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Effect of Olive Oil and Pomegranate Peels on Rats Suffering from Chronic Injury in the Liver

Dalia M. Talatt Abd EL-khalik.,[1] Ebtesam Fath M. Omar.[2]

Home Economic Department, Faculty of Specific Education, Fayoum University, Egypt[1]., Home Economic Department, Faculty of Specific Education, Alexandria University, Egypt[2].

Abstract: The aim of this study was to investigate the effect of olive oil and pomegranate (*Punica granatum* L.) peels on rats suffering from chronic injury in the liver. Forty-eight male albino rats (Sprague Dawley Strain) used in this study, the rats divided into two main groups. The first main group (6 rats) fed on basal diet (as a control negative group). The second main group (42 rats) treated with CCl_4 in paraffin oil (50% v/v 2 ml/kg) twice a week subcutaneous injection for two weeks to induce chronic damage in the liver. Then the second main group was divided into seven subgroups. Results showed that, injected rats with CCl4 induced significant increase in organs weights / body weight% including (liver and kidney), serum lipid profile except HDL-cholesterol, kidney functions, liver enzymes activity, bilirubin, glucose and nitric oxide (NO), while weight gain, serum protein, albumin and superoxide dismutase (SOD) decreased. Treating rats, which were, suffer from chronic injury on the liver with two level from pomegranate peels (3 and 6%), two dosage from olive oil (1 and 2 ml olive oil /kg b.w.) and the same dosage from olive oil with the same levels from pomegranate peels together improved all above parameters. the best results in these parameters recorded for the group treated with 6% pomegranate peels and 2 ml olive oil /kg b.w., followed by the groups treated with 3% pomegranate peels and 1ml olive oil /kg b.w. and 6% pomegranate peels, respectively. From these results, it could be concluded that, pomegranate peels and olive oil (alone or together) improved the sever disorders which result from the injection with CCl4.

Keywords: liver damage, liver enzymes, lipid profile, kidney function, glucose, superoxide dismutase, nitric oxide.

Introduction

Non-alcoholic fattv liver disease and non-alcoholic steatohepatitis occur in 10-24% of the general population **(Willner *et al.*, 2001)**. Hepatic fibrosis is a common result of chronic injury to the liver **(Wang *et al.*, 2010)**. Oxidative stress has been recognized as a fundamental factor in the pathological changes observed in various liver diseases **(Cederbaum and Lu, 2009)**.

Olive oil has traditionally been the principal oil of the Mediterranean diet. The mono unsaturated fatty acids (MUFA) diet prevents central body fat accumulation and decreases postprandial adiponectin expression induced by a carbohydrate rich diet in insulin-resistant subjects **(Paniagua *et al.*, 2007)**. Polyphenols present in olive oil, such as oleuropin, hydroxytyrosol, tyrosol and caffeic acid, have an important antioxidant and anti-inflammatory effect **(Covas *et al.*, 2006).** Previous studies carried out in fibrotic rats showed that olive oil, in contrast to polyunsaturated oils, could protect against the development of fibrosis (**Szende *et al.*, 1994)**. Decreasing total fat consumption and shifting to MUFAs found in olive oil (20 - 40% of total energy) or n-3 poly unsaturated fatty acids (PUFAs) found in fish oil (2 g/d) could lead to a decrease in postprandial lipidemia and steatosis **(Capanni *et al.*, 2006)**.

Antiatherogenic effect of olive oil is related to the antioxidant and anti-inflammatory effects exerted by various components, especially monounsaturated fatty acids (MUFA) and polyphenols **(Weinbrenner *et al.*, 2004)**. Phenolic compounds, especially hydroxytyrosol and oleuropein, dosedependently inhibit low density lipoprotein (LDL) and high density lipoprotein (HDL) oxidation in *vitro* and in *vivo*, repress superoxide-driven reactions, and break the chain-like propagation of lipid peroxides **(Visioli *et al.*, 2002)**. In this respect, **Fraser *et al.*, (2008)** reported that a modified Mediterranean diet, high in MUFAs, was associated with the lowest ALT levels in 6 months. They showed that these effects were independent of changes in body mass index (BMI).

Li *et al.*, (2006 [a & b]) reported that pomegranate peel extract appeared to have more potential as a health supplement rich in natural antioxidants than the pulp extract. High antioxidant content of pomegranate peel extract has a therapeutic effect on protecting liver from fibrosis and oxidative injury due to biliary obstruction **(Toklu *et al.*, 2007).** CCl4 produced significant elevation in triglycerides and cholesterol. Pomegranates are a source of polyphenols and other antioxidants **(Prakash *et al.*, 2008).** In this respect, **Sudheesh and Vijayalashmi, (2005)** concluded that flavonoids from pomegranate seem to possess a significant antiperoxidative activity.

Pomegranate peel contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid. The antioxidant effect of

flavonoids and polyphenols found in pomegranate enhanced the process of regeneration. This might be due to the destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of the damaged cell membrane **(Nasr *et al.*, 1996)**.

