Journal of Advances in Biology & Biotechnology
5(1): 1-13, 2016; Article no.JABB.21996
ISSN: 2394-1081

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Hepatorenal Protective Effects of Pomegranate (*Punica granatum*) Juice against Nicotine Induced Toxicity in Guinea Pigs

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2016/21996
Editor(s):
(1) James W. Lee, Department of Chemistry and Biochemistry, Old Dominion University, USA.
Reviewers:
(1) Anthony Cemaluk C. Egbuonu, Okpara University of Agriculture Umudike, Nigeria.
(2) Hala Fahmy Zaki, Cairo University, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/12122

Original Research Article

Received 14th September 2015
Accepted 15th October 2015
Published 6th November 2015

ABSTRACT

Pomegranate juice possess a marked antioxidant capacity with a high content in tannins, phenols and flavonoids which can directly or indirectly reduce oxidative damage by preventing the excessive generation of free radicals. The present work aimed to evaluate the effectiveness of pomegranate (*Punica granatum*) juice as a natural source of antioxidants to minimize the harmful effects of nicotine induced hepatorenal toxicity in Guinea pigs. In this study, twenty four adult male Guinea pigs were used for this study and divided into four groups. The first group was control group, the 2nd group was administered the pomegranate juice orally, the 3rd was the experimental and received intraperitoneal injection of nicotine (6 mg/kg body weight /day), the 4th one co-administered intraperitoneal injection of nicotine (6 mg/kg body weight /day) and pomegranate juice orally for 8 weeks. Blood samples were obtained for assessment of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyltransferase activities, total proteins, albumin, and globulin concentrations, albumin concentration/globulin concentration (A/G) ratio, urea, uric acid, creatinine, sodium ions, and potassium ions concentrations. In nicotine treated animals, the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ-glutamyl transferase activities, urea, uric acid, creatinine, and potassium ions concentrations were significantly ($p<0.05$), increased as compared to the control group. On the other hand, serum total

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proteins, albumin, globulin, sodium ions concentrations, and A/G ratio of nicotine treated Guinea pigs were significantly ($p<0.05$) decreased as compared to the control Guinea pigs. Co-administration of pomegranate juice significantly improved all biochemical parameters. It can be concluded that, nicotine had adverse effects on liver and kidney functions parameters in the blood serum. Pomegranate juice administration showed a remarkable amelioration of these abnormalities in nicotine treated male Guinea pigs. It is recommended that the heavy smokers should be advised to take pomegranate juice as a rich source of antioxidant to prevent the hepatorenal toxicity of nicotine. Further studies are necessary to elucidate exact mechanism of hepatorenal protection and potential usefulness of pomegranate juice as a protective agent against nicotine induced hepatorenal toxicity in clinical trials.

Keywords: Hepatorenal protective; hepato-toxicity; renal-toxicity; pomegranate juice; male Guinea pig; nicotine.

1. INTRODUCTION

Nicotine is the principal alkaloid contained in tobacco and it is believed to be the primary reason for cigarette smoking in many people particularly as they derive satisfaction and pleasant sensation from inhaling nicotine [1]. It is a highly toxic organic compound containing nitrogen and alkaloid [2]. People who smoke and also who are exposed to cigarette smoke indirectly by breathing the air in the same environment are exposed to nicotine induced oxidative stress [3,4], Oxidative stress would result in increased free radical injury in the tissue leading to extensive tissue damage with subsequent derangement of cell physiology [5]. As a consequence, these radicals interact with cell components such as lipids, proteins, DNA, RNA, carbohydrates and enzymes [3,4]. During smoking, nicotine is rapidly absorbed into the circulatory system where more than 80% is metabolized in the liver. Liver is an important organ that has many tasks, and is responsible for processing drugs and other toxins to remove them from the body. Nicotine increases the production of pro-inflammatory cytokins that would be involved in liver cell injury [6]. Also, nicotine is hepatotoxic [7-9], and nephrotoxic [10].

The worldwide incidence of chronic renal disease is increasing [11], but access to renal replacement therapy, either transplantation or dialysis is limited in several regions of the world due to a lack of financial and clinical resources [12]. Strategies to delay the onset of dialysis or to attenuate uremia often rely on dietary supplements [13].

