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The Protective Role of crude Polyphenolic Compounds Extracted from black Olive fruit (*Oleaeuropae*) on Liver Functions in Males Rats Treated with Hydrogen Peroxide

Layla Hashim Alol
Department of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University, Iraq.

Summary

This study was conducted to investigate the protective role of polyphenolic compounds extracted from olive (*Oleaeuropae*) to contrast the damaging effects of 1% hydrogen peroxide on liver functions in male rats. Crude polyphenolic compounds were extracted from fruits of black olive by 95% methanolic extraction method. Twenty adult male rats (200-220gm.) were randomly divided into four equals groups and treated daily for 30 days. Rats in the first group received tap water (orally)and considered as control group, animals of second group received 1% H2O2 in drinking water . The rats in the third group received 1% H2O2 in drinking water plus 200mg/kgB.W. of crude polyphenolic compounds while animals in the fourth group received 200mg/kg B.W. of crude polyphenoliccompounds. At the end of the experiment, blood samples were taken to investigate the activity of liver enzymes (Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), concentration of total serum bilirubin, as well as protein picture of blood serum by using agarose gel electrophoresis. Ultimately, animals of all groups were sacrified to examine the histopathological changes in liver. The results illustrated significant increase ($P<0.05$) in liver enzymes activity (AST,ALT) and total serum bilirubin in H2O2 treated group as compared with control. Although rats treated polyphenolic compounds of olive plus H2O2 showed significant decrement ($P<0.05$) in ALT activity and total serum bilirubin, while no significant alteration in (AST) activity was recorded in H2O2 treated group. The result also demonstrated significant decrease ($P<0.05$) in authority of ALT, total serum bilirubin in animals treated with polyphenolic compounds. Serum proteins showed a significant ($P<0.05$) decreament of albumin percentage and increment of globulins in H2O2 treated group as well as polyphenolic compounds treated group as compared with control group (G1) . However, no significant different in group treated with polyphenolic compounds as compared with control. Histological sections of liver illustrated clear impact of group treated with H2O2, manifested by necrosis of hepatic cells with infiltration of inflammatory cells while animals treated with polyphenolic compounds plus H2O2 revealed slight infiltration of inflammatory cells with proliferation of kupffer cells in liver. In infereance, the autcomes of this study documented the advantageous effect of crude polyphenolic compounds of olive apposite the noxious effect of H2o2 on liver function of adult males rats.

الدور الوقائي للمركبات المتعددة الفينول الخام المستخلصة من ثمار الزيتون الاسود *Oleaeuropae* على وظائف الكبد في ذكور الجرذان المعاملة ببروكسيد الهيدروجين

ليلى هاشم علول
فرع الفسلجة والادوية / كلية الطب البيطري / جامعة بغداد

الخلاصة

هدفت هذه الدراسة تقييم الدور الوقائي للمركبات المتعددة الفينول المستخلصة من ثمار الزيتون الاسود *Oleaeuropae* لازالة التاثير الضار الناتج من التجريع ببروكسيد الهيدروجين 1% في وظيفة الكبد في ذكور الجرذان . تم استخلاص المركبات المتعددة الفينول من ثمار الزيتون (المنزوعة النوى) باستخدام كحول ميثيلي اعقب ذلك الحصول على 20 غرام من المركبات متعددة الفينول للكيلو غرام الواحد من ثمار الزيتون (المنزوعة النوى) . تم استعمال عشرون جرذ ذكر بالغ (200-220 غم) قسمت عشوائيا الى اربع مجامبع متساوية عوملت يوميا لمدة ثلاثون يوما وكالاتي :- حيوانات المجموعة الاولى (G1) اعطيت مياه الشرب العادية واعتبرت سيطرة للمجموعة الثانية والرابعة ، اما حيوانات المجموعة الثانية (G2) اعطيت 1% من H2O2 فقط في مياه الشرب واعتبرت سيطرة للمجموعة الثالثة فضلا عن حيوانات المجموعة الثالثة (G3) فقد اعطيت 200ملغم / كغم من وزن الجسم من المركبات المتعددة الفينول اضافة الى

