Reducing of Hydrogen Peroxide Effects by Pomegranate Peel Extract in Vivo, in Female Wistar Rats

Dhuha Salah Noori[1], Agharid. A.Al-Rasheed[2], Muna Salah Rasheed[3], Bashiru Garba[4]

[1]Asst. Lec. Department of Food Science, College of Agriculture, Tikrit University, Iraq, [2]Lec. Department of Microbiology, College of Veterinary Medicine, Tikrit University, Iraq, [3]Asst. prof. Department of Biology, College of Science, Tikrit University, Iraq, [4]Asst. prof. Department of Veterinary Public Health & Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria

Abstract

In this study, the effect of pomegranate peel aqueous extract (PPEA) in reducing toxicities associated with hydrogen peroxide consumption was investigated using rats. This was achieved by assessing the serum biochemical parameters as well as histopathological analysis of the liver and stomach sections. Forty, 6-week-old female Wistar albino rats weighing 11.6± 4.4 g were divided equally into six groups. The rats were administered the pomegranate extract and challenged with H_2O_2 via the oral route for 28 days. To achieve this, positive control rats ingested H_2O_2 at 5 mg/ 100g body weight (b.w), while the negative control groups were given saline solution. Rats in the other three experimental groups were given H_2O_2 and treated with PPEA at 20, 40 mg and Vitamin at 25mg per 100g body weight respectively. H_2O_2 at 5 mg/100 g b.w induced injury to the liver and stomach tissues, while increasing levels of malondialdehyde (MDA), the Aspartate Transaminase (AST) besides Alanine Transaminase (ALT) and, reduced of Glutathione GSH) were observed in comparison with the control groups. Treatment with PPEA demonstrated protective effects by reducing the MDA, ALT and AST values and increase of GSH level as well as, improving the liver and stomach tissues to recover. Our results indicate that the administration of treatments against H_2O_2 using PPEA decrease the harmful effect orally administered H_2O_2 at 5 mg/100g b.w.

Keywords*: Hydrogen peroxide, pomegranate, pomegranate peel aqueous extract, antioxidants biomarkers, liver enzymes, rats*.

Introduction

Hydrogen peroxide (H_2O_2) is an oxidizing agent that is utilized in the production of various household items, including some broadly useful disinfectants and bleach, element of multiple teeth brightening products, treatment of wounds as well as for the purpose of sterilizing instrumentations[(1)]. H_2O_2 induces toxicities by destructive or corrosive harm, and the direct action of their oxidizing features by the formation and development of oxygen gas alongside lipid peroxidation through the reactive oxygen species ROS (Watt et al., 2004). On the other hand, ROS increases oxidative damage and causes adverse adjustments to cell components, such as proteins, lipids as well as DNAs [(2)].

Organisms have devised strategies to manage H_2O_2, which involvee endogenous protection accomplished by enzymes like superoxide dismutase, catalase and glutathione peroxidase. Also, the occurrence of antioxidants of low-molecular weight nature such as glutathione and α-tocopherol helps remove the ROS and reactive nitrogen species RNS [(3)]. The consumption of normal dietary antioxidants such as vitamins, protein, phenolic mixes, terpene, coenzyme Q, and nitrogen mixes behave as a free radical scavenger molecules by authorizing redox metals to thwart free radical leading to the generation of anti-inflammatory action [(4,5)].

Pomegranate Tunica granatum L. (Lythraceae), belongs to the Punicaceae family which have been

Corresponding author:
Agharid. A. Alrasheed.
agharidalrasheed@tu.edu.iq

portrayed as an antediluvian extraordinary plants that were utilized in a few frameworks of medication for relieving an assortment of sicknesses [6,3,7]. Pomegranate producing juices and an enormous measure of peels as bye-products and accounts for about half of the entire organic fruit mass [8]. The pomegranate peels (exocarp and mesocarp) are the non-edible part and have largely been used as a source of bioactive composites, for instance, hydrolysable tannins pedunculagin, punicalin, punicalagin, ellagic and gallic acids [9]. Punicalagin, a unique ellagitannin of pomegranate, is the main polyphenolic component of pomegranate peels and has a strong antioxidant activity [10].

