

Effect of Pomegranate Seed Oil against Hepatotoxicity- Induced by Sodium Fluoride in Adult Female Rats (Part II).

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Summary

This experiment was designed to evaluate the hepatoprotective effects of pomegranate seed oil against toxicity- induced by sodium fluoride and in normal rats. Twenty adult female Wistar rats were divided into four equal group and treated daily for 40 days as follows: Group C administered tap water and served as control , group T1 : received sodium fluoride 120ppm in drinking tap water, group T2: received both sodium fluoride 120ppm in drinking water and administered orally pometone (pomegranate seed oil) 30mg/kg B.W. and group T3 : administered pomegranate seed oil as in group T2 orally. Fasting blood samples were collected at 0, 20 and 40 days to estimation of some biochemical parameters and oxidative stress biomarkers . In addition, sections from liver were taken at the end of the experiment for histopathological study. The results revealed that SF (group T1) caused a significant increase in serum aminotransferases (serum alanine aminotransferase and aspartate aminotransferase) activities, total cholesterol ,total bilirubin and peroxy nitrite radical concentrations, while GSH concentration was a significantly decrease. PSO caused an alleviation to the hepatic dysfunction induced by sodium fluoride (group T2) manifested through significant elevation of GSH concentration, in addition, a significant reduction in serum transaminases activity, total cholesterol, peroxy nitrite radical and total bilirubin concentrations. In contrast, administration of pomegranate seed oil (group T3) showed no alterations in most of these parameters. Furthermore histopathological examination of liver tissues of group T1 manifested aggregation of mononucleated cells, proliferation of hepatocyte, cytoplasmic fat droplet and granulomatous lesion consists of aggregation of macrophage and lymphocyte. All these alteration in liver histology were modified by treatment of rats with pomegranate seed oil (group T2) and no pathological lesion was reported in group T3. On conclusion, this study documented the beneficial effect of pomegranate seed oil against the deleterious effects of SF on liver functions of adult female rats.

Keywords: Pomegranate seed oil, Sodium fluoride, liver functions tests, GSH, peroxy nitrite.

Introduction

Sodium fluoride was originally used in the 1930s as a wood preservative (1), in pesticides, various types of adhesives and glues (2). The adverse effects of sodium fluoride are possible at fluoride levels far above the recommended dosage (3). In contaminated areas, fluoridated water is the major source of fluoride, after absorption the fluoride cleared by the kidneys (4). The compounds of sodium fluoride in the various formulations have several caries-protective mechanisms (5) also fluoride reduces the decay of teeth enamel by remineralization of enamel and teeth (6).

Many evidence suggested that excessive fluoride intake may be contributing to a wide range of adverse health effects (7). As the

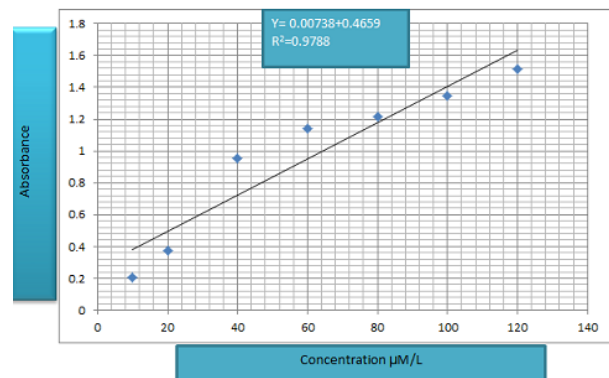
fluoride cross the cell membranes causing structural and functional changes leading to fluorosis of bones associated with bone cancer (8) and dental and skeletal fluorosis (9). At the same time, (10) explained that cattle suffering from signs of dental discoloration and bony lesions when browse in area contaminated with fertilizer. Evidently, fluoride inhibits some enzymes involved in metabolic pathways and fatty acid oxidation (11), as well as, fluoride cause change of lipid peroxidation (LPO) (12), lipids profile (13) and inhibits certain total antioxidants capacity with increase generation of oxygen free radicals (14). Moreover, some studies reported a decreased protein content in liver and serum of mice and rats exposed to sodium fluoride (15 and 16).