1% من بيروكسيد الهيدروجين في مياه الشرب وحيوانات المجموعة الرابعة (G4) اعطيت 200ملغم /كغم من وزن الجسم من المستخلص الخام المتعدد الفينول لثمار الزيتون . وفي نهاية التجربة ، تم سحب الدم وفصل المصل لغرض قياس فعالية انزيمات الكبد AST, ALT وقياس مستوى الصبغة الصفراء الكلي ومستوى الالبومين باستخدام تقنية AgaroseGel Electrophoresis ، تم التضحية بجميع الحيوانات لغرض دراسة التغيرات المرضية النسجية الحاصلة في نسيج الكبد . اظهرت النتائج وجود ارتفاع معنوي (P<0.O5) في فعالية انزيمات الكبد(AST, ALT) ومستوى الصبغة الصفراء للمجموعة المعاملة ب H2O2مقارنة بمجموعة السيطرة . اما المجموعة المعاملة بالمركبات الفينولية للزيتون مع بيروكسيد الهيدروجين فقد لوحظ فيها انخفاض معنوي (P<0.05) في فعالية انزيم الكبد ALT وانخفاض مستوى الصبغة الصفراء لكن لم يلاحظ وجود فرق معنوي في فعالية انزيم AST مقارنة بالمجموعة المعاملة بالمركبات الفينولية فقط في حين ليس هناك فرق معنوي في فعالية انزيم AST مقارنة بمجموعة السيطرة . اظهرت الفحوصات المرضية النسجية للكبد تاثيرا واضحا في المجموعة المعاملة ببروكسيد الهيدروجين حيث لوحظ تنخر في الخلايا الكبدية مع ارتشاح للخلايا الالتهابية وعندما جرعت حيوانات التجربة بالمركبات المتعددة الفينول مع بيروكسيد الهيدروجين لوحظ ارتشاح للخلايا الالتهابية مع وجود تكاثر خلايا كوفر في الكبد . وفي النهاية اثنتت نتائج هذه الدراسة التاثير المفيد لمركبات المتعددة الفينول الخام لثمار الزيتون الاسود ضد التاثير الضار لبيروكسيد الهيدروجين في وظائف الكبد في ذكور الجرذان البالغة .

Introduction

Olive fruits are remarkable source of antioxidant (1,2) and anti-inflammatory phytonutrients; most prominent are phenolic compounds (tyrosol&hydroxytyrosol) and several terpenes(especiallyoleuropinerythrodiol,uvaol,oleanolicacid,elenoic acid and ligstroid). Flavonoids including apigenin, luteoline, cyanidin and phytonutrient content of olives depend upon olive variety, stage of peonidin. The maturation and post harvesttreatment. Olive fruits are a good source of iron, vitamin E,copper, and dietary fiber (3). Olive oil contains monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) which are help in heart diseases (4). Some research showed that MUFAs benefit in insulin levels and blood sugar control (5). Polyphenolic compounds of olive benefit in cancer (6), antihistaminic (7), blood pressure (8),Alzheimer's (clogging of arteries) caused by cholesterol and saturated fatty acid for galls stones (9).

Materials and Methods

Extraction of polyphenolic compounds from olive fruits was carried out according to Markham method (10) by using 95% methanolic alcohol (1:9) and shaking the mixture, by using magnetic stirrer for 18 hrs. at room temperature, then filtrate with filter paper and concentrated the supernatant at 40^0 C in an incubator . They yield was brown pasty substance (creamy texture) that kept at -20 ^{0}C till use.

Twenty males,Swiss albino rats were used in this study. They were divided into four groups designated and treated as follows for 30 days: G1 (control group received tap water); G2 (animals received 1% H2O2); G3 (rats received 1% H2O2 + 200mg/kg of polyphenolic compounds of olive (11) ; G4 (animals received 200mg/kg of polyphenolic compounds only), which given orally for 30 days , at the end of experiment of period , blood samples were taken from anaesthetized rats using ketamine (kepro/ Holland) plus xylazine (Bayer/Germany). The rats were killed for histopathological examination and measuring the followings: Serum ALT (Alanin aminotransferase) using analytical kit Biomerix, France, AST(Aspartate aminotransferase) using analytical kit Linear , Spain) , Bilirubin using analytical kit (Biosystem , Spain) were determined by using the method of 12, 13 ,14 interval . Electrophoresis examination of serum proteins was carried out using agarose gel (Hellabiokit,Greece). Serum protein fractions were fixed and stained by amido black and the percent protein fractions were estimated byHellabioscan at 520 nm. The results were evaluated using ANOVA variance analysis and regression analysis using SPSS programme (15).