The probable therapeutic assets of pomegranate peel are enormous including its application in the treatment and counteraction for the progression of cancers [11], cardiovascular illness [3,12], diabetes [13] and antimicrobial activity [14], in addition to its antioxidant and anti-inflammatory properties [15]. However, there is limited information on ways to reduce the harmful effects of H_2O_2 above 3 mg/100g by natural bye-products. Therefore, this research is aimed at examining the potentials of aqueous extracts of pomegranate peel in reducing the effects of H_2O_2 following oral administration with dose of 5 mg/100 body weight. The study also seeks to evaluate the antioxidants properties of the pomegranate by analyzing liver functions biomarkers and as well as histopathological studies of liver and stomach organs.

Materials and Methods

Plant material collection and extraction

The pomegranate fruits were purchased from local markets at Salah Al-Din province, Iraq in the months of September–November which coincide with the peak of their season. The pomegranate peels (mesocarp and exocarp) PP was physically detached from washed fruits, slashed, sun dried and powered with a grinder. The pomegranate aqueous extract (PPAE) was prepared according to the method described by Qnais *et al.* (2007) [16] which used the fractional modification approach. One hundred and fifty grams (150 g) of dried PP powder was poured into 3000 mL of distilled water and boiled for duration of one minute while being agitated on a magnetic stirrer after which the blend was kept in a shaker at room temperature for 24h. The resultant solutions were sifted through the Whatman no.1 filter paper and the filtrate was dried at room temperature and stored in the refrigerator until required. About 2 g powder was obtained from a quantity of 150 g dried peels. The solution of the pomegranate peel aqueous extract (PPAE) was then prepared by thawing the resultant powder using physiologic salt solutions.

Animal Model

Forty female albino rats of Wistar strain, weighing 116.3 ± 4.4 gram were obtained from the central animal house of the Faculty of Veterinary Medicine, Tikrit University, Iraq. They were kept in the departmental animal house and maintained under standard environmental conditions with bedding of wood shaving, fed with standard commercially accessible pellets with potable water. The rats were assigned to groups after seven days of acclimatization.

The research study framework was based on the recommendation and approval of animal control and supervision committee of the Scientific Affairs at the Tikrit University in Iraq, in accordance with the OECD guidelines on laboratory animal usage in research.

Treatment

The under-test rats were placed into six groups (G1-G6) containing five rats each per group. All the groups were administered 2 ml of the pomegranate extract orally daily for 28 days. The LD50 dose of PPAE was determined by using the method described by Qnais et al. (2007) and Patel et al. (2008) [16,17]. While the route and dose of H_2O_2 was chosen depending on the toxic effects of hydrogen peroxide (H_2O_2) as earlier described by Kawasaki (1969) [18]. However, the group 1 G1 (negative control) was treated by administration of saline solution only. G2 was treated with 5 mg/100 g body weight (b.w) of hydrogen peroxide (H_2O_2), G3 were given 25 mg/100 g body weight (b.w) of vitamin (vit) C, G4 were treated with a combination of H_2O_2 and Vitamin C, while rats in G5 and G6 were treated with H_2O_2 and 20 and 40 mg/100g body weight (b.w) of pomegranate peels aqueous extract (PPAE) respectively. The rats were refused feed for 3–4 h before administration. General observations were made for any moribund animals twice each day. On the 29th day after the last treatment, the rats were anaesthetized using 200 mg/kg (b.w), i.p

of pentobarbital sodium in order to obtain samples of blood and relevant organs. Blood samples were taken by cardiac puncture following sacrifice. Serum samples were obtained after blood centrifugation and stored at -20°C until required for analysis.

Liver enzyme functions tests

The serum concentration of aspartate transaminase (AST) and alanine transaminase (ALT) were assessed by kits obtained from RANDOX Laboratories Ltd., United Kingdom, according to the manufacturers' instructions.

Biomarkers of oxidative stress estimation

Malondialdehyde (MDA) as a biomarker of oxidative stress

Spectrophotometric assay was applied to detect the level of oxidase stress marker, Malondialdehyde (MDA) in serum samples by quantifying thiobarbituric acid (TBA) reactivity as MDA. The TBA test is based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) leading to the formation of MDA-TBA2 that produce a deeply colored chromogen fluorescent red adducts following absorption at 532 nm.