The body is engaged in a constant battle against damaging chemicals called free radicals or pro-oxidants to counter the harmful effects of free radicals, the body manufactures antioxidants to chemically neutralize them. However, the natural antioxidant system may not always be equal to the task. Sources of free radicals, such as cigarette smoke may overwhelm this defense mechanism [14]. Nowadays, considerable attention has been devoted to medicinal plants particularly rich in polyphenols, mainly flavonoids and phenolic acids, which exhibit antioxidant properties due to their hydrogen-donating and metal-chelating capacities as potential chemopreventive agents [13,15].

Pomegranate (*Punica granatum* L.) is a long-lived and drought-tolerant plant. Arid and semiarid zones are popular for growing pomegranate trees [16]. They are widely cultivated in Iran, India, and the Mediterranean countries such as Turkey, Egypt, Tunisia, Spain, and Morocco [16,17]. The pomegranate fruit is berry-like with a leathery rind enclosing many seeds surrounded by juicy arils [18]. Pomegranate is an important source of bioactive compounds and has been used for folk medicine for many centuries [19]. This fruit is rich in polyphenols, flavonoids, anthocyanins, punicic acid, ellagitannins, alkaloids, fructose, sucrose, glucose, simple organic acids, and other components and has antiatherogenic [19], antihypertensive, anti-inflammatory [16,20], hepatoprotective [21,22], and renoprotective [13,23,24] properties. *Punica granatum* is used as a medicinal plant, and its fruit concentrate has been used for the prevention and treatment of liver diseases [21]. Also, it can be used in the prevention and treatment of several types of cancer, cardiovascular disease, osteoarthritis, rheumatoid arthritis, and other diseases. In addition, it improves wound healing and is beneficial to the reproductive system. Pomegranate can induce its beneficial effects through the influence of its various bioavailable

constituents and metabolites on gene expression [16]. The antioxidant capacity of pomegranate juice was shown to be three times higher than that of red wine and green tea, based on the evaluation of the free-radical scavenging and iron reducing capacity of the juices [25]. Among the antioxidants, punicalagin and ellagic acid have been identified [26]. Punicalagins possess two isomeric forms in pomegranate: α and β. Punicalagin is an ellagitanin in which the gallagic acid and ellagic acid are linked through a molecule of glucose [27]. Punicalagins and ellagic acid are also responsible for the antioxidant activity and healthy benefits of pomegranates [28]. Pomegranate contains tannins, phenols and flavonoids which can directly or indirectly reduce oxidative damage by preventing the excessive generation of free radicals [29]. The evidence reporting the amelioration by pomegranate juice in nicotine induced hepatorenal toxicity in Guinea pigs are hardly found. So, the present work aimed to evaluate ameliorating effect by pomegranate juice in nicotine induced hepatorenal toxicity in Guinea pigs.

2. MATERIALS AND METHODS

2.1 Chemicals

Nicotine hydrogen tartrate salt [1-methyl-2- (3-pyridyl) pyrrolidine-bitartrate salt] was purchased from Sigma-Aldrich (St. Louis, MO, USA). The drug was dissolved in physiological saline (0.9% sodium chloride) and injected subcutaneously daily with 6 mg, nicotine / kg body weigh for 8 weeks. Nicotine 6 mg/kg body weight was prepared by mixing 60 mg of nicotine in 10 ml normal saline. A total of 1 ml /Kg body weight of the nicotine. The selection of the nicotine dose (6 mg/kg body weight) in the present study was based on approximate the plasma levels reported in heavy smokers [30] and previous published studies [31,32], where the toxic effects of nicotine was confirmed.

2.2 Plant Material and Pomegranate Juice Preparation

Pomegranates *(Punica granatum)* fruits were collected from market of Surman city, West Libya. The plant material was authenticated in botany department, faculty of science, Alejelat, Zawia University, on the basis of taxonomic characters and by direct comparison with the herbarium specimens available at the herbarium of the botany department.

Ten kg of pomegranates were washed and manually peeled, without separating the seeds. Juice was obtained using a commercial blender, filtrated with a Buchner funnel and immediately diluted with distilled water to volume of 1:3 and stored at −20ºC for no longer than 2 months [20,29].

2.3. Animals

Twenty four adult male Guinea pigs (*Cavia porcellus*) weighting 450-600 gm were used for this study. The animals were obtained from animal house unit in the faculty of veterinary medicine, Tripoli University, Libya. The animals were housed in a room under standard conditions of ventilation, temperature (25±2ºC), humidity (60-70%) and light/dark condition (12/12). The animals were provided with tape water *ad libitum* and fed with the standard commercial chow. All animal procedures were performed in accordance with the Ethics Committee of Zawia University and in accordance with the recommendations for the proper care and use of laboratory animals (NIH publication No. 85–23, revised 2007).