Results

Extraction of polyphenolic compounds from olive fruits (*Oleaeuropae):-* The result of this study revealed that out of each kilogramof seedless olive approximately 20 gm crudepolyphenolic compounds wereobtained and the formation of green bluishcolour with 1% of ferric chloride solution which confirmed the presence of phenoliccompound

Alanine aminotransferase (ALT) activity (units / ml):- Table (1) and Figure (A) demonstratedthemean of values of serum ALT. There was significant (P<0.05) increase in the activity of this enzyme in H2O2 treated group (G2)as compared tothe controlgroup (G1).Activity of the enzyme had showed significant (P<0.05) decrement in H2O2 pluspolyphenolic compounds (G3), as well as treated groupolyphenolic compounds (G4) as compared with control group.

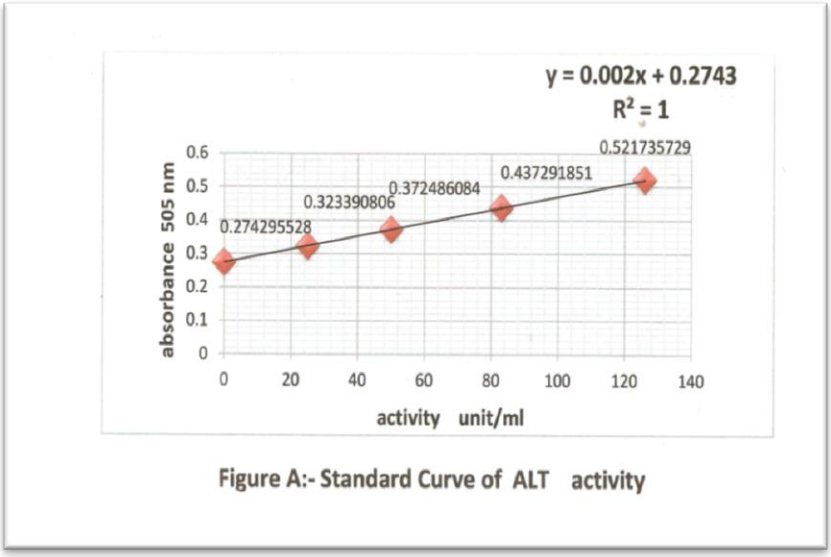


Figure A:- Standard Curve of ALT activity

Aspartate aminotransferase (AST) activity (U/L):- In table (1) AST activity showed significant (P<0.05) increase in H2O2 group (G2) and in H2O2 plus polyphenolic compounds (G3) as compared with control (G1) and polyphenolic compounds group (G4).

Table (1):- Effect of H2O2 and polyphenolic compounds with H2O2 on Alanine aminotranseferase (ALT) activity units /ml and on Aspartate aminotransferase (AST) activity U/L in adult males rats.

parameters / Groups	ALT (units / ml)		AST (U /L)	
G1 (CONTROL)	37.52 ± 4.37	B	89.86 ± 5.26	B
G2 (1% H2O2 in drinking water)	77.03 ± 4.70	A	146.10 ± 1.13	A
G3 (1% H2O2 + 200 mg /kg B.W. of polyphenolic compounds	23. 76 ± 6.85	C	138.05 ± 7.98	A
G4 (200mg/kg B.W. of polyphenolic compounds)	24.05 ± 3.52	C	91.30 ± 3.94	B
L.S.D.	13.27		13.2	

Values are represented as mean ± SE (n= 5 rats /group).
Capital letters denotes differences between groups (P<0.05).