Glutathione (GSH)

Reduced glutathione (GSH) in serum was evaluated with reference to the Burtis method and Ashwood (2007) utilizing Ellman's reagent [19]. The assay is based on the decrease of the Ellman's reagent by sulfhydryl groups into 5, 5′-dithiobis (2-nitrobenzoic acid), which has a strong yellow color. The solution was weighted at 412 nm utilizing the Japanese spectrophotometers brand Shimadzu.

Histopathological assessment

Fresh liver and stomach organs from sacrificed animals were collected in sample bottles containing 10% formalin. The tissues from the harvested organs were processed into 4 to 5 µm thickness. The gut tissue segments were adhered on glass slides, de-parafinized, and embedded in a solution of Hematoxylin and Eosin stains. The stained sections were then viewed under a microscope at 40 X magnifications.

Statistical analysis

The recorded data from all the six groups were analyzed using one way analysis of variance (ANOVA) and statistical significance was deduced with the aid of Duncan's numerous range test (DMRT) on SPSS (version 25). Statistical significance was determined at $p < 0.05$. All the values were expressed as mean ± standard deviations (S.D).

Results

Effects on liver enzymes

The serum levels of AST and ALT were different among animals in the treatment and non-treatment groups (Fig.1). In group G2, rats were found to exhibit substantial ($p < 0.05$) levels of serum AST (by 104.5%) and ALT (by 58.8%) in comparison with control group G1. Similarly, rats treated with vitamin C at 25 mg/100 g b.w (G4) and PPAE at 40 mg/100g b.w (G6) showed significant ($p < 0.05$) reduction in both enzymes AST (by 28.8%; 20% respectively) and ALT (by 49.4%; 41.1% respectively) in comparison with G2 group, indicating the anti-inflammatory effect of PPAE at 40/100g b.w against H_2O_2 effects (Fig1, A & B) .

Oxidative stress parameters

Serum GSH activity level of rats in group (G2) showed a significant ($p < 0.05$) decrease (by 65.5%) and increase in MDA level (by 73.6%) in comparison with control group G1. Furthermore, treatment with vitaminC in G4 and PPAE in G6 revealed statistically signicant increase ($p < 0.05$) in the activity of GSH action (by 84%; 89% respectively) while MDA level decreased by by 52% and 68.8% respectively in comparison with group (G2) as shown in Figure 1 C & D, which shows the antioxidant activity of PPAE in reducing the harmful effect of H_2O_2 at 5mg/100g b.w.

