

دور زيت بذور الرمان ضد التسمم الكبدي الناجم عن فلوريد الصوديوم في في اناث الجرذان المعامله

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المستخلص

لقد تم تصميم هذا العمل لتقييم التأثير الواقي للكبد لزيت بذور الرمان (PSO) ضد التسمم الناجم عن فلوريد الصوديوم (SF) في إناث الفئران البالغة. تم تقسيم عشرين من إناث الفئران البالغة الى أربع مجاميع متساوية وعوملت يوميا لمدة 40 يوما على النحو التالي: المجموعة C اعطيت ماء الصنبور، وعدت كمجموعه سيطرة، مجموعة T1: أعطيت فلوريد الصوديوم (120ملغم / لتر) مع مياه شرب الحنفية، مجموعة T2: اعطيت كل من فلوريد الصوديوم (120ملغم / لتر) مع مياه شرب الحنفية و زيت بذور الرمان بتركيز 30 ملغم /كغم من وزن الجسم اما المجموعة T3: زيت بذور الرمان بتركيز 30 ملغم /كغم من وزن الجسم. تم جمع عينات الدم بعد تجويع الحيوانات للأيام 0، 20 و 40 يوما من التجربة لتقدير بعض القياسات البيوكيميائية والمؤشرات الحيوية الاكسدة. اضافة إلى ذلك، في نهاية التجربة تم اخذ عينات من الكبد للدراسة النسيجة المرضية. اظهرت النتائج أن فلوريد الصوديوم في المجموعة T1 تسبب في زيادة كبيرة في نشاط الأنزيمات الناقله للأمين (ALT و AST)، وتركيز الكولسترول الكلي، البيليروبين الكلي، والبيروكسي نايتريت في مصل الدم، بينما كان تركيز الكلوتاثاين منخفضا. على النقيض من ذلك تسبب زيت بذور الرمان في التخفيف من وطأة القصور الوظيفي الكبدي الناجم عن فلوريد الصوديوم في مجموعة T2 حيث تجلى ذلك من خلال ارتفاع كبير في تركيز الكلوتاثاين، بالإضافة إلى انخفاض كبير في نشاط الأنزيمات الناقله للأمين، وتركيز الكولسترول الكلي، والبيروكسي نايتريت والبيليروبين الكلي في المصل. في المقابل، أدى زيت بذور الرمان (مجموعة T3) استعادة ما يقارب اغلب هذه المعايير إلى مستويات قربه او أدنى من المستويات الطبيعيه. فضلا عن ذلك اظهر الفحص النسيجي للكبد في المجموعه T1 جميع الخلايا المتعددة النواة، تكاثر خلايا الكبد، قطرات دهنيه في الهيولى مع تجمع الخلايا اللمفاوية والبلعمية. كل هذه التغييرات تم تحويلها في انسجة كبد المجموعه T2 نتيجة لمعاملة الفئران بزيت بذور الرمان. في حين لم تلاحظ افات مرضية واضحة في المجموعه T3. يستنتج من نتائج هذه الدراسة الني وثقت الأثر المفيد لزيت بذور الرمان ضد الآثار الضارة لفلوريد الصوديوم على وظائف الكبد في اناث الفئران البالغة.

الكلمات المفتاحيه : زيت بذور الرمان ، فلوريد الصوديوم ، اختبارات وظائف الكبد ، الكلوتاثاين ، بيروكسي نايتريت،الجرذان .

Introduction

Sodium fluoride (SF) was originally used in the 1930s as a wood preservative (18), in pesticides, various types of adhesives and glues (46). The adverse effects of SF are possible at fluoride levels far above the recommended dosage (13). In contaminated areas, fluoridated water is the major source of fluoride, after absorption the fluoride cleared by the kidneys (68). The compounds of sodium fluoride in the vari-

ous formulations have several caries-protective mechanisms (21)) also fluoride reduces the decay of teeth enamel by remineralization of enamel and teeth (64).

Recent evidence suggested that excessive fluoride intake may be contributing to a wide range of adverse health effects (70). As the fluoride cross the cell membranes causing structural and functional changes leading to fluorosis of bones associated with bone cancer (62) and dental and skeletal fluorosis (54) . At the same time, (50) explained that cattle and buffalo suffering from signs of dental discoloration and bony lesions when browse in area contaminated with fertilizer. Evidently, fluoride inhibits some enzymes involved in metabolic pathways and fatty acid oxidation (28) ,as well as, fluoride cause change of lipid peroxidation (LPO) (71), lipids profile (56) and inhibits certain total antioxidants capacity with increase generation of oxygen free radicals (1) . Moreover, some studies reported that SF caused a decreased protein content in liver and serum of mice and rats exposed to SF (5 and 30).