Total Serum Bilirubin : Table (2) showed a significant (P<0.05) increase in the value of total serum bilirubin in H2O2 treated group (G2) as compared to control group, while in groups (G3) and (G4), the results clarified a significant (P<0.05) decrement in serum between groups as compared to the groups (G1) and (G2).

Table (2):- Effect of 1% H2O2 and oral intubation phenolic compounds of olive fruit for one month on total serum bilirubin (mg/dl) in males rats.

Groups / TSB	G1 (control)	G2 1%H2O2	G3 1% H2O2+ 200mg/kg phenolic compounds	G4 200mg/kg of phenolic compounds
Total serum bilirubin mg/dl	0.5703±0.05 B	0.7903±0.03 A	0.0942±0.04 D	0.1674±0.04 C

L.S.D. =0.13, values are represented as mean ± SE (n=5 rats /group)
G1:-group received tap water for 30 days.
G2:-group received 1%H2O2 in drinking water for 30 days.
G3:-group received 1% H2O2+ 200mg /kg of phenolic compounds of olive orally for 30 days.
G4:- group received 200mg/ kg B.W. of polyphenolic compounds of olive orally for 30 days.

Serum protein electrophoresis:- The results of table (3) and figure (B) indicated significant (P<0.05) decrease in % albumin fraction in H2O2 group (G2) and H2O2 plus polyphenolic compounds (G3) as compared with control group (G1) and (G4), while the results ofglobulines fractions showed significant (P< 0.05) increase in α1 globulin in H2O2 group (G2) as compared with other group , in addition to significant (P<0.05) increment of γ–globulines in H2O2 group (G2) and H2O2 plus polyphenolic compound (G3) as compared tocontrol group and G4.

Table(3):- Agarose protein electrophoresis (protein fraction %) in serum of rats received 1% H2O2 in drinking water & intubated orally with 200mg/kg B.W. of polyphenolic compounds of olive .

Groups	Albumin%	1-globulin% α	2-globulin%α	-globulin%β	-globulin%γ
G1	73.19±1.63 A	3.15±0.42 B	3.34±0.55 B	15.10±0.26 A	5.30±1.29 B
G2	60.80±5.11 B	21.59±5.92 A	1.80±0.34 C	6.10±2.14 B	12.10±2.73 A
G3	59.50±4.19 B	4.80±1.37 B	6.20±1.50 A	15.80±1.20 A	13.40±3.94 A
G4	71.00±1.14 A	3.20±0.44 B	5.19±0.94 A	14.50±0.31 A	5.90±0.71 B
L.S.D.	8.80	5.86	2.39	2.81	6.24

Values are represented as means ± SE (n= 5 rats/group)
G1: group received tap water orally for 30 days.
G2: group received 1% H2O2 in drinking water for 30 days.
G3: group received 1%H2O2 + 200mg/kg B.W. of phenolic compounds of olive orally for 30 days.
G4: group received 200mg/kg of phenolic compounds orally for 30 days.

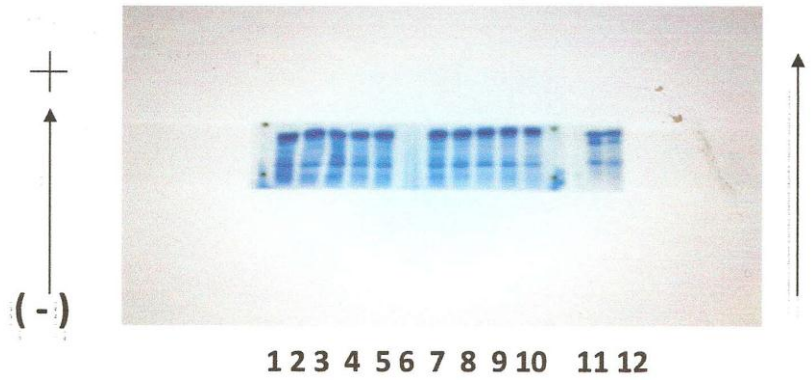
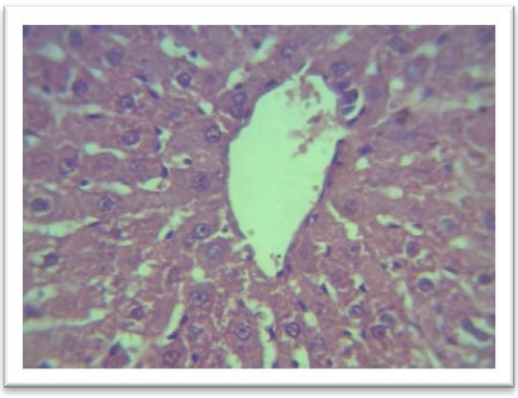