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 (17) including: flavonoids, proanthocyanidins and hydrolysable tannins, sterols, triterpenoids, and alkaloids (18). Besides it was reported that pomegranate seed oil is a major source of polyunsaturated fatty acids (PUFAs) with a low saturated fatty acid which is an important for therapeutic uses (19). However, pomegranate juice supplementation has been shown to alleviating the coronary heart disease (20), Alzheimer's disease (21), against diarrhea and intestinal parasites (22), anti-cancer (23) and used as anti-inflammatory, hepatoprotective activities, improved lipid profile and glucose metabolism (24,25 and 26), as well as a cardioprotective effects of PSO in methionine overload rabbits was reported (27). Therefore, this study was aimed to investigate the hepatoprotective effect of pomegranate seed oil in normal and sodium fluoride administered female rats

Materials and Methods

Twenty female adult Wistar rats, weighed (219.5 g. - 250.1 g.) were used in this investigation. Animals 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 acclimation 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 ppm in tap water, group T2: rats were subjected to sodium fluoride 120 ppm 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 was isolated and frozen at -18 °C until

determination of: serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities calorimetrically (28) using enzymatic kit (Bio system, Spain), total cholesterol was measured using enzymatic assay kit (29), total bilirubin (TB) was measured using bilirubin kit (Bio System, Spain) (30), peroxy nitrite radical was evaluated as described by (31) and reduced glutathion (GSH) was determined by (32) using standard GSH curve (Fig.1). At the end of experiment rats were anesthetized, sacrificed, then livers were preserved in 10% neutral formalin buffer solution. Several liver sections were prepared stained with Hematoxylin – Eosin stain (33). 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 \pm SE and a significant were tested at $P < 0.05$ (34).



Figure, 1: Standard curve of different GSH concentrations.

Results and Discussion

A marked increase ($p < 0.05$) in serum aminotransferases (ALT and AST) activity were showed after 20 and 40 days post sodium fluoride administration in T1 group as compared with control, T2 and T3 groups (Fig. 2,A and B). Comparing to group T1, exposure of female rats to sodium flourides in drinking water concurrently with oral intubation of PSO for 40 day caused a 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 significant ($p < 0.05$) increase in these enzymes activities during two experimental periods as compared to the control group. Treatment of

normal rats to sodium fluoride for 20 and 40 successive days elicited a significant increase ($p < 0.05$) in serum total cholesterol concentration group T1 as compared to group T2 (SF plus PSO), group T3 and control group. Meanwhile, at the end of the experiment rats treated with sodium fluoride and PSO (T2) exhibited a significant ($p < 0.05$) decrease in total cholesterol concentration as compared to group T1 (Fig. 2C). Administration of PSO (group3) to female rats caused a significant ($p < 0.05$) decrease in total cholesterol concentration at 20 and 40 days of the experiment as compared with other treated groups.

A significant ($p < 0.05$) increase in the mean values of serum total bilirubin concentration at 20 and 40 day in T1 group as compared with control, T2 and T3 groups. Meanwhile, oral intubation of rats with pomegranate seed oil concurrently with sodium fluoride along the experimental period caused a significant ($p < 0.05$) decrease in this parameter in group T2 comparing to T1 and control groups (Fig. 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 (Fig. 2E), serum peroxynitrite radical concentration recorded a significant ($p < 0.05$) elevation in group T1 versus baseline in control, T2 and T3 groups. While serum peroxynitrite concentration reduced significantly ($p < 0.05$) in group treated with SF+ PSO (T2) at 20 and 40 day of the experimental 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. (Fig. 2F) clarified the a significant ($p < 0.05$) decrease GSH concentration in group treated with SF (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 differences between T2 and T3, which indicate the beneficial effect of PSO against sodium fluoride.

Liver sections of rats received SF (group T1) showing aggregation of mononuclear cells (MNCs) around the bile duct associated with necrosis of surrounding hepatocyte (Fig. 3) with polymorphonuclear cells (PMNCs) in

congested blood vessels (Fig. 4) and mild cytoplasmic fat droplets in hepatocyte with vacuolation around dark nuclei granulomatous lesion was also seen (Fig. 5) as compared to control (Fig. 6). Whereas liver sections from rats treated with SF plus PSO (group T2) showing moderate infiltration of MNCs in liver parenchyma with granulomatous lesion consists mainly of macrophage were reported (Fig. 7 and 8), as well as, aggregation of macrophages with slight Kupffer proliferation was reported. However, in this area treated of rats with PSO showed appearance of megakaryocytes accompanied with moderate proliferation of Kupffer cells (Fig. 9), besides, no pathological lesions were noted in other sections (Fig. 10).