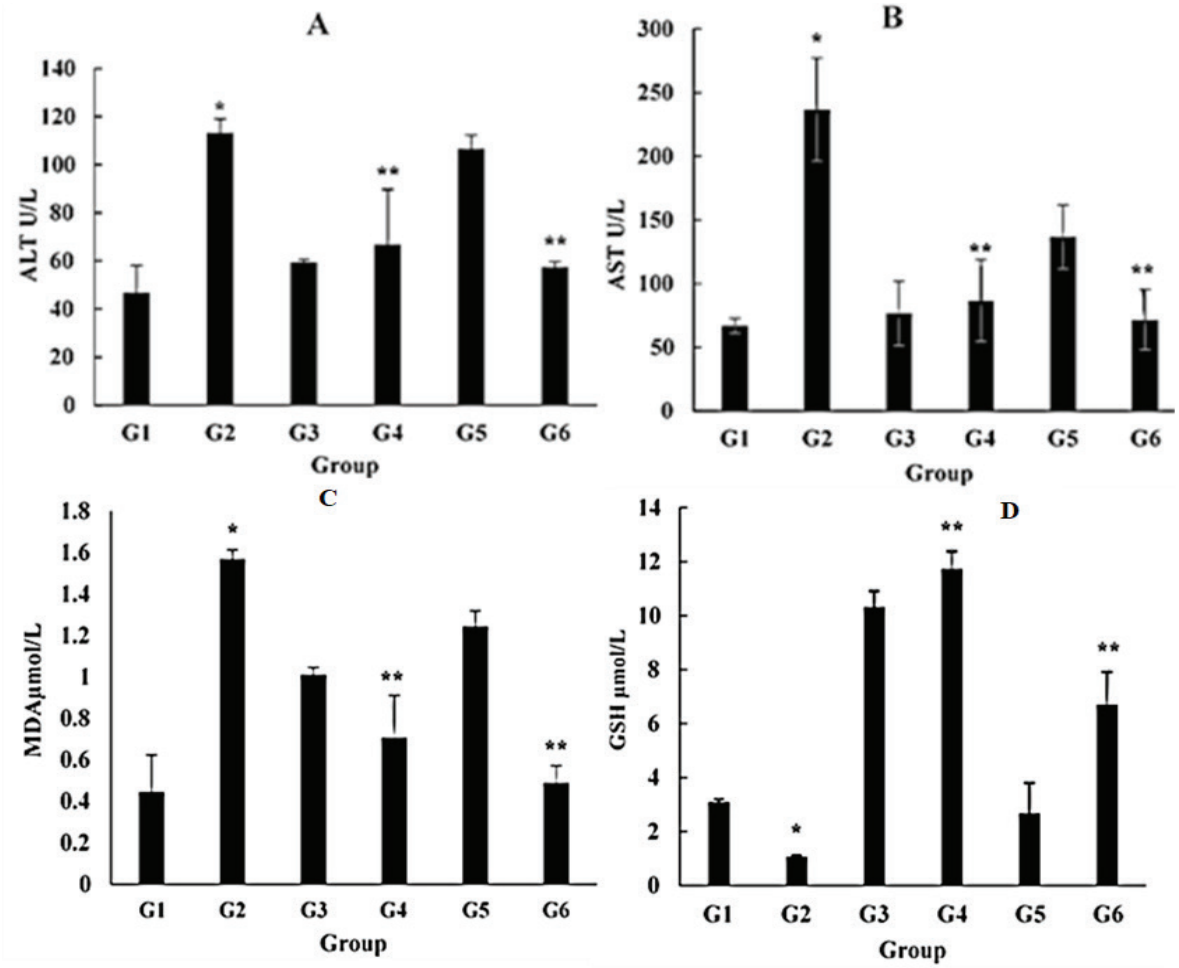

Figure 1. 2A, 2B, 2C and 2D indicates effects of PPAE and vitamin C on the AST and ALT activities as well as the oxidative stress parameters MDA and GSH in the serum of rats exposed to H_2O_2. The data was presented as means ±SD, (n = 5) animals. With asterisk* indicating statistical significance ($p < 0.05$) in comparison with the control group G1; while double asterisk indicate substantial increase ($p < 0.05$) in relation to the oxidative stress measure in group 2 G2.**

Histopathological examination

Microscopic examination of histological liver sections in the control group G1 (Fig. 2. A) showed a normal histological architecture. Normal hepatocytes (arrow) were organised in a number of several types of plates within the areas of the central vein (CV) and sinusoidal (S) plates. However, liver sections of rats in group G2 showed a marked damage (Fig 2B). The liver showed loss of the normal architecture, an invasion of inflammatory cells, with cellular infiltrations like neutrophils and Kupffer cells in the portal area, and hepatocytes indicating necrosis. Oral administration of the vitamin C 25 mg/100 g b.w and PPEA at 40 mg/100g b.w showed protection activity from the harmful effects of H_2O_2 (Fig 2 D, F). Remarkable change and reduction of inflammation and congestion in H_2O_2 intoxicated liver following treatment with PPEA at 40mg/100g b.w in comparison to group G2 was also observed. The observed regeneration in the hepatocytes (arrow) and binucleated liver cells was also shown.

The inner gastric surface of the stomach of the rats in the control group G1 (Fig 2 A), showed a normal histological structure of the gastric mucosa, where normal surface columnar epithelium (SCE), parietal and chief cells (PCs) as well as the gastric pits (GP) were observed. On the other hand, ingestion of 5 mg/100 g (b.w) dose of H_2O_2 for 28 days has shown destruction of the mucosal architecture, dissociation and erosion of

the mucosa and infiltration of inflammatory cell (arrow) (Fig 2B). Oral treatment with vitamin C at 25 mg/100 g b.w and PPEA at 40 mg/100g b.w reveal slight reduction of ulceration in the gastric mucosa of the stomach (Fig 2 D, F). Additionally, the gastric mucosa of the rats treated with PPEA at 40 mg/100g (b.w) show observable reappearance of the epithelial layers compared to group G2.