2.4. Experimental Design

After one week of acclimation, the animals were randomized and divided into four groups (6 Guinea pigs for each) as follow: Group I (Control group): The animals received intraperitoneal injection of saline (0.5 ml/day) for 8 weeks.

Group II (Pomegranate juice group): The animals received pomegranate juice supplied on dark water bottles and renewed every 2–3 days [20,29] for 8 weeks.

Group III (Nicotine treated group): The animals received intraperitoneal injection of nicotine only (6 mg/kg body weight /day) for 8 weeks.

Group IV (Nicotine/pomegranate juice co-administered): The animals received intraperitoneal injection of nicotine (6 mg/kg body weight /day) and received pomegranate juice as group II for 8 weeks.

At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed under light ether anesthesia, then rapidly dissected and blood samples were drawn by cardiac puncture. The samples were collected in clean dry tubes and centrifuged at 3000 rpm for 15 minutes then serum was separated and kept

in a deep freezer at -20ºC until biochemical measurements were carried out.

2.5 Biochemical Analysis

The activities of Alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in serum according to the methods described by Reitman and Frankel [33]. Serum alkaline phosphatase (ALP) activity was determined according to Kind et al. [34]. Serum γ-GT activity was determined according to the method of Szas [35].

Serum total proteins concentration was determined according to Biuret method explained by Weichselbaum [36]. Serum albumin concentration was determined according the method of Doumas et al. [37]. Serum globulin concentration was determined according to the formula: Globulin = total protein – albumin.

The ratio of serum albumin concentration /globulin concentration (A/G) was determined as albumin/globulin level. Serum urea measurement was based upon the cleavage of urea with urease [38]. Serum uric acid was determined [39]. Serum creatinine was measured without protein precipitation [40]. Sodium concentration in serum was determined by colorimetric method according to Trinder [41], and Maruna [42]. Potassium concentration in serum was determined by turbidimetric tetraphenylborate method according to Hoeflmayr [43].

2.6. Statistical Analysis

The values were presented as means ± SD of different groups. One-way analysis of variance (ANOVA) was carried out. For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (SPSS version 17.0).The results were considered statistically significant when $p < 0.05$.

3. RESULTS

Guinea pigs that received intraperitoneal injection of nicotine only (6 mg/kg body weight /day) for 8 weeks had significantly ($p<0.05$), increased the serum alanine aminotransferase, aspartate amino-transferase, alkaline phosphatase and γ- glutamyl- transferase activities, urea, uric acid, creatinine, and potassium ions concentrations parameters as compared to the control Guinea pigs. Co-administration of nicotine with pomegranate juice were significantly ($p<0.05$) prevented the changes recorded in serum liver function serum enzymes activities, and serum kidney function parameters as compared with control group (Fig. 1-4 & 9-11). On the other hand, serum total proteins, albumin, globulin, sodium ions concentrations, and A/G ratio of nicotine treated Guinea pigs were significantly ($p<0.05$) decreased as compared to the control Guinea pigs (Fig.5-8 &12). Co-administration of nicotine with pomegranate juice were significantly ($p<0.05$) prevented the changes recorded in serum total proteins, albumin globulin, sodium ions concentrations, and A/G ratio as compared with control group.

4. DISCUSSION

Nicotine which is a major toxic component of cigarette smoke has been shown to produce diffuse damage to endothelium and plays a major role in the development of numerous human disease or disorders [44]. Overproduction of reactive oxygen metabolites and a reduction in antioxidant mechanisms have been reported due to acute or chronic smoke exposure [45].