Pomegranate (*Punica granatum L.*) is used in folkloric medicine for treatment of different diseases and has gained an attention in complementary and alternative medicine due to pomegranate have a wide range of phytochemicals (31) which including: flavonoids, proanthocyanidins and hydrolysable tannins, sterols, triterpenoids, and alkaloids (15). Besides (20) reported that pomegranate seed oil (PSO) is a major source of polyunsaturated fatty acids (PUFAs) with a low saturated fatty acid which is an important for therapeutic uses . However, pomegranate juice (PJ) supplementation has been shown to alleviating the coronary heart disease (61) , Alzheimer's disease (59), against diarrhea and intestinal parasites (11), to prohibit prostate cancer metastasis(69) and used as anti-inflammatory, hepatoprotective activities ,improved lipid profile and glucose metabolism (26,41 and 43) as well as a cardioprotective effects of PSO in methionine overload rabbits (2). Therefore, this study was aimed to investigate the hepatoprotective effect of pomegranate seed oil through alleviating the deleterious effects of sodium fluoride in female rats.

Materials and Methods

Twenty female adult Wistar rats weighed 219.5 - 250.1 g. were used in this investigation . Animals were obtained from the animal house of the College of Veterinary Medicine , University of Baghdad. They were housed in plastic cages in a conditioned room (22-25 °C) in the animal house of the College of Veterinary Medicine - University of Baghdad. They were left for two weeks for adaptation with the experimental conditions. Animals had free access to water and standard pellets diet along and were divided randomly into four equal groups (5/group), rats were treated daily as follows for 40 days :Group C: rats were administered distilled water, serving as control group, group T1: rats were received sodium fluoride 120 mg/liter in tap water , group T2: rats were subjected to sodium fluoride 120 mg/liter in tap water plus administered PSO (Pometone-Vita) 30mg/kg BW. orally and rats in group T3 rats received pometone 30mg/kg BW. orally. Fasting blood samples was drawn by cardiac puncture from anesthetized rats (by using ketamine 90 mg/kg B.W and xylazin 40

mg/kg B.W.) at 0, 20 and 40 days of the experimental in gel tubes, then serum samples were isolated and frozen at -18 °C until determination of: serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined calorimetrically (53) using enzymatic kit (Bio system, Spain) , total cholesterol (TC) was measured using enzymatic assay kit according to (17) total bilirubin (TB) was measured using bilirubin kit (Bio System, Spain) according to (51), peroxynitrite radical was evaluated as described by (66) and reduced glutathion (GSH) was determined according to (12) using standard GSH curve (figure-1). At the end of experiment rats were anesthetized, sacrificed , then livers were preserved in 10% neutral formalin buffer solution . Several tissue sections were prepared from the liver of individual rats and stained with Hematoxylin – Eosin stain according to (36).

Statistical analysis

Collected data were subjected to Two-Way (ANOVA) followed by Least significant differences test (LSD) using of SAS® software (SAS, 13). All values are expressed as mean ±SE and a significant were tested at $P < 0.05$ (60) .

Standard GSH Curve:

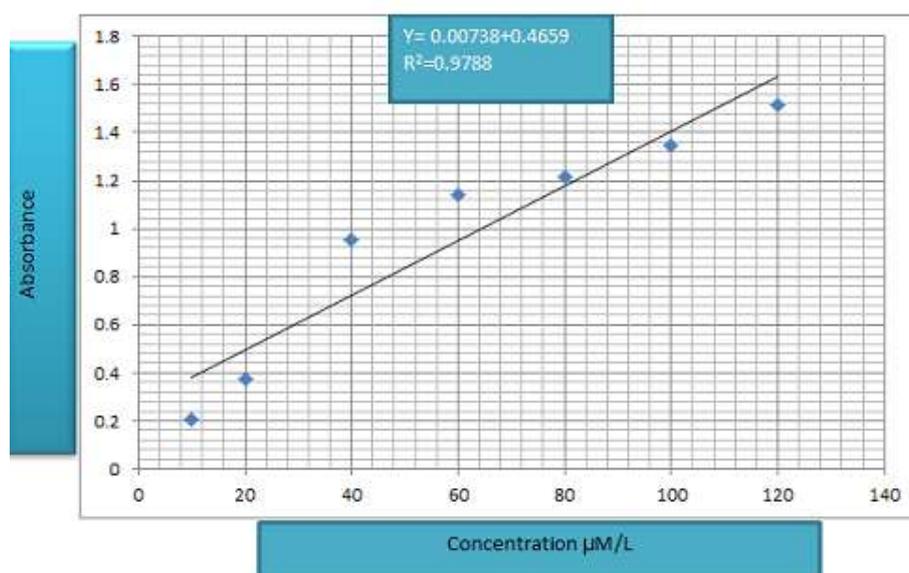


Figure (1): Standard GSH curve

Results

A markedly increase ($P < 0.05$) in serum aminotransferase (ALT and AST) activity were showed after 20 and 40 days post SF administration in T1 group as compared with control, T2 and T3 groups (figures - 2A and 2B). Comparing to group T1, exposure of female rats with SF in drinking water concurrently with oral intubation of PSO for 40 day caused a marked significant ($P < 0.05$) decrease in serum ALT and AST activity along the experiment. The result also revealed that rats received PSO alone afforded a slight significant ($P < 0.05$) increase in these parameters during two experimental periods compared to the control group. Treatment of normal rats with SF (group T1) for 20 and 40 successive days elicited a significant increase ($P < 0.05$) in serum TC concentration as compared to group T2, T3 and control groups. Meanwhile, at the end of the experiment rats treated with SF and PSO exhibited a significant ($P < 0.05$) decrease in TC concentration as compared to group T1 (figure- 2C). Administration of PSO to female rat caused a significant ($P < 0.05$) decrease in TC concentration at 20 and 40 days of the experiment as compared with other treated groups.

A significant ($P < 0.05$) increase in serum total bilirubin concentration was reported at 20 and 40 day in T1 group as compared with control, T2 and T3 groups. Meanwhile, oral intubation of rats with PSO concurrently with SF along the experimental period caused a significant ($P < 0.05$) decrease in this parameter in group T2 comparing to T1 and control groups (figure-2D). With exception of T2, PSO caused a significant ($P < 0.05$) differences in serum TB in T3 group after 40 day of the experiment as compared to control and T1 groups. As shown in figure-2E, serum peroxynitrite radical concentration recorded a significant ($P < 0.05$) elevation in group T1 versus baseline in control, T2 and T3 groups. Besides, serum peroxynitrite concentration reduced significantly ($P < 0.05$) in group T2 at two treated periods compared to T1 group. While a low significant reduction ($P < 0.05$) in this parameter was observed in group T3 at the end of the experiment. Figure (2F) clarified a significant ($P < 0.05$) decrease GSH concentration in group T1 at days 20 and 40 of the experiment as compared with control and other treated groups. In the same figure, the results showed a non-significant ($P > 0.05$) differences between T2 and T3 as compared between each other's, which indicate the beneficial effect of PSO against SF.

Liver sections of rats received SF(group T1) showing aggregation of mononuclear cells (MNCs) around the bile duct associated with necrosis of surrounding hepatocyte (figure-4) with polymorphonuclear cells (PMNCs) in congested blood vessels (figure-5) and mild cytoplasmic fat droplets in hepatocyte with vaculation around dark nuclei granulomatous lesion was also seen (figure-6) as compared to control (figure-3). Whereas liver sections of rats treated with SF plus PSO showing moderate infiltration of MNCs in liver parenchyma with granulomatous lesion consists mainly of macrophage were reported (figure-7,8). As well as, aggregation of magrophages with slight Kupffer proliferation was reported. However, in this area treated of rats with PSO showed appearance of megakaryocytes accompanied with moderate prolifera-

tion of Kupffer cells (figure-9) ,besides no pathological lesions were noted in other sections (figure10) .