Abdel Moneim *et al.*, (2011) reported that pomegranate juice (PJ) and methanol extract of pomegranate peel (MEPP) reduced lipid peroxidation and nitric oxide in both liver and kidney tissue homogenate on the other hand these treatments recorded significant increase in superoxide dismutase and catalase activities of rats. The researchers reported also pomegranate has a potent anti-oxidative effect.

Carbon tetrachloride (CCl4) treatment is frequently used in rats to produce an experimental model to study liver fibrosis **(Safer *et al.*, 2012)**. The elevation serum enzymes Aspartate Amine Transaminase (AST), Alanine Amine Transaminase (ALT) and Alkaline Phosphatase (ALP) levels has been attributed to the hepatocellular degeneration and impairs different enzymatic systems **(Kim *et al.*, 1990).**

Therefore, the present study was carried out to assess the effects of olive oil and pomegranate peels on rats suffering from chronic injury in the liver induced by CCl4

Materials and Methods

Materials

Casein, vitamins, minerals, cellulose, carbon tetrachloride CCl4, DL-methionine and choline chloride were purchased from El-Gomhoreya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

Starch, soy oil, Pomegranate (*Punica granatum* L.) and olive oil were obtained from local market in Cairo. Egypt.

Forty-eight male albino rats (Sprague Dawley Strain) 150 ± 10 g were obtained from the laboratory animal colony, Ministry of Health and Population, Helwan, Cairo, Egypt.

Kits for biochemical analysis were obtained from Gamma Trade Co. for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods

Chemical composition of Pomegranate (*Punica granatum* L.)

Moisture, protein, fat, fiber and carbohydrate were determined in Pomegranate (*Punica granatum* L.) according to **A.O.A.C. (1990)**.

Determination of total polyphenolic content

Determination of total polyphenol content (TPC) by the Folin-Ciocalteu method. Total polyphenol content was carried out as described by **Ragaee *et al.*, (2006)** and **Dvorakova *et al.*, (2008)**. Total polyphenol content was carried out. Using gallic acid as an equivalent (GAE). The

reaction medium was comprised of 50 µL of Folin-Ciocalteu reagent, 200µL of 20% sodium carbonate, 740µL of distilled water and 10 µL of sample or standard solution. The absorbance was measured at 725 nm and total polyphenol content was calculated. The absorbance value was measured with a UV/VIS-Spekol 1300 spectrophotometer .The calibration analytical parameters were calculated with Excel and resulted in R2 = 0.999.

Fatty acid composition of olive oil

Gas liquid chromatography technique (GLC) was employed to identify the fatty acids composition of olive oil.

Extraction and methylation of fatty acids

The olive oil was saponified with methanolic KOH (20% W/V) for 24 hr. at room tempreture. The unsaponifiable matter was extracted three times with diethyl ether. The aqueous layer (soap) was acidified with HCL (1:1 V/V) and the liberated fatty acids were extracted with potrolum ether (40-60 ^{o}C). The fatty acides were washed several times with dislilled water, then dried over anhydrous sodium sulfate. The free fatty acids fraction were methylated with an ethereal solution of diazomethane and subjected for analysis by Gas Liquid Chromatographyic (GLC) for the identification of methylated fatty acids.

Separation of fatty acids methyl esters by GLC

The fractionation of fatty acids methylesters was conducted using a coiled glass column (15×14mm) packed with 10% polyethylene glycoladipate (PEGA). The separation conditions were;

- The column temperature was programmed at 8 ºC / min, initial temperature was 70 ºC , and final temperature was 190 ºC with a final time of 30 minutes.
- Chat speed was 2 min/cm.
- Detector temperature was 300 ºC.
- Injection temperature was 270 ºC.
- Flow rates of gasses were; nitrogen 3 ml/ min. and hydrogen 33 ml/ min. and air 330 ml/min.

Identification and determination of fatty acids

Peak identification was performed by comparing the relative retention time of each peak with those of standard materials. Fatty acids was calculated as present of the total identified acids after measuring the peak areas **(Gunstone *et al.*, 1994)** and **(Yeshajahu, 1994).**

Biological Studies

Experimental Animal Design

Forty-eight male albino rats Sprague Dawley Strain weighing (150 ± 10) were housed in a health condition and fed on basal diet (BD) for one week for adaptation according to **Reeves *et al.*, (1993).** After this week, the rats were divided into two main groups as follows: The first main group (6 rats) fed on BD used as a control negative group. The

second main group (42 rats) subcutaneously injected with CCL_4 in paraffin oil (50% v/v 2 ml/ kg bwt.) twice week for two weeks, to induce chronic damage in the liver **(Jayasekhar *et al.*, 1997)**. After this period serum AST, ALT and ALP were determined in the first and second main groups to insure the induction.