Elevation in serum aminotransferases 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 (35 and 36). Moreover, the fluoride toxicity was found to cause a significant increase in aminotransferase activity, as reported in cattle (37) and in goat (38), so these elevations 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 (39). However, an increase of apoptosis is consequent to the exposure of fluoride, had been reported in various mammalian cells (40 and 41) 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 (42), which was accompanied with a decrease in the antioxidant status of liver leading to impairment of its function (43). The hepatoprotective activity of PSO was reflected via a decrease in the ALT and AST levels in group T2 as compared with SF treated rats and returned to normal range. This result in agreement with others (44 and 45). 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 (46).

A significant elevation in serum TC in SF treated group T1 might indicate hepatotoxicity with degenerative and hepatic cells necrosis, This was agreement with other researchers (14 and 47). It had 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 (48), moreover, fluoride intoxication in rats caused an increased in the activity of HMG-CoA reductase due to deficiency of insulin (49) leading to excessive production and accumulation of cholesterol resulting in the formation of foam cells (50). . Furthermore, administration of SF caused an increase in LPO (12) which might be an 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 (51). In agreement with (27 and 52), serum TC was decreased in PSO treated group indicating its hypolipidemic effects through decreased total and LDL-cholesterol versus baseline (53) .

An increase in serum bilirubin concentration in SF treated rats might be due to the destruction of R.B.Cs and /or damage of liver tissues (54). Because cell membranes of the erythrocytes are sensitive to the presence of free radicals thus, used as an oxidative stress biomarker (55). 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 (56) and an increase in MDA level of these tissues (57).Therefore, liver injury and hemolysis of RBCs (58) in group T1 may lead to hyperbilirubinemia .

Overall, this study findings showed that PSO supplementation caused a significant decrease in serum bilirubin in 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 (59) exhibited a protective effects against oxidative damage and

considered to be as hepatoprotective (61 and 61), improve the intestinal barrier functions in obstructive jaundice (62) and protects liver from fibrosis due to biliary obstruction (63) through augmented the antioxidant defense mechanism and increases the erythrocyte activity (64). 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 (65).

The results also showed changes in biomarkers of oxidative stress as evidenced by a decrease of reduced glutathione and an increase in peroxy nitrite concentrations. Fluoride is known to reduce intracellular GSH levels and inhibit various enzymes that require GSH as a cofactor (66). Besides, (14) 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 (67) associated to an increase of LPO (56). This deterioration overlaps with the elimination of H₂O₂ and LPO products, cause 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 (68). 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 (69) .

Peroxy nitrite is an oxidant agent, and its formation in vivo had been ascribed to the reaction between radicals of superoxide and nitric oxide causing destruction of cellular constituents, dysfunction of cellular processes, then induction of cell death through both necrosis and apoptosis with depletion of anti-oxidant enzymes (70 and 71). so, such results could be responsible for histopathological changes of liver. 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 (27 and 44) via reducing the LPO (72) which having an effect

on the scavenging capacity of superoxide anion and hydrogen peroxide (73 and 74). Thus, it could be concluded that the the

beneficial effect of PSO against the deleterious effects of SF on liver functions of adult female rats.