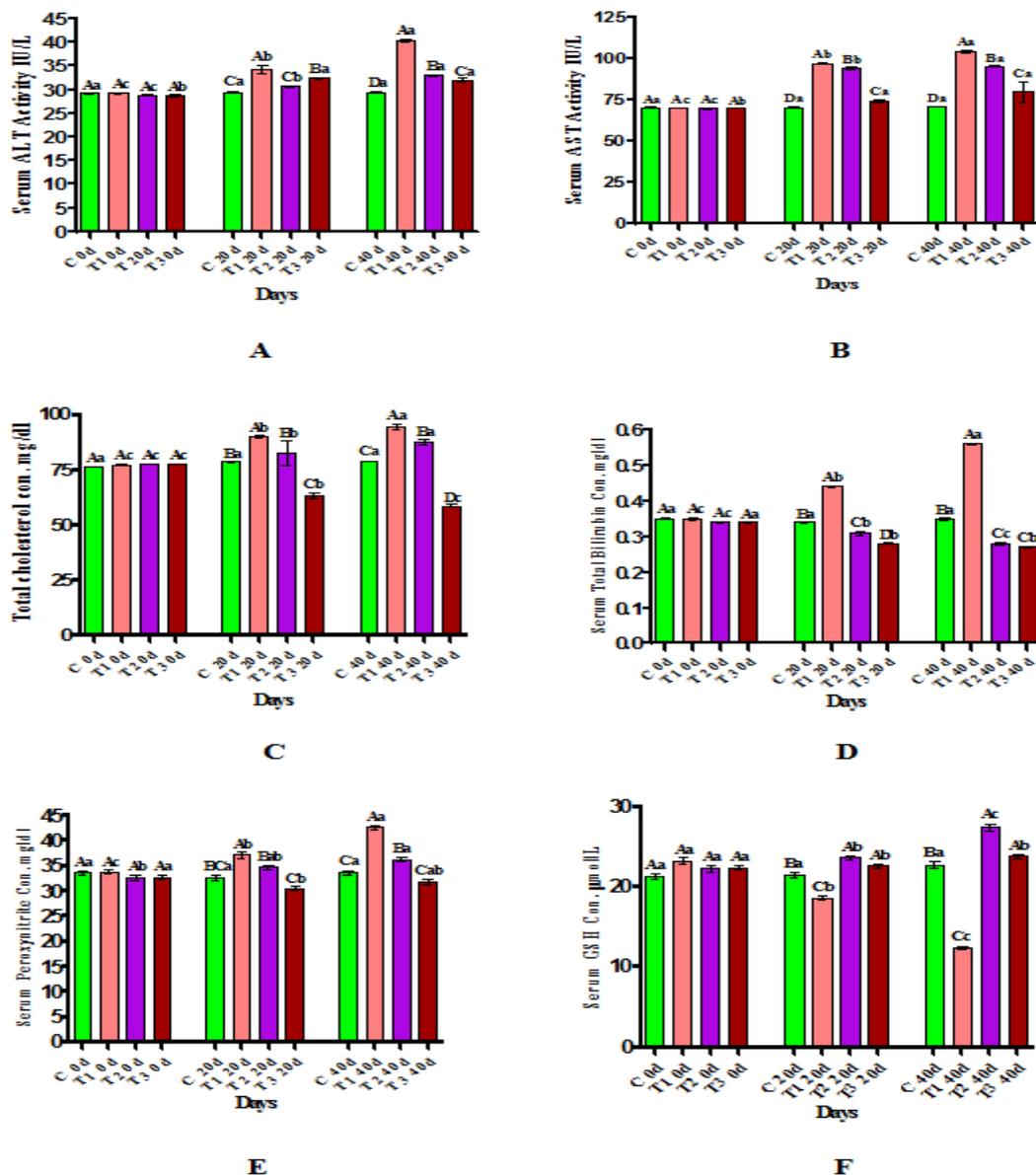


Figure (2) : Effect of pomegranate seed oil (PSO) on serum biochemical tests in female rats treated with sodium fluoride.

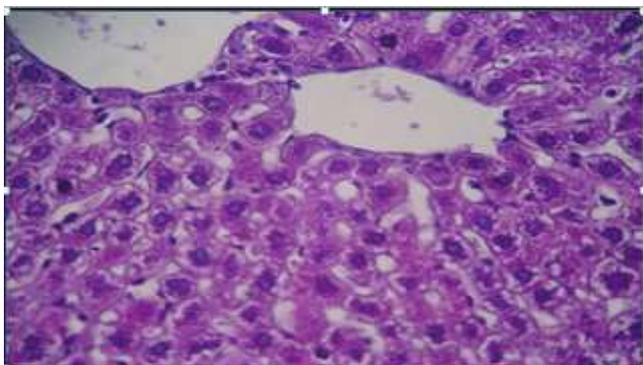


Figure-3: Photomicrographs showing histology of liver tissue from control rat. Note normal characteristics feature of the liver (H -E X 40).