The rats in the second main group were divided into seven subgroups as follow: *Subgroup (1)*: was fed on the BD used as a positive control group. *Subgroup (2 and 3):* fed on BD supplemented with 3% and 6% dried pomegranate peels. *Subgroup (4 and 5):* fed on BD and treated each rat in each subgroup with (1ml/kg b.wt. and 2 ml/kg b.wt daily) olive oil, respectively. *Subgroup (6):* fed on BD supplemented with 3% dried pomegranate peels and treated daily with 1 ml olive oil/kg b.wt. *Subgroup (7):* fed on BD supplemented with 6% dried pomegranate peels and treated daily with 2 ml olive oil/kg b.wt. At the end of the experiment (4 weeks), the animals were fasted overnight, and then the rats were anaesthetized and sacrificed. Blood samples were collected from each rat. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. serum glucose according to **(Trinder, 1959)**, cholesterol **(Allain *et al.*, 1974)**, triglycerids **(Foster and Dumns, 1973)**, low density lipoprotein LDL-c and VLDL-c **(Friedwald *et al.*, 1972)**, uric acid **(Fossati *et al.*, 1980)**, urea nitrogen **(Patton and Crouch 1977)**, creatinine **(Bohmer, 1971)**, aspartate amino transaminase (AST) and alanine amino transaminase (ALT) **(Reitman and Frankel, 1957)**, Alkaline Phosphatase (ALP) **(Belfield and Goldberg 1971),** liver content of superoxide dismutase (SOD) acitvity **(Oyanagui, 1984)** and nitric oxide (NO) **(Nagi *et al.*, 2010).**

Liver and kidney were separated from each rat and weighted to calculate organs to body weight percentage.

The statistical analysis was carried out by using SPSS, PC statistical software (version 10.0; SPSS Inc, Chieago, USA). The results expressed as mean ± SD. Data analyzed by one-way analysis of variance (ANOVA). The Differences between means were tested for significance using least significant difference (LSD) test at ($P<0.05$) **(Steel and Torri, 1980)**.

Results and Discussion

Chemical composition of pomegranate *(Punica granatum* L.*)* peel:

Pomegranate peel and total polyphenols was analyzed and illustrated in Table (1). The moisture, protein, fat, ash, fiber, and carbohydrates of Pomegranate peel were 10.66, 3.31, 1.9, 3.00, 12.10 and 69.03 g/100g, respectively. The total polyphenols content of Pomegranate peel was 60.72 mg/g. In this respect, **Rowayshed *et al.*, (2013)** reported that, the pomegranate fruits peel powder is considered a good source of crude fibers, ash and carbohydrates, Therefore, pomegranate fruit peels and seeds powders should be utilized in fortification of foodstuffs.

The chemical composition reported was in accordance with **Li *et al.*, (2006[a])** who reported that the contents of total phenolics and flavonoids were higher in peel of pomegranate *(Punica granatum* L.*)* peel extract than in pulp extract. The large amount of phenolics contained in peel extract may cause its strong antioxidant ability.

Table (1). Chemical composition of pomegranate

Components	(g/100g DW)
Moisture	10.66
Protein	3.31
Fat	1.9
Ash	3.00
Fiber	12.1
Carbohydrates	69.03
Total polyphenols	**60.72**

Fatty acid composition of olive oil

Gas liquid chromatography technique (GLC) was employed to identify the fatty acids composition of olive oil. Fatty acid composition of olive oil was recorded in Table (2).

Table (2). Fatty acid composition of olive oil

Fatty acid	Value(%)
Lauric (12:0)	0.10
Myristic (14:0)	0.04
Palmitic (16:0)	15.2
Palmitoleic (16:1)	2.10
Stearic (18:0)	2.40
Oleic (18:1)	61.46
Linoleic (18:2)	17.20
Linolenic (18:3)	0.50
Arachidic (20:0)	1
SFA	**18.74**
MUFA	**63.56**
PUFA	**17.70**

In simillar study, **Scano *et al.*, (1999)** found that, fatty acids present in olive oil are palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic. Myristic, heptadecanoic and eicosanoic acids are found in trace amounts. Greek, Italian, and Spanish olive oils are low in linoleic and palmitic acids and they have a high percentage of oleic acid. Also olive oil is

composed of 71% oleic and 1% palmitoleic (monounsaturated fats); 10% linoleic and 1% linolenic (polyunsaturated fats); and 13% palmitic, 3% stearic, and 1% arachidic (saturated fats) **(ISEO, 2016)**.

Effect of olive oil and pomegranate peels on feed intake, body weight gain (%) and some organs weight/body weight% of rats suffering from chronic injury in the liver

Results in Table (3) illustrate the effect of two levels of pomegranate peels (3% and 6%), two dosage of olive oil (1 ml./kg b.wt. and 2 ml. / kg b.wt.) and their tested materials together (pomegranate peels and olive oil) on daily feed intake (g/day/each rat), body weight gain (%) and some organs (liver and kidney) weight/ body weight % of rats suffering from chronic injury in the liver.

The mean value of feed intake in healthy rats (control –ve) showed non-significant differences, as compared to chronic liver disease group (positive control group). High level of pomegranate peels led to significant ($P \leq 0.05$) decrease in feed intake, as compared to the positive control group. On the other hand, treating rats, which were suffering from chronic liver disease with the two dosage from olive oil, caused non-significant changed in feed intake, as compared to the control groups. The result in this table showed non-significant changed in the mean value of feed intake between the group fed on diet containing 6% pomegranate peels and treated with 2 ml olive oil /kg b.wt., as compared to the negative control group.