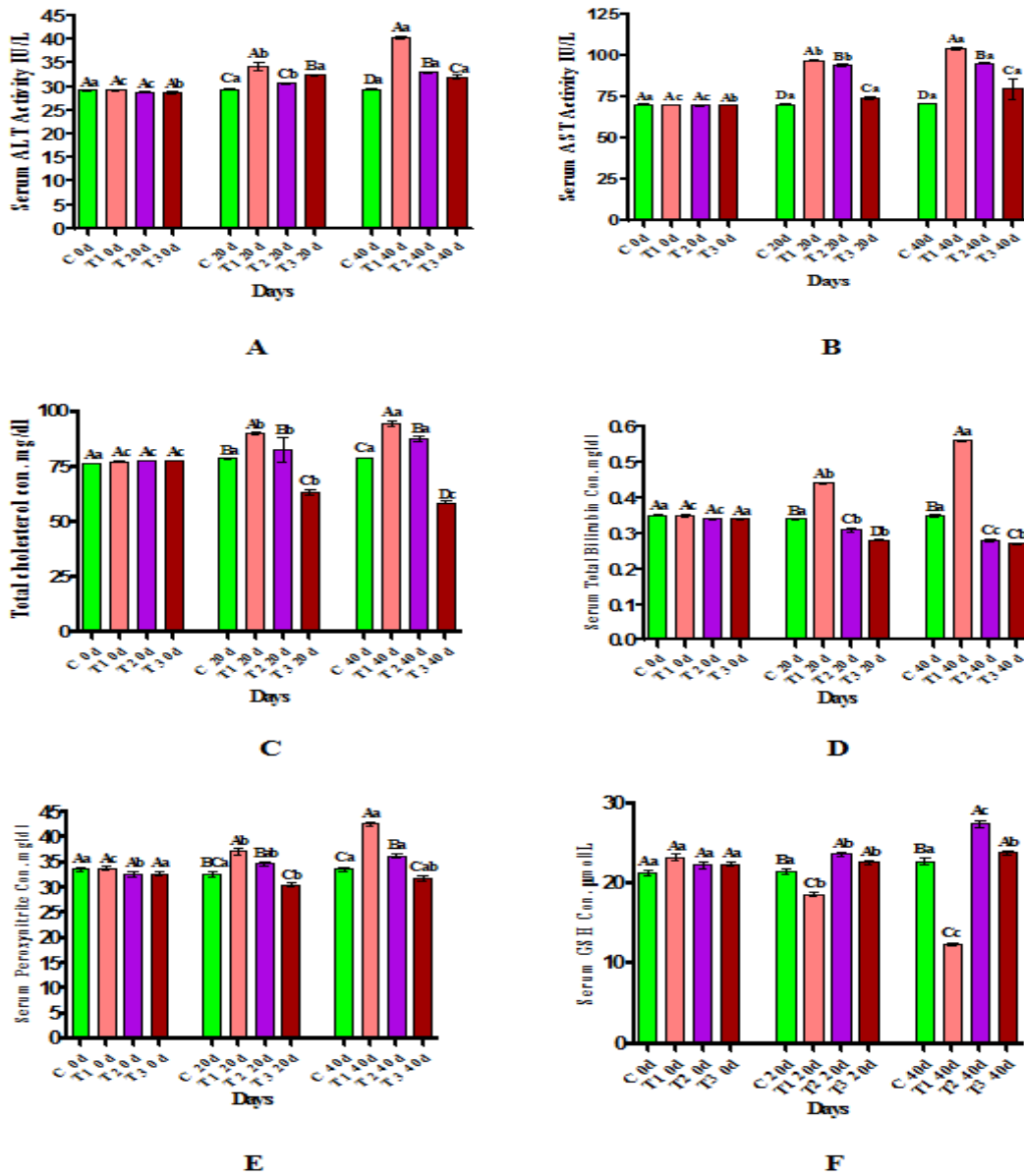
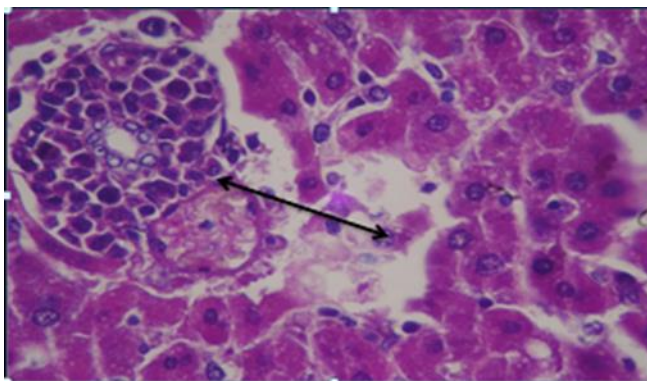
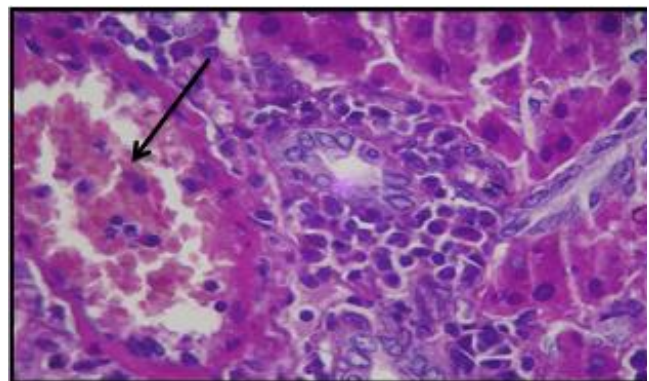


Figure (2): Effect of pomegranate seed oil (PSO) on serum biochemical tests in female rats treated with sodium fluoride.