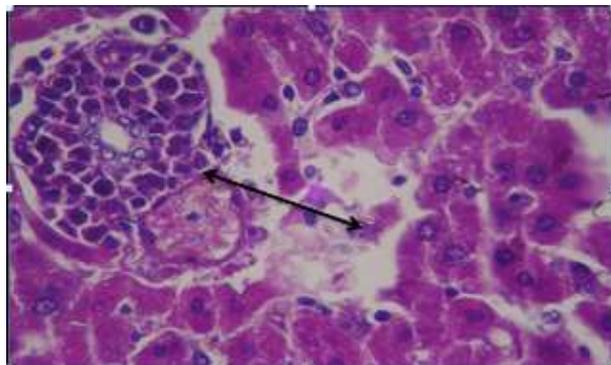


Figure-4 : Photomicrographs showing histology of liver tissue from group T1 showed MNCs aggregation around bile duct with necrosis of surrounding hepatocytes \longleftrightarrow (H/E X 40)

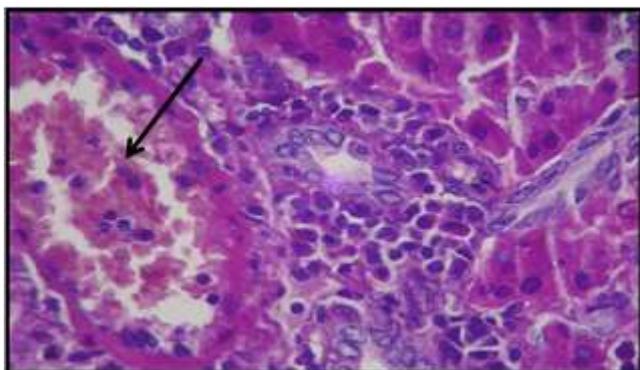


Figure-5: Photomicrographs showing histology of liver tissue from group T1 showed presence of PMNCs in the dilated and congested blood vessels mainly in portal area (H /E X 40). \longrightarrow

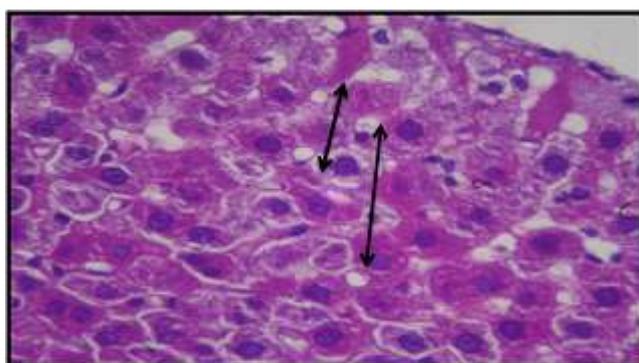


Figure-6: Photomicrographs showing histology of liver tissue from group T1 showed cytoplasmic fat droplet in the hepatocyte seen around dark nuclei \longleftrightarrow (H -E X 40).

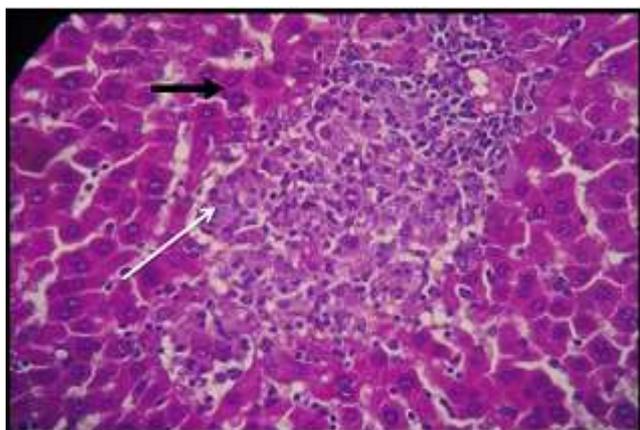


Figure-7: Photomicrographs showing histology of liver tissue from group T2 showed moderate MNCs infiltration in liver parenchyma consist mainly of macrophage → (H-E X 10).