Concerning body weight gain (%) of the positive control group results showed a significant ($P \leq 0.05$) decrease, as compared to the negative control group. The two levels of pomegranate peels recorded significant ($P \leq 0.05$) decrease in BWG% , as compared to the positive control group. Treating chronic liver disease rats with (1 ml olive oil/kg b.wt.) showed non-significant differences in BWG%, as compared to the positive control group, while, (2 ml. olive oil/kg b.wt.).The mean value of BWG% significant ($P \leq 0.05$) increased, as compared to the positive control group. Feeding rats, which were, suffer from chronic liver disease on pomegranate peels and treated with olive oil (together) led to significant ($P \leq 0.05$) increase in BWG% , as compared to the positive control group.

All treated groups with the two levels of pomegranate peels (3 and 6%), two dosage of olive oil (1 ml./kg b.wt. and 2 ml. / kg b.wt.) and their tested materials together (pomegranate peels and olive oil) showed significant ($P \leq 0.05$) decrease in liver weight/body weight%, as compared to the positive control group. All tested groups showed non-significant differences in kidney weight/body weight %, as compared to the positive control group, on the other hand, the groups which treated with pomegranate peels (3 and 6%) recorded non-significant change in this organ, as compared to the negative control group.

In the simillar study **Murthy *et al.*, (2002)** reported that the decrease of weight gain in injected rats with CCL_4 might be due to gastrointestinal toxicity. **Cerd´a *et al.*, (2003)** suggest that pomegranate peel may prevent the accumulation of white adipose tissue (WAT) in high fat diet - induced obese rats. On the other hand, **Ludwing, (2000)** reported that, pomegranate peel consists of high dietary fiber; therefore, the increase consumption of fiber is associated with decreased body weight and reduction in other cardiovascular risk factors. Data in this table showed that, the groups that treated with the two dosages of olive oil increased the weight gain, as compared to the positive control group; this may be due to increase the caloric intake.

Table (3). Effect of olive oil and pomegranate peels on feed intake, body weight gain (%) and some organs weight/body weight% of rats suffering from chronic injury in the liver

Groups	Feed intake (g/day/rat)	BWG%	Liver weight / body weight%	Kidney weight / body weight
Negative control group (-ve)	17.30± 0.71 $^{b c}$	27.49± 2.361 ^{a}	2.91± 0.11 $^{d e}$	0.58± 0.06 ^{b}
Positive control group (+ve)	16.60± 0.56 $^{c d}$	18.76± 1.607 ^{c}	3.23± 0.11 ^{a}	0.68± 0.05 ^{a}
3% Pomegranate Peels	16.06± 0.48 $^{d e}$	16.38± 1.459 ^{d}	3.04± 0.13 $^{b c d}$	0.63± 0.03 $^{a b}$
6% Pomegranate Peels	15.48± 0.27 ^{e}	14.23± 1.639 ^{e}	2.94±0.13 $^{c d e}$	0.62± 0.02 $^{a b}$
1 ml olive oil /kg b.w.	17.22± 0.99 $^{b c}$	18.52± 0.920 ^{c}	3.10± 0.08 $^{a b}$	0.65± 0.07 ^{a}
2 ml olive oil /kg b.w.	17.30± 0.63 $^{b c}$	21.32± 0.820 ^{b}	3.08± 0.09 $^{b c}$	0.64± 0.03 ^{a}
3% Pomegranate Peels and 1 ml olive oil /kg b.w.	18.32± 1.03 ^{a}	21.87± 0.554 ^{b}	2.94± 0.12 $^{c d e}$	0.66± 0.02 ^{a}
6% Pomegranate Peels and 2 ml olive oil /kg b.w.	17.64± 0.76 $^{a b}$	22.32± 1.531 ^{b}	2.85± 0.06 ^{e}	0.64± 0.02 ^{a}

All results are expressed as mean $\pm$ SD. Values in each column which have different superscript letters are significant different at $p \leq 0.05$.

Effect of olive oil and pomegranate peels on lipid profile of rats suffering from chronic injury in the liver

The effect of two levels olive oil, pomegranate peels and (pomegranate peels and olive oil) together on serum cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-c), low and very low density lipoprotein-cholesterol (LDL-c and VLDL-c) mg/dl of rats suffering from chronic injury in the liver presented in Table (4).

Injected rats with CCL_4 (positive control group) led to significant ($P \leq 0.05$) increase in serum cholesterol, triglycerides, LDL-c and VLDL-c, while this treatment induced decrease the mean value of serum HDL-c significantly ($P \leq 0.05$), as compared to non-injected rats (negative control group). Treating chronic liver disease groups with the two levels from pomegranate peels, two dosage from olive oil and the two levels from pomegranate peels and olive oil together, the mean values of all lipid parameters significant ($P \leq 0.05$) decreased, except HDL-c which showed significant ($P \leq 0.05$) increase, as compared to the positive control group.