The present study demonstrated that nicotine treatment caused significant increases in the serum ALT, AST, ALP and γ-GT activities and decreases in the serum total proteins, albumin, globulin concentrations, A/G ratio and indicating impaired liver function. Similar results were also reported by Jang et al. [46] and Sharif et al. [47]. Fahim et al. [48] reported rise in both hepatic ALT and AST levels following i.p nicotine injection (1 mg/Kg) for 3 weeks in mice. Another study observed over expression of ALP level and other genes involved in osteoblast maturation and differentiation in osteoblasts in response to subtoxic nicotine administration in humans [49]. Also, Mahmoud and Amer [50] found that significant elevations in the activities of ALT, AST, and alkaline phosphatase in liver homogenate of nicotine treated rats compared with control group. These results may be attributed to the state of hypoxia of the parenchyma for contracting fibrous tissue and the increased permeability of hepatic cell membrane due to nicotine treatment which release ALT enzyme into the circulation. The increased level of ALT is marked as liver parenchymal cell destruction induced by nicotine treatment. The elevation in serum ALT activity observed in the present study may reflect

hepatotoxic potency of subchronic exposure of nicotine on liver. This effect could be an essential process for the liver to restore the balance of different free amino acids that might have been disturbed throughout recovering mechanisms. It has been established that the liver is the sole source for the synthesis of albumin, fibrinogen and most of Alpha and β globulins, while the immunoglobulin are formed in the lymphoid tissues by the plasma cell [51]. Accordingly, the liver affected by nicotine may suffer from dysfunctions and this may modify the synthesis and metabolism of proteins. This might explain the significant decrease observed in the serum albumin, globulin and total proteins in Guinea pigs treated with nicotine. The results are also in accordance with the work of Sershen et al. [52] who found that, injection of nicotine produced inhibition of protein synthesis.

The elevated serum levels of urea and creatinine indicate reduced ability of the kidney to eliminate the toxic metabolic substances [53]. Nicotine and its metabolites are eliminated from kidney, these organs are adversely influenced by nicotine. Membrane lipids are vital for the maintenance and integrity of cell function, the breakdown of membrane phospholipids and lipid peroxidation due to the generation of free radicals are expected to change membrane structure, fluidity, transport and antigenic properties, all of which play an important role in the pathogenesis of organ disorders [54]. Indeed, increasing evidence suggests that chronic cigarette smoking adversely influences the prognosis of nephropathies [54,55].

In the present study, the serum urea, creatinine and uric acids were significantly increased in Guinea pigs treated with nicotine compared with control animals suggesting an impairment of kidney function. The observed alterations in renal function parameters are in line with the reports by these findings are agreement with the results of other studies [10 & 56-59]. This is agree with the findings of Ahmed et al. [60] who found that the levels of creatinine and urea were significantly higher in smoker group when compared with the control group. There is evidence that an increase in renal retention of uric acid can occur in cases of acute or chronic renal disease/ failure [61]. Several mechanisms may be operative in inducing renal vasoconstriction and vascular damage. Nicotine increases plasma levels of vasoconstrictors including catecholamines, arginine, vasopressin and endothelin-1 [62]. Cigarette smoke damages endothelial cells, and nicotine induces smooth muscle cell proliferation [63]. Other study attributed the renovascular resistance to activation of the sympathetic nervous system [64]. These effects could be attributed to changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [65].

The present study shows that, treatment of Guinea pigs with nicotine were caused a significant decrease in serum sodium ions and increase in serum potassium ions concentrations compared with control group. This is in agreement with Hozayen et al. [66] who found that, the administration of aspartame showed a highly significant decrease in serum sodium concentration and increasing in potassium concentration when compared to normal rats, this action may be due to inhibition of Na+, K+- ATPase activity. The Na+, K+- ATPase is a complex membrane protein that utilizes ATP to transport three Na+ ions out of cells and two K+ ions in against their concentration gradients [67].

Co-administration of nicotine with pomegranate juice were significantly ($p<0.05$) prevented the changes recorded in serum ALT, AST, ALP and ɣ-GT activities, total proteins, albumin, globulin concentrations, and A/G ratio as compared with control group. This is in agreement with many authors [68 - 73]. The results are consistent with Darwish et al. [74] who found that administration of pomegranate Juice in parallel with aspartame protected rats from aspartame-induced oxidative injury and ameliorated liver function. Also, administration of yoghurt supplemented with pomegranate juice is acceptable as functional food alleviates the harmful effect of CCl_4-induced liver injury and offers a pleasant and effective route in increasing the total phenolic content and antioxidant intake in our daily diet [73]. Antioxidant properties in pomegranate are due to polyphenols, including ellagic acid in the free form linked to glycosides, galutanins, anthocyanins (cyanidin, delphinidi, pelargonidin glycosides) and other flavonoids (quercetin, kaempferol and luteolin glycosides) [75,76]. In our study, pomegranate juice alleviates the harmful effect of nicotine induced hepatotoxicity may be due to antioxidant properties.