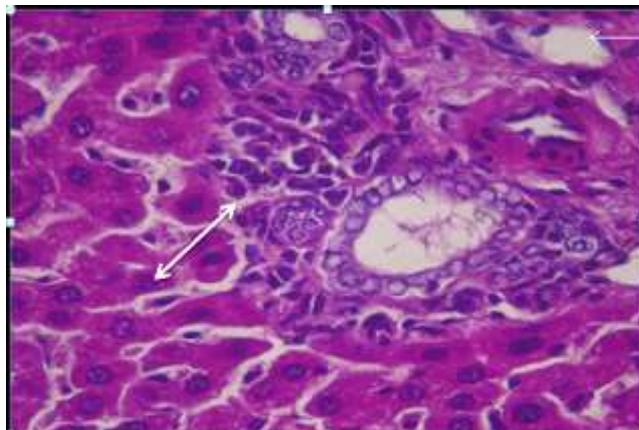


Figure-8: Photomicrographs showing histology of liver tissue from group T2 showed focal MNCs periductal aggregation consist mainly of macrophage with slight kupffer cells proliferation (H-E X 40). ↔

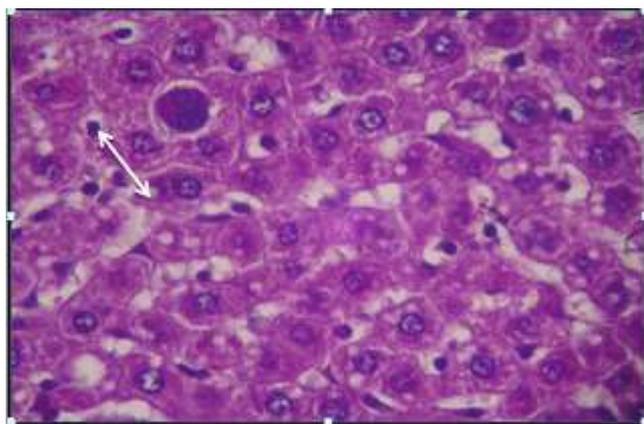


Figure-9: Photomicrographs showing histology of liver tissue from group T3 showed proliferation of kupffer cell accompanied with appearance of megakaryocyte in hepatic parenchyma ↔ (H/E X40)

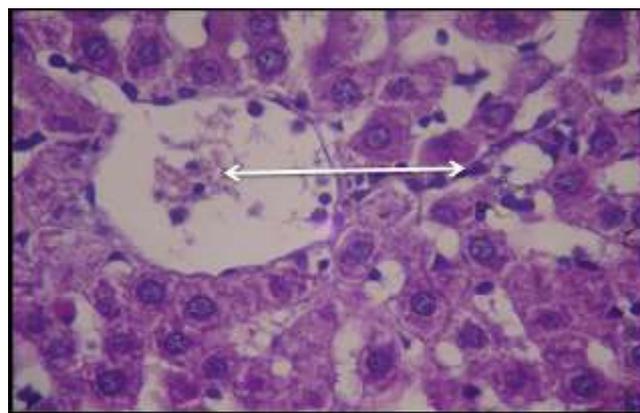


Figure-10: Photomicrographs showing histology of liver tissue from group T3 showed few MNCs proliferation in the dilated sinusoid and central vein ↔ (H-E X 40).

Discussion

Elevation in serum aminotransferases (ALT and AST) coupled with a decrease in GSH level and changes in hepatic functions was observed in group T1 as compared with other groups. The catalysis of aminotransferase reactions is considered to be a marker of hepatocellular dysfunction. The result of the current study is in agreement with other studies such as (35 and 47). Moreover, the fluoride toxicity was found to cause a significant increase in aminotransferase activity, as reported in Cattle (39) and in goat (58). SO these elevation could be due to a secondary event following SF induced LPO of hepatocyte membranes with the subsequent increase in the leakage

of these biomarkers from the liver tissue (4). However, an increase of apoptosis consequent to the exposure of fluoride has been reported in various mammalian cells (25 and 29) as well as, the increase in the cytochrome C release from mitochondria and the activation of both the intrinsic and extrinsic pathway of cell death have been reported in SF exposure (8), which was accompanied with a decrease in the antioxidant status of liver leading to impairment of its function (7). The present study revealed the hepatoprotective activity of PSO via a decrease in the ALT and AST level in group T2 as compared with SF treated rats and returned to normal range. This result in agreement with (42 and 48). Thus, it could be concluded that PSO protects hepatocyte from the dangerous effects of SF and decrease leakage of these enzymes may be due to a specific modulation of hepatocytes and/or enzymes by its phytochemical compounds (3).