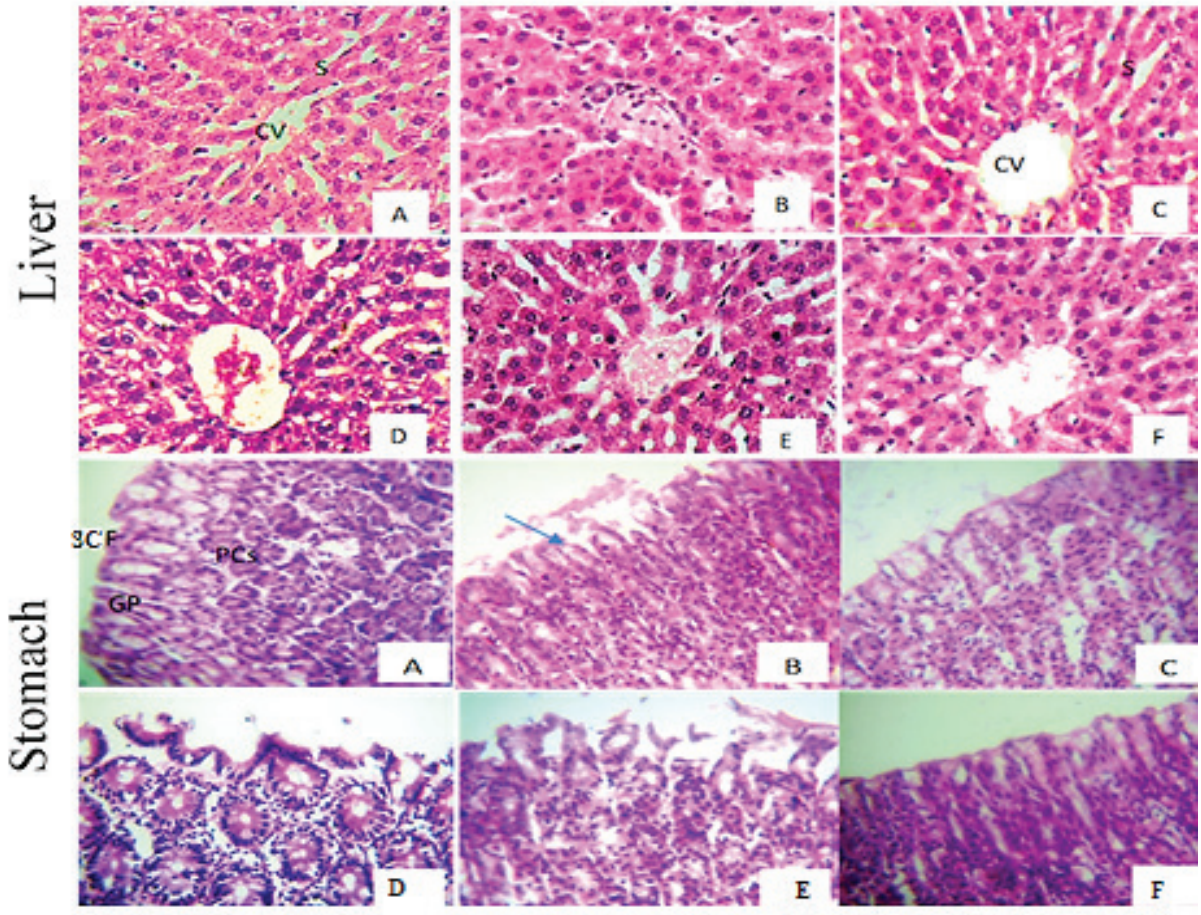

Figure 2. Microphotographs of the liver and stomach sections from the control and treatment groups stained by H & E and magnification, x400. G1 (A) control; G2 (B) 3 mg/100g H_2O_2; G3 (C) 25 mg/100g (b.w) vitamin C; G4 (D) vitamin C+H_2O_2; G5 (E) 20 mg/100 g (b.w) PPAE +H_2O_2 and G6 (F) 40 mg/100g (b.w) PPAE +H_2O.

Discussion

The marked increase in H_2O_2 within cells can bring about oxidative stresses as well as induce cellular destruction [21]. H_2O_2 in the presence of transition metals generates the often highly toxic reactive oxygen species (ROS) that provoke damage to multiple cellular organelles and process, leading to lipids peroxidation by influencing the irons redox cycling (30). Glutathione, is one of the major thiol-containing compound with low molecular weight within animals cells that are essential in many biochemical and pharmacological reactions due to the ease with which they are oxidized and the speed with which they can be regenerated, and they also successfully rummages ROS as antioxidants agent [22]. Because malondialdehyde (MDA) is one of the significantly formed aldehydes after lipids hydroperoxides failure, it is viewed as a decent biomarker contributing to free radical damages in pathologies associated with oxidative stress [23].