The mean values of serum cholesterol, triglyceride, LDL-c and VLDL-c decreased gradually with increasing the levels of tested materials. While HDL-c increased gradually. The highest improvement in lipid profile recorded for the group, which treated with (6% pomegranate peels and 2 ml olive oil/kg b.wt.) with each other. This treatment decreased the mean value of serum (cholesterol, triglyceride, LDL-c and VLDL-c) by about 29.19, 44.68, 53.81 and 44.68% respectively, than that of the positive control group, respectively. While HDL-c increased by about 88.66%.

Moreover the current study was in general agreement with **Esmaill *et al.*, (2006)** who reported that, intake of the concentrated pomegranate juice decreased LDL-c and the ratio of LDL-c/high density lipoprotein cholesterol (HDL-c) in patient suffering from diabetes. On the other hand, **Rosenblat *et al.*, (2006)** reported that pomegranate juice consumption resulted in a significant reduction in serum lipid peroxides and thiobarbituric acid reactive substances (TBARS) by 56% and 28% respectively.

Eating about 2 tablespoons (23 g) of olive oil daily may decrease the risk of coronary heart disease due to the monounsaturated fat in olive oil **(FDA 2014)**. Consuming olive oil decreased total and low-density lipoprotein (LDL) cholesterol compared to butter **(Engel and Tholstrup, 2015)**,

which may decrease risk of a heart attack or stroke. In addition, (about 2 tablespoons of extra-virgin olive oil per day) in adults over 50 years of age decreased total and LDL cholesterol within 6 weeks **(Haban *et al.*, 2004).**

Table (4). Effect of olive oil and pomegranate peels on lipid profile of rats suffering from chronic injury in the liver

Groups	Lipid Profile (mg/dl)				
	Cholesterol	Triglyceride	HDL-c	LDL-c	VLDL-c
Negative control group (-ve)	80.33 ± 4.33 [e]	39.55 ± 3.13 [d]	48.67±2.83 [a]	23.75 ±2.32 [g]	7.91 ± 0.63 [d]
Positive control group (+ve)	141.70 ± 6.16 [a]	77.87 ± 5.11 [a]	23.49 ± 2.14 [f]	102.64±5.49 [a]	15.57± 1.02 [a]
3% Pomegranate Peels	122.58 ± 4.32 [b]	59.57± 3.82 [b]	35.58 ± 1.56 [d]	75.09 ± 2.86 [c]	11.91 ± 0.76 [b]
6% Pomegranate Peels	110.18 ± 5.21 [c]	49.58 ± 3.81 [c]	45.56 ± 3.03 [b]	54.70 ± 3.55 [e]	9.92± 0.76 [c]
1 ml olive oil /kg b.w.	127.04 ± 4.51 [b]	60.48 ± 1.89 [b]	32.44 ± 1.64 [e]	82.50 ± 3.35 [b]	12.10 ± 0.38 [b]
2 ml olive oil /kg b.w.	115.22 ± 5.73 [c]	53.62 ± 3.04 [c]	48.78 ± 3.37 [a]	57.52 ± 4.88 [d e]	10.72 ± 0.61 [c]
3% Pomegranate Peels and 1 ml olive oil /kg b.w.	110.37 ± 4.24 [c]	51.00 ± 3.86 [c]	39.98 ± 1.33 [c]	60.19 ± 2.96 [d]	10.20 ± 0.77 [c]
6% Pomegranate Peels and 2 ml olive oil /kg b.w.	100.33 ± 4.26 [d]	43.07 ± 2.89 [d]	44.31 ± 1.19 [b]	47.41 ± 3.95 [f]	8.61± 0.58 [d]

All results are expressed as mean + SD. Values in each column which have different superscript letters are significant different at $p \leq 0.05$.

Effect of olive oil and pomegranate peels on liver enzymes and bilirubin levels of rats suffering from chronic injury in the liver

Table (5) illustrate the effect of olive oil and pomegranate peels on liver enzymes including (AST), (ALT) , (ALP) and bilirubin levels of rats suffering from chronic injury in the liver**.** The mean values of AST, ALT, ALP and bilirubin significant ($P \leq 0.05$) increase in the positive control group, as compared to the negative control group. These increases were about 148.61%, 316.86%, 101.59% and 37.08%, respectively.