The results showed a significant elevation in serum TC in SF treated group (T1) indicated hepatotoxicity with degenerative and hepatic cells necrosis. The result of this study agrees with that of other researchers (1 and 33). It has been found that fluoride inhibit the activity of lipases enzymes such as triglyceride lipase, unspecific esterase and pyrophosphates leading to changes in lipid metabolic profile (14), moreover, fluoride intoxication in rats caused an increased in the activity of HMG-CoA reductase due to deficiency of insulin (55) leading to excessive production and accumulation of cholesterol resulting in the formation of foam cells (16). Furthermore, administration of SF caused an increase in LPO (71) which might be important determinants of altered lipid metabolism and associated with the hyperlipidemia. Hence, abnormal enzyme activities seem to be one of the major factors responsible for the rise in serum cholesterol and triglycerides (38). In agreement with (2 and 44), serum TC was decreased in PSO treated group indicating its hypolipidemic effects through decreased total and LDL-cholesterol versus baseline (19).

An increase in serum bilirubin concentration in SF treated rats could be due to the destruction of R.B.Cs and /or damage of liver tissues (52). Because cell membranes of the erythrocytes are sensitive to the presence of free radicals thus, used as an oxidative stress biomarker (24). Excessive evidence demonstrated that SF initiates and produce LPO (as mentioned above) of plasma membrane leading to hepatocytes injury accompanied with alteration in antioxidant enzymes (72) and an increase in MDA level of these tissues (57). Therefore, liver injury and hemolysis of RBCs (22) in group T1 may lead to hyperbilirubinemia. However, the mechanism by which SF caused an increase in serum bilirubin still unknown. Overall, this study findings that PSO supplementation caused a significant decrease in serum bilirubin in T2 and T3 treated groups versus control. Many studies reported that pomegranate is an important source of anthocyanin, ellagic acid, gallic acid, vitamin C and flavonoids (9) exhibited a protective effects against oxidative damage and considered to be as hepatoprotective (32 and 73), improve the intestinal barrier functions in obstructive jaundice (62) and protects liver from fibrosis due to biliary obstruction (65) through augmented the antioxidant defense mechanism and increases the erythrocyte activity (34). Herein, the ameliorative effect of PSO against SF toxicity may be maintained

the structural integrity of liver cells membrane, documented by the absence of histopathological changes in treated groups (6).

The results also showed changes in biomarkers of oxidative stress as evidenced by a decrease of reduced glutathione and an increase in peroxynitrite concentrations. Fluoride is known to reduce intracellular GSH levels and inhibit various enzymes that require GSH as a cofactor (67). Besides, (1) reported that, a reduction in GSH content and in the activity of antioxidant enzymes in the liver of rats exposed to SF indicating an impaired function of the hepatic antioxidant defense system (27) associated to an increase of LPO (72). This deterioration overlaps with the elimination of H₂O₂ and LPO products and causes their accumulation in the cells leading to the damage of cell membranes and may affect the activity of these enzymes which constituting the cell anti-oxidative system (37). Besides, fluoride inhibits glucose-6 phosphates dehydrogenase (G6PD) by an oxidative damage, and then subsequent decrease of pentose phosphate pathway flow could make the cell unable to maintain the normal GSH/GSSG ratio, which is lowered by fluoride (10).

Peroxynitrite is an oxidant agent, formation of peroxynitrite *in vivo* has been ascribed to the reaction of the free radical superoxide with the free radical nitric oxide (63). So after long periods of time will resulted in destruction of cellular constituents leading to the dysfunction of cellular processes, then induction of cell death through both necrosis and apoptosis (49) lead to the depletion of anti-oxidant enzymes (63). So, such results could be responsible for histopathological changes. As shown in the current study, the antioxidant protective activity of PSO was documented in groups T2 and T3 appeared to an improvement of oxidant/antioxidant status (2 and 48) via reducing the LPO (45) which having an effect on the scavenging capacity of superoxide anion and hydrogen peroxide (40). Thus, it could be concluded that the presence of polyphenolic compounds in the pomegranate could be act as a potent antioxidant agents and may be responsible to maintain membrane integrity and prevent liver injury against SF.

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