In this study, we demonstrated that oral administration of hydrogen peroxide (H_2O_2) with a dose of 5 mg/100 g body weight (b.w) result in increase of MDA and depletion of GSH levels in the serum of rats (group 2). As reported by Kawasaki et al. 1969 [18], when

rats are exposed daily, by oral gastric tube, to above 3 mg/100 g of H_2O_2 solutions, it causes significant blood chemistry changes. Findings of this study were similar to previous reports by Ganie *et al*. [24], and Rahim *et al*. [25] who both reported a considerable increase of MDA level in H_2O_2 diagnosed rats, indicating the likelihood of decreased fundamental creation, and bigger volume of lipoid peroxidation and induction of oxidative stress. However, treatment with PPEA with dose 400 mg/kg probably reduced the oxidative stress as indicated by the inhibition of lipid hydroperoxides due to depletion of MDA and increase GSH levels. Besides, the parameters were seen as near their normal levels in the serum of vitamin C treated group (G5). Both Osman *et al.* [26], and Cichoz-Lach *et al*. [27] demonstrated that the lipids peroxidation levels decrease in the pomegranate extract handled animals groups as compared to the control groups. Pomegranate peels are rich in antioxidants polyphenolic mixtures which has a protective effect from the harmful reactive oxygen species (ROS), through destruction of free radicals or by preventing the generation of these species.

Similarly, the antioxidant property of vitamin C is dependent on the decrease of lipid peroxidation through the removal of free radicals action [28]. Increase of MDA and depletion of GSH in the serum of rats of group G2 as evidenced by the significant increase observed in the levels of liver (AST and ALT) enzymes activities resulted in chronic hepatic injury following oral administration of H_2O_2. Rahim *et al.* [25], and Abozid *et al.* [29] indicated that oxidative stress induced by H_2O_2 bring about hepatic injury as depicted by the raise in the liver enzymes activities in the blood. Moreover, microscopic examine of the liver section of rats in group 2 showed damage in liver cells as result of H_2O_2 toxicity. Free radicals that invade the liver cell compartments cause changes in its composition and functions as result of oxidative stress [30,31]. The present research work realized a manifest impact of PPEA (400 mg/kg) diminished ALT and AST enzymes and produced a remarkable reduction of damage in liver cells. Toklu *et al*. [32] examined the impact of chronic organization of PPEA initiated by bile conduit legation within rats and got that serum ALT and AST were tremendously diminished. Which, indicate activity of pomegranate peel to preserve the auxiliary uprightness of the hepatocellular layer and liver cell design by particular phenolic mixes that may potentiate the defensive impacts against ROS [33]. Also, vitamin C protects the lipids and lipoproteins in the hepatocellular membranes versus oxidative harm brought about by dangerously toxic free radical scavenging activity [31].

After 28 days, ulceration of the gastric mucosa was observed in rats at group 2. Once ingested, H_2O_2 promptly interacts with tissue catalase such as mucous layers. This exothermic response quickly decays H_2O_2 to water, warmth, and oxygenated and is effortlessly changed over to hydroxyl radical which cause harm to numerous cell parts or even cell death [34]. On other hand, following treatment with PPEA, a slight reduction of stomach mucosal damage was noted. Inflammatory bowel disease is favorably influenced by consumption of several dietary natural plant products including polyphenolic compounds. Metabolism of polyphenol compounds such as ellagic acid in pomegranate peel by gut microbiota result in the formation of bioactive urolithins A, B, C, and D. Urolithin A (UA) is the most dynamic and compelling gut metabolite and goes about as a powerful calming element to oxidizing agents [10].

Conclusion

In conclusion, prolonged consumption of food contaminated with chemicals such as hydrogen peroxide can cause toxicity at a certain site of the body. Therefore, reducing this effect will require the intake of natural bio products such pomegranate peel extract which has enhanced antioxidative potentials, and can act as a free radical scavenger in addition to its anti-inflammatory action.

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way.