Table (5). Effect of olive oil and pomegranate peels on liver enzymes and bilirubin levels of rats suffering from chronic injury in the liver

Groups	Liver enzymes (U/l)			Bilirubin (g/dl)
	AST	ALT	ALP	
Negative control group (-ve)	60.61 ± 2.99 [g]	21.36± 1.68 [g]	83.90 ± 3.64 [g]	2.51 ± 0.13 [f]
Positive control group (+ve)	150.68 ± 5.31 [a]	89.04 ± 3.11 [a]	169.14 ± 4.64 [a]	3.44± 0.16 [a]
3% Pomegranate Peels	132.82 ± 3.23 [c]	78.82 ± 2.48 [c]	144.96 ± 3.92 [c]	3.07± 0.11 [c d]
6% Pomegranate Peels	111.21 ± 5.04 [e]	67.41 ± 4.21 [e]	123.56 ± 4.18 [e]	2.88± 0.12 [e]
1 ml olive oil /kg b.w.	142.26 ± 2.11 [b]	83.61± 3.43 [b]	151.63 ± 2.29 [b]	3.25± 0.10 [b]
2 ml olive oil /kg b.w.	118.82 ± 2.97 [d]	72.42 ± 5.02 [d]	129.35 ± 4.89 [d]	3.12± 0.18 [b c]
3% Pomegranate Peels and 1 ml olive oil /kg b.w.	119.76 ± 1.74 [d]	67.74 ± 2.80 [e]	131.90 ± 2.18 [d]	2.93± 0.11 [d e]
6% Pomegranate Peels and 2 ml olive oil /kg b.w	103.11 ± 3.08 [f]	55.57 ± 4.07 [f]	112.45 ± 3.23 [f]	2.79± 0.09 [e]

All results are expressed as mean + SD. Values in each column which have different superscript letters are significant different at P≤0.05.

All treated groups showed significant ($P \leq 0.05$) decrease in AST, ALT, ALP and bilirubin, as compared to the positive control group. On the other hand, the mean values of liver enzymes and bilirubin decreased gradually with increasing the levels of pomegranate peels, olive oil and (pomegranate peels and olive oil) together. The data in this table recorded that, the two levels of pomegranate peels improved the mean values of liver enzymes and bilirubin than olive oil. The highest decrease in liver enzymes and bilirubin were found in the group which fed on diet containing 6% pomegranate peels and treated with 2 ml olive oil (with each other), followed by the group treated with 6% Pomegranate Peels.

These results in line with **Wei *et al.*, (2015)** whom stated that, the extracts of pomegranate peels (EPP) and seeds (EPS) have protective effects against liver fibrosis induced by CCl_4, and its mechanisms might be associated with their antioxidant activity. Pomegranate contains large amounts of polyphenols and flavonoid, so that the antioxidant capacity are obvious among in pomegranate fruit, juice and peel **(Aviram *et al.*, 2008)**. EPP have effective effect against liver fibrosis in rats. Experimental observations indicated that punicagranatum peel methanolic extract reversed thioacetamide-induced liver fibrosis, and significantly decreased the activity of liver enzymes, bilirubin and serum hepatocyte

growth factor levels. These effects could be attributed to its antioxidant properties, antifibrotic and antiapoptotic activity **(Salwe *et al.*, 2015)**.

Jalali *et al.*, (2017) reported that, diet containing (10 and 20% olive oil) decreased the level of ALT enzyme on the other hand the diet containing 20% olive oil reduced total serum cholesterol level in rats. **Bahrololumi *et al.*, (2014)** examined the effects of virgin olive oil on serum aminotransferases through a normal fat diet among 50 patients with non- alcoholic fatty liver disease (NAFLD). A significant decrease in ALT and AST was observed in the intervention group, as compared to the controls. Olive oil containing high amount of mono-unsaturated fatty acid.

Effect of olive oil and pomegranate peels on kidney functions of rats suffering from chronic injury in the liver

The results in Table (6) showed the effect of olive oil, pomegranate peels and (olive oil and pomegranate peels) on serum (uric acid, urea nitrogen, creatinine, total protein and albumin) of rats suffering from chronic injury in the liver. Injected rats with CCl_4 (positive control) induced significant increase ($P \leq 0.05$) in serum uric acid, urea nitrogen and creatinine, while total protein and albumin recorded significant ($P \leq 0.05$) decrease, as compared to non-injected rats (negative control group).

Treating rats, which were, suffer from chronic liver disease with olive oil, pomegranate peels and (olive oil and pomegranate peels) led to significant ($P \leq 0.05$) decrease in all parameters, except protein and albumin levels, as compared to the positive control group. These results revealed that, kidney functions improved gradually with increasing the levels of olive oil, pomegranate peels and (olive oil and pomegranate peels). Feeding rats, which suffer from chronic liver disease on diet containing 6% pomegranate peels and treated daily with 2 ml olive oil/kg b.wt. recorded the best results in kidney functions, followed by the group fed on diet containing 6% pomegranate peels.

The results coincided with that reported by **Abdel-Moneim and El-Khadragy, (2013)** whom suggested that pomegranate has established antioxidant properties that might have counteracted the oxidant effects of CCL_4. Administration of PG juice and PG peel methanol extract in rats with chronic renal failure significantly decreased serum levels of

creatinine, blood urea nitrogen, uric acid **(El-Habibi, 2013).** These effects are assumed to be related to the antioxidant property of pomegranate, through scavenger of free radical FR released because of oxidative damage **(Singh *et al.*, 2011)**. Olive oil could have a beneficial role against mercuric chloride induced oxidative and renal stress in rat **(Necib *et al.*, 2013)**.