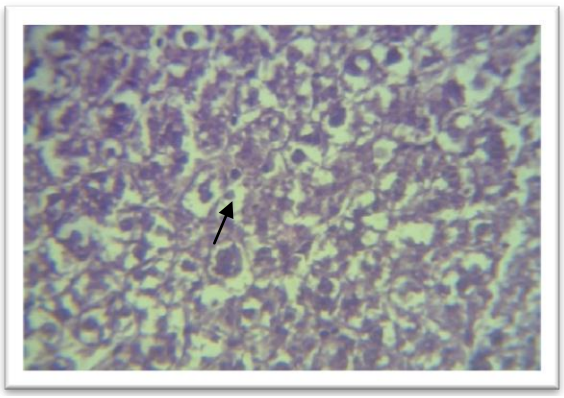
Figure (B) :- Agarose Gel Electrophoresis of blood serum at PH 8.6, size of sample 5µl.

1, 2, 3:- Rats received 1% H2O2 + polyphenolic compounds of olive (200mg/kg) orally for 30 days 4, 5,6:-Rats received polyphenolic compounds of olive (200mg/kg) orally for 30 days . 7, 8, 9 :- Rats received tap water.10, 11,12:- Rats received 1% H2O2 in drinking water .

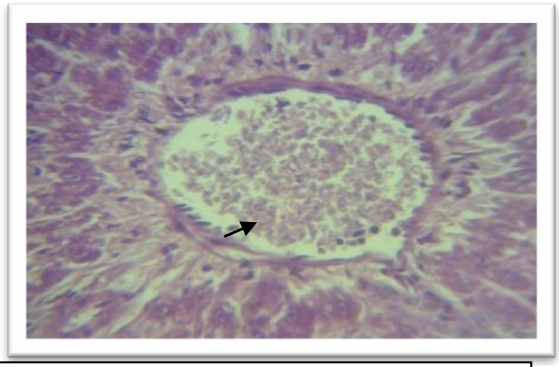
Histopathological findings in liver:-Tissue sections showed normal histological structure in control group (Fig. 1), the light microscopy examination of rats treated with 1% H2O2 showed vacuolar degeneration of hepatocytes with pyknoticnuclei (Fig. 2). Other areas undergo severe coagulativenecrosi leading to destructive hepatic parenchyma with blood oozing to the necrotic area (Fig. 3). The central veins were dilated and congested containing inflammatory cells within their lumina with proliferation of endothelial lining cells and fibrous thickening of their walls and the adjacent centrilobular area (Fig. 4). On the other hand treatment with 1% H2O2 and polyphenolic compounds showed fibrosis which also lead to the thickening of glisson capsule (Fig. 5) and atrophy of hepatocytes (Fig. 6) severe periportal fibrosis with mononuclear inflammatory cells infiltration and severe congestion of portal vein was also seen (Fig. 7). While , hepatic tissue section of group treated with polyphenol of olive showed formation of early granulomatous reaction consists of mononuclear cells within hepatic parenchyma (Fig. 8) , and adjacent the dilated and congested blood vessels (Fig. 9) , while group treated with 1%H2O2 plus polyphenolic compounds of olive showed infiltration of inflammatory cells mainly mononuclear cells within dilated and congested central veins and sinusoids accompanied with proliferation of kupffer´s cells (Fig.10) .