Figure,3 : Photomicrographs showing histology of liver tissue from group T1 showed MNCs



Figure,4: Photomicrographs showing histology of liver tissue from group T1 showed presence

aggregation around bile duct with necrosis of surrounding hepatocytes(H/E X 40).

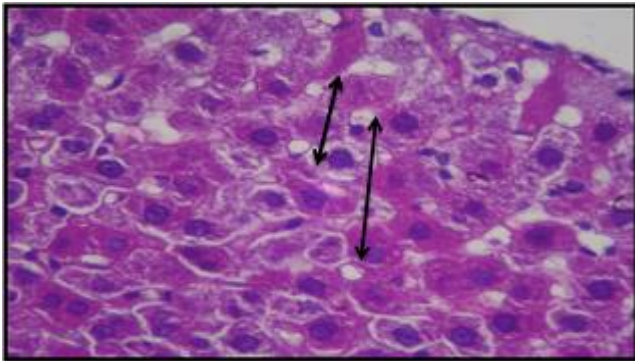
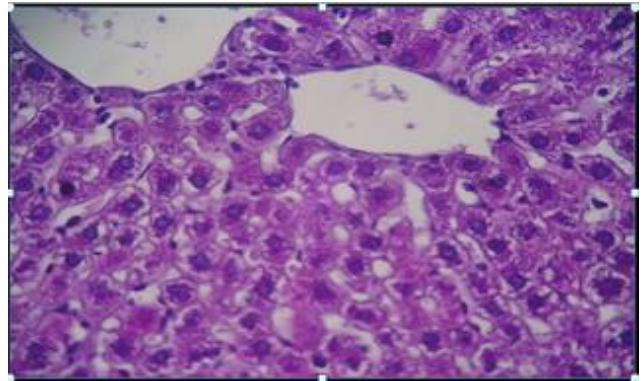
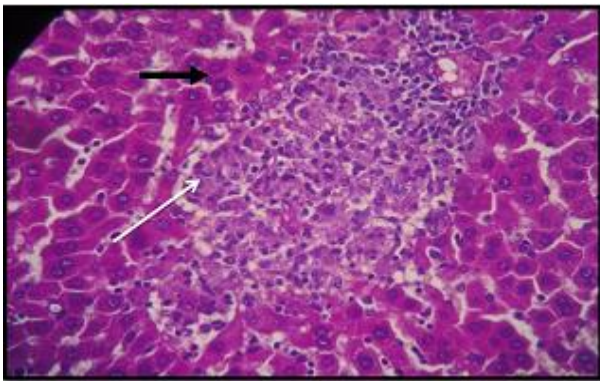


Figure-5: Photomicrographs showing histology of liver tissue from group T1 showed presence of PMNCs in the dilated and congested blood vessels mainly in portal area (H /E X 40).

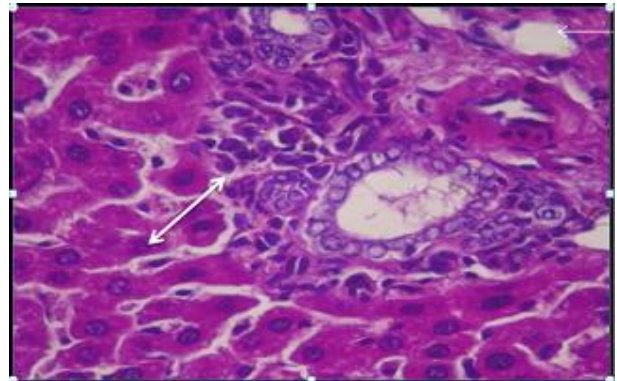
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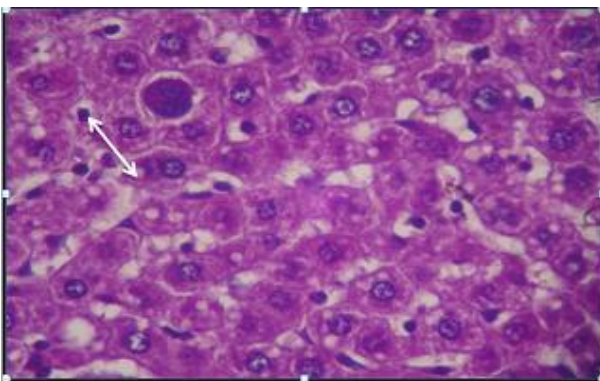
Figure,6: Photomicrographs showing histology of liver tissue from control rat. Note normal characteristics feature of the liver (H -E X 40).