Table (6). Effect of olive oil and pomegranate peels on kidney functions of rats suffering from chronic injury in the liver

Groups	Kidney functions (mg/dl)			Protein (g/dl)	Albumin (g/dl)
	Uric acid	Urea nitrogen	Creatinine		
Negative control group (-ve)	1.36 ± 0.09 ^{f}	27.20 ± 2.07 ^{g}	0.58 ± 0.04 ^{g}	6.12 ± 0.36 ^{a}	2.45 ± 0.10 ^{a}
Positive control group (+ve)	2.77± 0.09 ^{a}	61.75 ± 4.08 ^{a}	1.92± 0.08 ^{a}	4.63 ± 0.28 ^{e}	1.67± 0.15 ^{e}
3% Pomegranate Peels	2.31± 0.07 ^{c}	49.05 ± 1.35 ^{c}	1.45± 0.08 ^{c}	5.19±0.18 $^{c d}$	2.11± 0.12 $^{c d}$
6% Pomegranate Peels	1.90± 0.09 ^{d}	39.05 ± 1.44 $^{e f}$	1.01± 0.08 $^{e f}$	5.45 ±0.10 $^{b c}$	2.39± 0.09 $^{a b}$
1 ml olive oil /kg b.w.	2.51± 0.09 ^{b}	53.26 ± 2.85 ^{b}	1.58± 0.07 ^{b}	5.02± 0.27 ^{d}	2.05 ± 0.12 ^{d}
2 ml olive oil /kg b.w.	2.00± 0.09 ^{d}	41.65 ± 1.45 $^{d e}$	1.09± 0.10 $^{d e}$	5.31± 0.10 ^{c}	2.25± 0.10 $^{b c}$
3% Pomegranate Peels and 1 ml olive oil /kg b.w.	2.01± 0.07 ^{d}	43.05 ± 1.07 ^{d}	1.15± 0.06 ^{d}	5.47 ±0.09 $^{b c}$	2.33± 0.11 $^{a b}$
6% Pomegranate Peels and 2 ml olive oil /kg b.w	1.80± 0.03 ^{e}	36.20 ± 2.55 ^{f}	0.94± 0.06 ^{f}	5.72± 0.10 ^{b}	2.45± 0.14 ^{a}

All results are expressed as mean + SD. Values in each column which have different superscript letters are significant different at $p \leq 0.05$.

Effect of olive oil and pomegranate peels on serum glucose, superoxide dismutase and nitric oxide of rats suffering from chronic injury in the liver

Treating rats which were suffer from chronic liver disease with pomegranate peels, olive oil and their combination shown several changes of serum glucose, superoxide dismutase (SOD) and nitric oxide (NO) (Table 7).

The mean value of serum glucose increased ($P \leq 0.05$) significantly in the positive control group, as compared to the negative control group. All treated groups showed significant ($P \leq 0.05$) decreased in serum glucose, as compared to the positive control group. The highest decrease in serum glucose was found in the group treated with the high levels from

pomegranate peels and olive oil together, followed by the group treated with 6% pomegranate peels, respectively.

Table (7). Effect of olive oil and pomegranate peels on serum glucose, superoxide dismutase and nitric oxide of rats suffering from chronic injury in the liver

Groups	Glucose mg/dl	Superoxide dismutase (SOD) U/ml	Nitric oxide (NO) µmol/l
Negative control group (-ve)	82.61 ± 4.43 ^{g}	69.89 ± 3.45 ^{a}	2.14 ± 0.11 ^{g}
Positive control group (+ve)	149.98 ± 3.84 ^{a}	21.48± 1.17 ^{g}	13.69 ± 0.98 ^{a}
3% Pomegranate Peels	118.82 ± 4.53 ^{c}	30.13 ± 2.03 ^{f}	10.02 ± 0.37 ^{b}
6% Pomegranate Peels	102.12 ± 2.74 ^{e}	40.13 ± 2.20 $^{c d}$	7.84± 0.52 ^{e}
1 ml olive oil /kg b.w.	124.77 ± 4.71 ^{b}	33.31 ± 1.88 ^{e}	9.28± 0.35 ^{c}
2 ml olive oil /kg b.w.	109.08 ±2.87 ^{d}	42.91 ± 2.64 ^{c}	8.65± 0.24 $^{c d}$
3%Pomegranate Peels and 1 ml olive oil/kg b.w.	106.61 ± 3.73 $^{d e}$	37.87 ± 1.19 ^{d}	8.53 ± 0.68 $^{d e}$
6% Pomegranate Peels and 2 ml olive oil /kg b.w	97.03 ± 3.08 ^{f}	45.88 ± 2.57 ^{b}	6.89± 0.52 ^{f}

All results are expressed as mean + SD. Values in each column which have different superscript letters are significant different at p≤0.05.