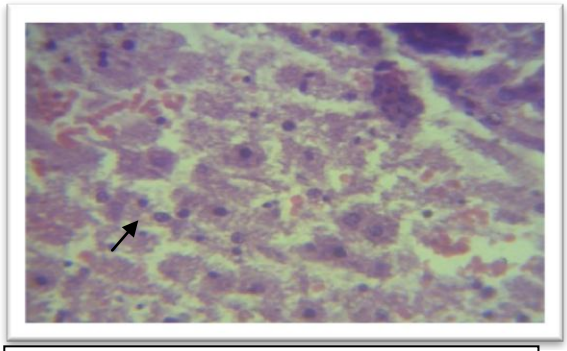
Figure(1):-section in liver of rat in (G1) showing normal histological structure (H& E 400X)



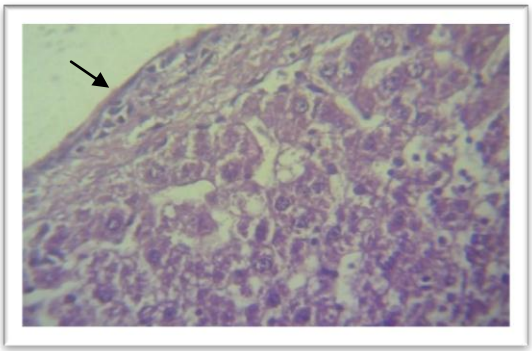
Figure(2):-section in liver in (G2) showing vacuolar degeneration of hepatocytes with pyknotic nuclei(H&E 400X)



Figure(3):-section in liver of rat in (G2)showing severe coagulative necrosis leading to destruction of parenchyma with blood oozing to the necrotic area (H & E 400X)



Figure(4):-section in liver in (G2)showing severe dilated and congestion of central vein with fibrous thickening of the wall and adjacent centrilobular region (H&E 400)



Figure(5):-section in liver in G3 showing fibrous thickening of glisson capsule (H & E 100X)

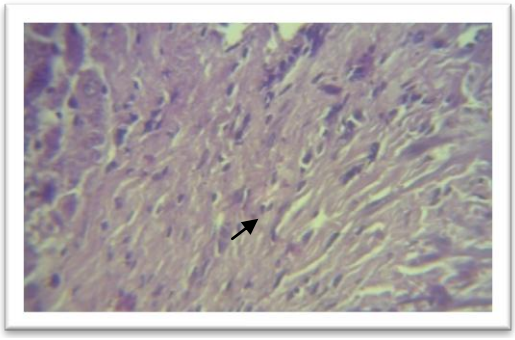
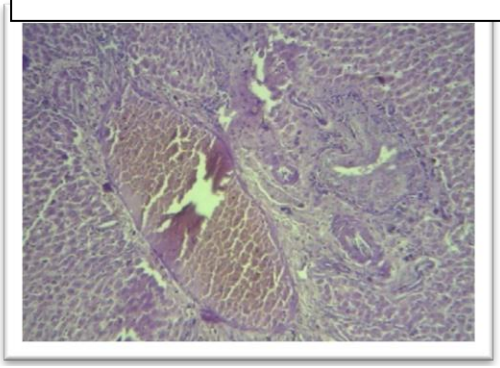
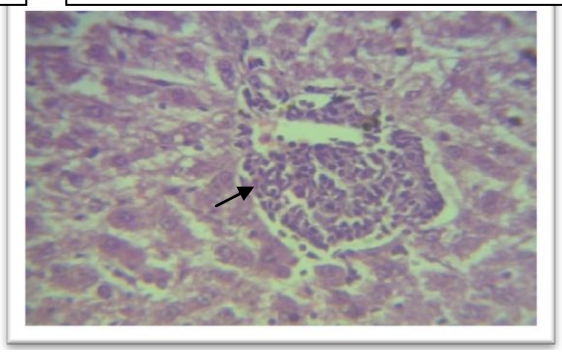


Figure (6):-section in liver in G3 showing marked fibrosis of hepatic parenchyma leading to atrophy of hepatocytes (H&E 400X).