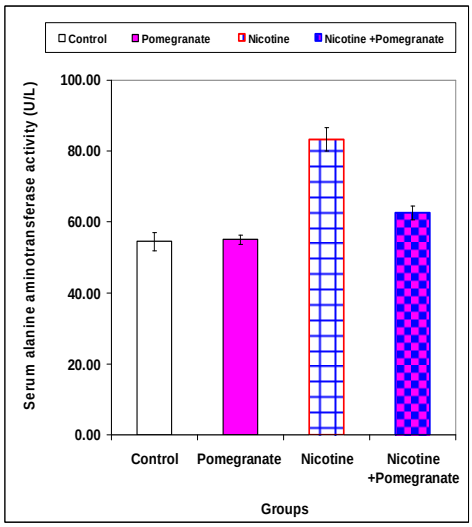

Fig. 1. Serum alanine aminotransferase activity in different animals groups

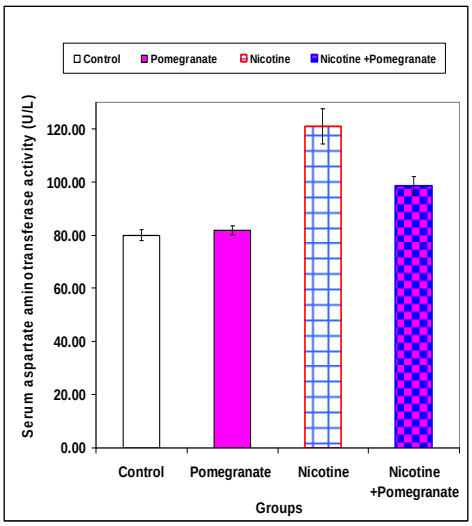

Fig. 2. Serum aspartate aminotransferase activity in different animals groups

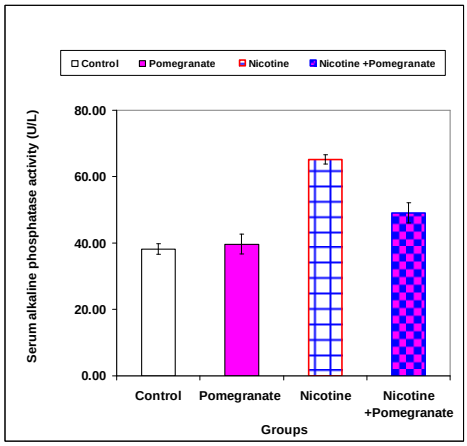

Fig. 3. Serum alkaline phoasphatase activity in different animals groups

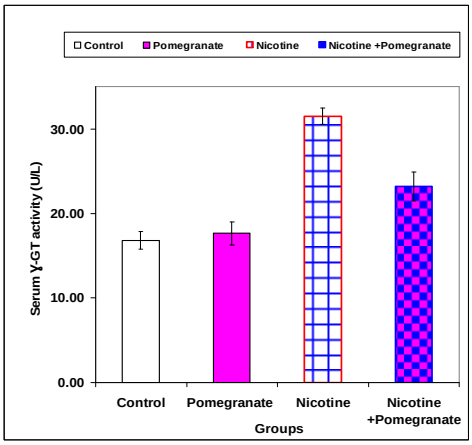

Fig. 4. Serum Ɣ-glutamyltransferase activity in different animals groups

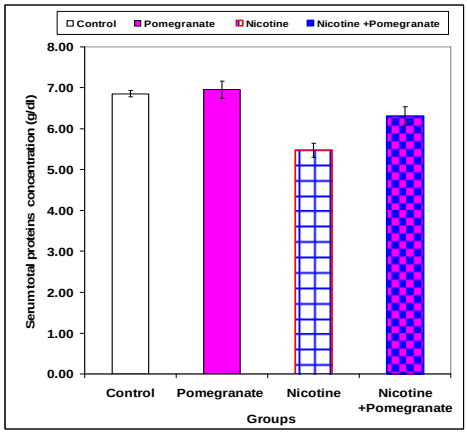

Fig. 5. Serum total proteins concentration in different animals groups

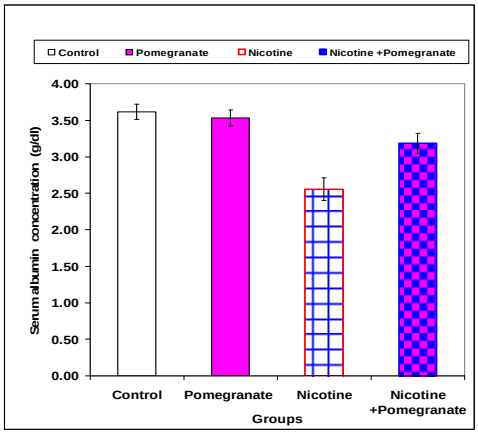

Fig. 6. Serum albumin concentration in different animals groups

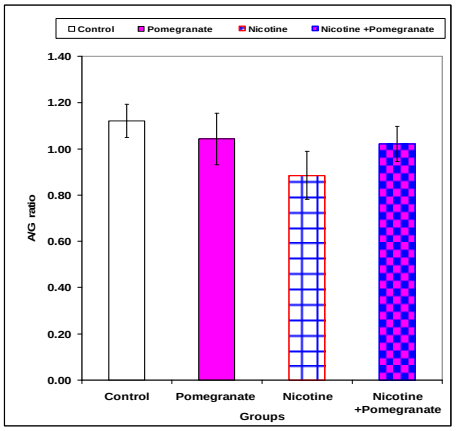

Fig. 7. Serum globulin concentration in different animals groups

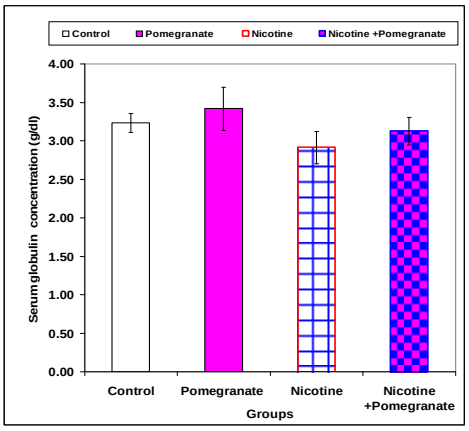

Fig. 8. Serum A/G ratio in different animals groups

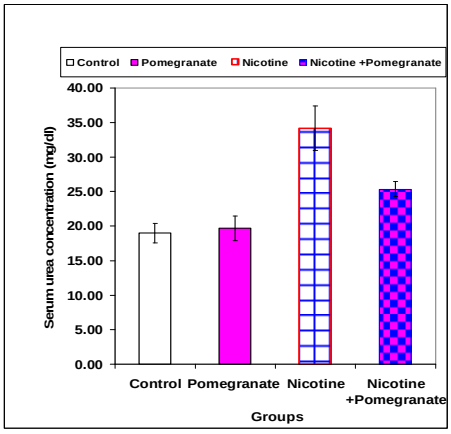

Fig. 9. Serum urea concentration in different animals groups

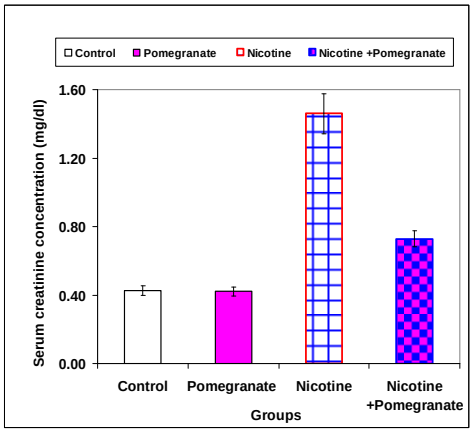

Fig. 10. Serum creatinine concentration in different groups

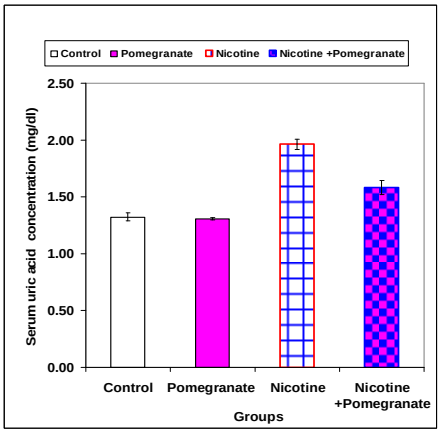

Fig. 11. Serum uric acid concentration in different animals groups

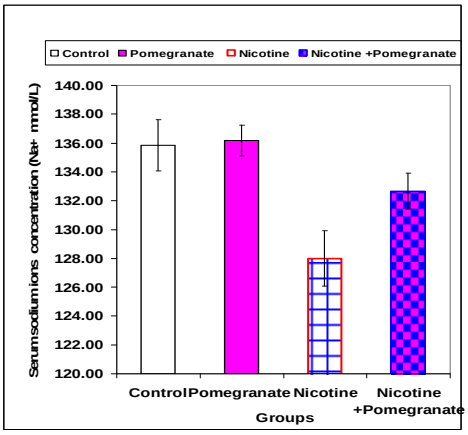

Fig. 12. Serum sodium ion concentration in different groups

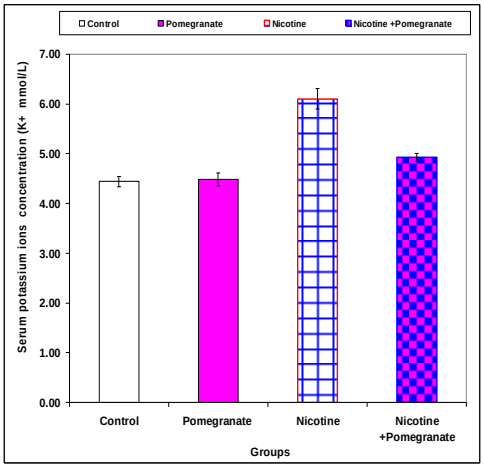

Fig. 13. Serum potassium ion concentration in different animals groups

In the current study, co-administration of pomegranate juice to animals treated with nicotine were significantly decreased the serum urea, creatinine and uric acid compared with nicotine treated group. This is in agreement with Ali and Saeed [77] who found that co-treatment