Conflict of Interest: The authors declare that there is no conflict of interests regarding the publication of this article.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Dhuha Salah Noori, Agharid A. Alrasheed: Conceptualization; **Dhuha Salah Noori; Agharid A. Alrasheed, Muna Salah Rasheed:**

Methodology; **Dhuha Salah Noori, Agharid A. Alrasheed, Muna Salah Rasheed, Bashiru Garba:** Writing- Original draft preparation. **Agharid A. Alrasheed, Bashiru Garba**: **Dhuha Salah Noori, Muna Salah Rasheed**: Writing- Reviewing and Editing.

References

1. Watt B. E., Proudfoot A. T., Vale J. A. Hydrogen peroxide poisoning. *Toxicological Reviews*. 2004*;23*(1): 51–57.
2. He L., He T., Farrar S., Ji L., Liu T., Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cellular Physiology and Biochemistry*. 2017*;*44(2): 532–553.
3. Jurenka J. Therapeutic applications of pomegranate (Punica granatum L.): a review. *Alternative Medicine Review*. 2008;13(2).
4. Zhang Y.-J., Gan R.Y., Li S., Zhou Y., Li, A.N., Xu D.P., Li H.B. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*. 2015;20(12): 21138–21156.
5. Uttara B., Singh A. V, Zamboni P., Mahajan R. T.. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*. 2009;7(1), 65–74.
6. Lansky. Therapeutic applications of pomegranate (Punica granatum L.): a review. *Alternative Medicine Review*. 2008;13(2).
7. Mustafa M.A., AL-Samarraie M.Q., Ahmed M. T. Molecular techniques of viral diagnosis, Science Archives. 2020;1(3): 89-92.
8. Mphahlele R. R., Pathare P. B., Opara U. L.. Drying kinetics of pomegranate fruit peel (cv. Wonderful). *Scientific African*. 2019;5:e00145.
9. Fischer U. A., Jaksch A. V, Carle R., Kammerer D. R.. Influence of origin source, different fruit tissue and juice extraction methods on anthocyanin, phenolic acid, hydrolysable tannin and isolariciresinol contents of pomegranate (Punica granatum L.) fruits and juices. *European Food Research and Technology*. 2013;237(2): 209–221.
10. Zhao R., Long X., Yang J., Du L., Zhang X., Li J., Hou, C. Pomegranate peel polyphenols reduce chronic low-grade inflammatory responses by modulating gut microbiota and decreasing colonic tissue damage in rats fed a high-fat diet. *Food & Function*. 2019;10(12):8273–8285.
11. Dikmen, M., Ozturk, N., & Ozturk, Y.. The antioxidant potency of Punica granatum L. Fruit peel reduces cell proliferation and induces apoptosis on breast cancer. *Journal of Medicinal Food*. 2011; 14(12): 1638–1646.
12. Mustafa M.A., AL-Samarraie M.Q .SECONDARY MENOPAUSE and its RELATIONSHIP to HORMONAL LEVELS AMONG WOMEN at SALAH AL-DIN HOSPITAL . *European Journal of Molecular & Clinical Medicine* . 2020; Volume 7, Issue 09, PP 96-104.
13. Middha S. K., Usha T., Pande V. A review on antihyperglycemic and antihepatoprotective activity of eco-friendly Punica granatum peel waste. *Evidence-Based Complementary and Alternative Medicine*. 2013.
14. Hayouni E. A., Miled K., Boubaker S., Bellasfar Z., Abedrabba M., Iwaski H., Oku H., Matsui T., Limam F., Hamdi M. Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds. *Phytomedicine*. 2011;18(11), 976–984.
15. Labsi M., Soufli I., Khelifi L., Amir Z.-C., Touil-Boukoffa C. A preventive effect of the combination of albendazole and pomegranate peel aqueous extract treatment in cystic echinococcosis mice model: an alternative approach. *Acta Tropica*. 2019;10:50-50.
16. Qnais E. Y., Elokda A. S., Ghalyun Y. Y. A., Abdulla F. A. Antidiarrheal activity of the aqueous extract of Punica granatum (pomegranate) peels. *Pharmaceutical Biology*. 2007;45(9), 715–720. https://doi.org/10.1080/13880200701575304
17. Patel C., Dadhaniya P., Hingorani L., Soni M. G. Safety assessment of pomegranate fruit extract: acute and subchronic toxicity studies. *Food and Chemical Toxicology*. 2008;46(8).
18. KAWASAKI C., KONDO M., NAGAYAMA T., TAKEUCHI Y., NAGANO H.. Effect of hydrogen peroxide on the growth of rats. *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)*. 1969;10(2): 68-72_1.
19. Bruns D. E., Ashwood E. R., Burtis C. A. Fundamentals of molecular diagnostics. Elsevier Health Sciences. 2007
20. Abdel-Hafeez E. H., Ahmed A. K., Abdellatif M. Z. M., Kamal A. M., Toni N. D. M. The Efficacy

of Pomegranate (Punica granatum) Peel Extract on Experimentally Infected Rats with Blastocystis Spp. *Journal of Infectious Diseases & Preventive Medicine*. 2016; 2–6.