Figure(7):-section in liver in (G3) showing severe periportal fibrosis with infiltration of mononuclear cells and hyperplasia of bile ductules with severe congestion of portal vein (H&E 100X)



Figure(8):-section in liver in (G4)showing formation of early granuloma within hepatic parenchyma (H&E 400X)

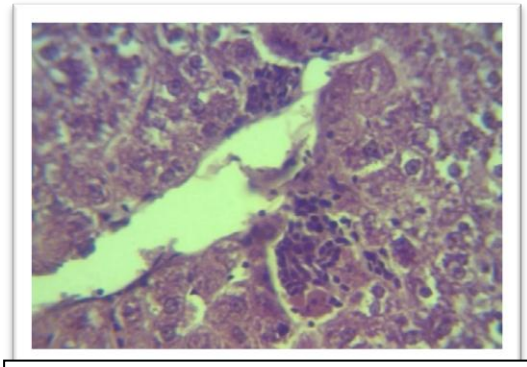


Figure (9):-section in liver in (G4) showing granuloma reaction adjacent the dilated and congested blood vessel ↗(H & E 400X).

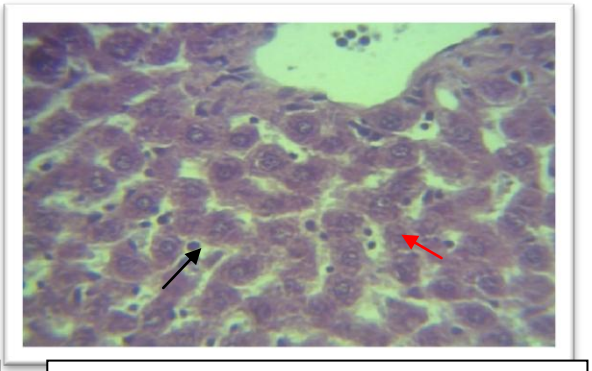


Figure (10):- section in liver in (G4) Showing infiltration of mononuclear cells within the dilated and congested central vein↗ andsinusoids with proliferation of kupffer s cells (H& E 400X) ↗

Discussion

Extraction of polyphenolic compounds from olive fruits (*Oleaeuropae*):-The result of polyphenolic compounds from olive fruits recorded in this study was approximately the same as that recorded by (16).

Significant increase of serum ALT, AST activity after H2O2 exposure may due to the effects of H2O2 as oxidative factor that decrease of cellular basal metabolic rate, increase irritability, causing destructive changes of liver (17).

Efficacy of polyphenolic compounds to remove the toxic effect of ALT, AST may be due to oxidant activity of crude polypheols manifested by eliminating the formation of FR s., inhibition of cell destruction and prevent enzyme leakage (18). In addition Umran (16) reported similar findings in normal and cancer cells lines in mice.

Total Serum Bilirubin : Significant increase in serum bilirubin in H2O2 treated group may be due to toxic effect of H2O2 exposure including hemolytic crises, characterized by increased serum bilirubin levels and intrahepatic cholestasis (reduction in bile flow) in rats, these findings may produce hyperbilirubinemia (19). While the animals which received polyphenolic compounds of olive showed decrease in the level of serum bilirubin , that may due to the protective effects and antioxidant properties of polyphenolic compounds against liver injury (7, 20) .

Serum protein electrophoresis: Disturbance in the ratio of albumin to globulins is an important finding indicating the abnormalities in liver function (21). Synthesis of albumin occurs in liver, a decrease in the level of albumin in H2O2 treated group & H2O2 plus polyphenolic compounds of olive may due to hepatic abnormality and is a serious indication of liver dysfunction (22). Besides significant elevation in α2 , β , γ-globulin in (G3) group and α2 , β , γ – globulin in (G4) may due to hepatocyte protective action of antioxidants (polyphenolic compounds of olive) against free radicals damage , where many antioxidants including polyphenols can protect cell membrane of hepatocyte from lipoprotein oxidation , and regulation of liver function (23) .

Histopathological changes :- liver sections documented the oxidative damage of H2O2 and confirmed the protective effect of olive fruit polyphenols , where inflammatory cells infiltration and proliferation of kupffer cells , as a result of increased expression of proteins in cells in polyphenols (G4) group is importance in the removal of toxic effect of oxidant (3, 24).

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