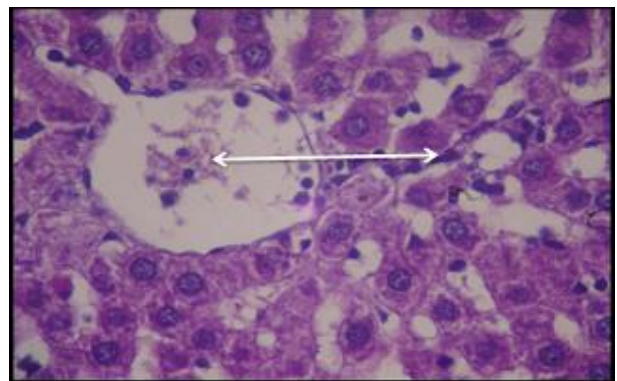
Figure,7: Photomicrographs showing histology of liver tissue from group T2 showed MNCs infiltration in liver parenchyma consist mainly of macrophage (H -E X 10).



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 inflammatory cells in blood vessels with proliferation of kupffer cells(H-E X 40).

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تأثير زيت بذور الرمان (PSO) ضد السمية الكبدية - الناجم عن فلوريد الصوديوم في الجرذان البالغة من الإناث (الجزء الثاني)

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الخلاصة

تم تصميم هذه التجربة لتقييم الدور الوقائي لزيت بذور الرمان (PSO) في حماية الكبد ضد السمية الناجمة عن فلوريد الصوديوم (SF) في الجرذان. قسمت عشرون من اناث الجرذان البالغات إلى أربع مجموعات متساوية وعوملت يوميا لمدة 40 يوما على النحو التالي: مجموعة السيطرة C اعطيت ماء الحنفية، المجموعة الثانية T1 تلقت فلوريد الصوديوم 120 جزء في المليون في مياه الشرب، المجموعة الثالثة T2 اعطيت كلا من فلوريد الصوديوم 120 جزء في المليون في ماء الشرب وزيت بذور الرمان عن طريق الفم 30 ملغم / كغم من وزن الجسم في حين جرعت المجموعة الرابعة T3 زيت بذور الرمان شفويا بنفس الجرعة كما في T2. بعد تصويم الحيوانات، جمعت عينات الدم للأيام 0 و 20 و 40 يوما لتقدير بعض المعايير الكيموحيوية وبعض المؤشرات الحيوية للاجهاد التأكسدي. بالإضافة إلى ذلك، أخذت أجزاء من الكبد في نهاية التجربة للدراسة النسيجية. أوضحت النتائج أن SF (المجموعة T1) تسبب في زيادة معنوية في نشاط ALT، AST، الكوليسترول الكلي، البيليروبين الكلي والتريغليسيريد الكلي وتركيز جذر البيروكسي نائترت، في حين كان هناك انخفاض ملحوظ في تركيز GSH. تسبب PSO في التخفيف من الخلل الكبدية الناجم عن فلوريد الصوديوم في المجموعة T2 حيث تجلى ذلك من خلال الارتفاع المعنوي في تركيز GSH، بالإضافة إلى ذلك، لوحظ انخفاض معنوي في نشاط انزيمات الترانساميناسات في المصل، الكوليسترول الكلي، تركيزات البيليروبين والراديكالية والبيليروبين الكلي. في المقابل، أدى تناول زيت بذور الرمان لوحده في المجموعة الرابعة الى عدم حدوث تغييرات في مستوى المعايير المدروسة وكانت قريبه إلى مستويات السيطرة أو أدنى. علاوة على ذلك، فإن فحص الأنسجة الكبدية للمجموعة T1 أظهرت وجود خلايا أحادية النواة (MNCs)، تكاثر الخلايا الكبدية، قطيرات الدهون السيتوبلازمية، والآفات الحبيبية المتكونه من تكاثر البلعم والخلايا اللمفاوية. تم تعديل كل هذه التغييرات النسيجية في الكبد عن طريق معاملة الفئران بزيت بذور الرمان (مجموعة T2) وتم الإبلاغ عن أي آفة الباثولوجية واضحة في المجموعة T3 تلقت PSO. في الختام، وثقت هذه الدراسة تأثير مفيد PSO ضد الآثار الضارة لفلوريد الصوديوم على وظائف الكبد في اناث الجرذان .

الكلمات المفتاحية: زيت بذور الرمان، فلوريد الصوديوم، اختبارات وظائف الكبد، GSH، بيروكسينيتريت.