21. Veal E. A., Day A. M., Morgan B. A.. Hydrogen peroxide sensing and signaling. *Molecular Cell*. 2007;26(1): 1–14.

.22 Xie Q., Guo F.-F., Zhou W.. Protective effects of cassia seed ethanol extract against carbon tetrachloride-induced liver injury in mice. Acta Biochimica Polonica. 2012; 59(2).

23. Mateos R., Lecumberri E., Ramos S., Goya L., Bravo L.. Determination of malondialdehyde (MDA) by high-performance liquid chromatography in serum and liver as a biomarker for oxidative stress: Application to a rat model for hypercholesterolemia and evaluation of the effect of diets rich in phenolic antioxidant. *Journal of Chromatography B*. 2005;827(1): 76–82.

24. Ganie S. A., Haq E., Hamid A., Masood A., Zargar M. A.. Long dose exposure of hydrogen peroxide (H2O2) in albino rats and effect of Podophyllum hexandrum on oxidative stress. *Eur Rev Med Pharmacol Sci*. 2011;15(8): 906–915.

25. Rahim S. M., Taha E. M., Mubark Z. M., Aziz S. S., Simon K. D., Mazlan A. G. Protective effect of Cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. Systems Biology in Reproductive Medicine. 2013;59(6): 329–336.

26. Osman M., Ahmed M., Mahfouz S., Elaby S.. Biochemical studies on the hepatoprotective effects of pomegranate and guava ethanol extracts. NY Sci J. 2011;4(3):27–41.

27. Cichoz-Lach H., Michalak A., Of T., Supplements V., Ward J. F., Blakely W. F., Joner E. I., Zeyuan D., Bingyin T., Xiaolin L., Jinming H., Yifeng C.. Effect of green tea and black tea on the blood glucose, the blood triglycerides, and antioxidation in aged rats. *Journal of Agricultural and Food Chemistry*. 2014;46(3): 3875–3878. https://doi.org/10.3748/wjg.v20.i25.8082

28. Saraswathy G. R., Maheswari E., Santhrani T.. Effect of vitamin C supplementation on phenytoin induced hepatotoxicity. *Global J Pharmacol*. 2010;4(3): 127–135.

29. Abozid M. M. Antioxidant and Protective Effects of Green Tea against H2O2 induced Liver Injury in Rats. October. 2018

30. De Salvia R., Fiore M., Aglitti T., Festa F., Ricordy R., Cozzi R., *et al*,.. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Pesticide Biochemistry and Physiology*. 1997;14(1): 143–166. https://doi.org/10.1016/j.aoas.2013.01.005

31. Saraswathy G. R., Maheswari E., Santhrani T., Osman M., Ahmed M., Mahfouz,S., Elaby S., Ashoush I. S., *et al*.. Effects of aqueous extracts from Quercus ilex l. root bark, Punica granatum l. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. *Evidence-Based Complementary and Alternative Medicine*. 2013;4(1): 42–45. https://doi.org/10.1016/j.aoas.2013.01.005

32. Toklu H. Z., Sehirli O., Sener G., Dumlu M. U., Ercan F., Gedik N., Gökmen V., Ibrahium M. I.. Efficiency of pomegranate peel extract as antimicrobial, antioxidant and protective agents. *World Journal of Agricultural Sciences*. 2010;6(4): 338–344. https://doi.org/10.1211/jpp.59.9.0014

33. Singh R. P., Chidambara Murthy K. N., Jayaprakasha G. K.. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry*. 2002;50(1): 81–86.

34. Chetsawang J., Govitrapong P., Chetsawang B.. Hydrogen peroxide toxicity induces Ras signaling in human neuroblastoma SH-SY5Y cultured cells. *BioMed Research International*, 2010.