. CCl_4-induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement. Altern. Med., Vol. 12. 10.1186/1472-6882-12-178.
16. Zhen, M.C., Q. Wang, X.H. Huang, L.Q. Cao and X.L. Chen *et al*., 2007. Green tea polyphenol epigallocatechin-3-gallate inhibits oxidative damage and preventive effects on carbon tetrachloride-induced hepatic fibrosis. J. Nutr. Biochem., 18: 795-805.
17. Olaleye, M.T., A.E. Amobonye, K. Komolafe and A.C. Akinmoladun, 2014. Protective effects of *Parinari curatellifolia* flavonoids against acetaminophen-induced hepatic necrosis in rats. Saudi J. Biol. Sci., 21: 486-492.
18. Kaur, G., Z. Jabbar, M. Athar and M.S. Alam, 2006. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chem. Toxicol., 44: 984-993.
19. Jurenka, J.S., 2008. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. Altern. Med. Rev., 13: 128-144.