of aqueous extract of pomegranate (*Punica granatum*), attenuated gentamicin-induced renal oxidative damage in rats. The nephroprotective effect of pomegranate extracts may be related to different mechanisms. One of these mechanisms is the antioxidant property of P. throug scavenger of free radicals released as a consequence of oxidative damage as reported in numerous studies [24,78]. Aviram et al. [79], and Yasoubi et al. [80], confirmed that the antioxidants, polyphenols are rich in pomegranate and they are more potent, on a molar basis, than many other antioxidants, like vitamins C and E and coenzyme Q10. Pomegranate is an important source of anthocyanins, hydrolysable tannins punicalagin and punicalin [81] ellagic and gallic acids [82] and also contains vitamin C [83]. Also, El-Habibi, [13] reported that, the obtained improvement in renal physiology of adenine-treated rats co-administered with pomegranate juice can be attributed to its high phenolic content and the mechanism of action may be through induction of various antioxidant enzymes and scavenging reactive oxygen species. Furthermore, another mechanism may be through anti-inflammatory and different signaling pathways [13]. A recent studies by Huang et al. [23] and Singh et al. [24] who reported that the renoprotective effects of pomegranate involve the activation of nitric oxide-dependent and peroxisome proliferator-activated receptor (PPAR-γ) signaling pathway. The protective role of nitric oxide (NO) in different models of renal failure has been documented [84], including glycerol-induced renal failure [85] and nephrolithiasis induced by ethylene glycol [86]. These studies have demonstrated that levels of NO are decreased in glycerol-induced renal failure and different agents have shown to produce renoprotection by increasing the NO production [13].

In the present study, co-administration of pomegranate juice to animals treated with nicotine were significantly decreased the serum potassium ions and increased sodium ions concentration compared with nicotine treated group. This is in agreement with Hozayen et al. [66] who reported that, the treatment of aspartame administered rats with rosemary extract induced a significant increase in serum sodium and decrease in potassium levels in comparison with corresponding groups. This may be due to the antioxidant properties of extracts of rosemary leaves. In the present study, these biochemical observations were suggested that pomegranate juice significantly attenuated nephrotoxicity and hepatotoxicity by the way of its antioxidant, radical-scavenging, and antiapoptotic effects.

5. CONCLUSION

The present study, concluded that, nicotine had adverse effects on the liver and the kidney functions parameters in the blood serum. Pomegranate juice administration showed a remarkable amelioration of these abnormalities in nicotine treated male Guinea pigs. It is recommended that the heavy smokers should be

advised to take pomegranate juice as a rich source of antioxidant to prevent the hepatorenal toxicity of nicotine. Further studies are necessary to elucidate exact mechanism of hepatorenal protection and potential usefulness of pomegranate juice as a protective agent against nicotine induced hepatorenal toxicity in clinical trials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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