The mean value of superoxide dismutase (SOD) significant (P≤ 0.05) decreased in rats suffer from chronic liver disease (positive control group), while nitric oxide (NO) increased significantly, as compared to the negative control group. Superoxide dismutase significant (P≤ 0.05) increased in all treated groups, while nitric oxide significant (P≤ 0.05) decreased with these treatments. Superoxide dismutase increased gradually with increasing the levels of pomegranate peels, olive oil and (pomegranate peels and olive oil), while nitric oxide decreased gradually. The best results in serum glucose, SOD and NO recorded for the group, which treated with 6% pomegranate peels and 2 ml olive oil /kg b.w. together.

The present results confirmed the data reported by **Parmar and Kar, (2007)** whom found that the administration of 200 mg/kg of pomegranate peel extract normalized all the adverse changes induced by alloxan, a widely used compound for inducing diabetes mellitus since it increases the serum levels of glucose and α-amylase activity and the rate of water consumption and lipid peroxidation in hepatic, cardiac, and renal tissues, while decreasing serum insulin levels

These effects are due to the inhibition of α-glucosidase in the gut mucosa. Several *in vitro* studies in cultured cells have shown that polyphenols may increase glucose uptake by peripheral tissues, which would diminish glycemia (**Scalbert *et al.*, 2005**). The mechanisms include inhibition of gluconeogenesis (**Waltner-Law *et al.*, 2002**), adrenergic stimulation of glucose uptake (**Cheng and Liu, 2000**), and stimulation of insulin release by pancreatic β-cells (**Ohno *et al.*, 1993**).

Consuming olive oil may help prevent type 2 diabetes (T2D) (**Guasch-Ferre *et al.*, 2015)**. A population study in Spain showed that those who consumed olive oil compared to sunflower oil had less risk of impaired glucose regulation **(Soriguer *et al.*, 2013)**. **Nakbi *et al.*, (2010)** showed that extra virgin olive oil and its extracts protect against oxidative damage of hepatic tissue by preventing excessive lipid peroxidation to increase mono-unsaturated fatty acid (MUFA) composition and by maintaining serum marker enzymes and hepatic antioxidant enzyme activities at near normal concentrations.

Finally, it could be concluded that pomegranate peels and olive oil improved the disorders which result from chronic injury in the liver which induced by injection with CCl4.Therefore, we are common ……. to use such food ….. i.e. olive oil as ….. in our daily dishes and beverages.

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تأثير زيت الزيتون و قشور الرمان على الفئران التي تعاني من إصابة مزمنة في الكبد

داليا محمد طلعت عبد الخالق [1] , ابتسام فتح محمود عمر[2]

قسم الاقتصاد المنزلي ، كلية التربية النوعية، جامعة الفيوم1 ، قسم الاقتصاد المنزلي ، كلية التربية النوعية، جامعة الاسكندرية [2]

الملخص العربى

تهدف هذه الدراسة لتقييم تأثير زيت الزيتون و قشور الرمان على الفئران التي تعاني من إصابة مزمنة في الكبد. استخدم في هذه الدراسة 48 فأر ذكر من نوع الألبينو من فصيلة (الاسبراجو داولي)،تم تقسيمهم إلى مجموعتين رئيسيتين. المجموعة الرئيسية الأولى (6 فئران) تم تغذيتهم على غذاء أساسي واستخدمت كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية (42 فأر) تم حقنهم تحت الجلد بمادة رابع كلوريد الكربون المختلط بزيت البرافين (50% حجم /حجم) بجرعة 2 مللى /كيلو جرام وزن فأر وذلك مرتين أسبوعيا لمدة أسبوعين لإحداث إصابة مزمنة في الكبد. المجموعة الرئيسية الثانية تم تقسيمها إلى 7 مجموعات فرعية (6 فئران لكل مجموعة). استمرت هذه التجربة 4 أسابيع , أظهرت النتائج أن حقن الفئران برابع كلوريد الكربون قد أحدث زيادة معنوية في وزن الأعضاء (الكبد والكلى) منسوباً للنسبة المئوية لوزن الجسم وصورة الدهن باستثناء الليبوبروتينات عالية الكثافة ووظائف الكلى وإنزيمات الكبد والبلوروبين والجلوكوز وأكسيد النيتريك بينما انخفض وزن الجسم المكتسب و بروتين الدم و الألبيومين و سوبر أكسيد ديسميوتيز. وقد وجد أن الفئران المصابة بإصابة مزمنة في الكبد التي تم تغذيتها على مستويين من قشور الرمان (6,3%) , جرعتين من زيت الزيتون (1 مل/ كجم من وزن الجسم و 2 مل / كجم من وزن الجسم) ونفس المستوى من زيت الزيتون ونفس المستوى من قشور الرمان معاً قد عمل على تحسن كل القياسات المرتفعة. أظهرت النتائج أن أعلى تحسن في التقديرات سُجل لمجموعة الفئران التي تم تغذيتها على 6% قشور الرمان و 2مل زيت الزيتون لكل كجم من وزن الجسم معاً. تلتها المجموعات التي تم تغذيتها على (3% قشور الرمان و 1 مل زيت الزيتون لكل كجم من وزن الجسم) و 6% قشور الرمان على التوالي. من هنا نستنتج أن قشور الرمان و زيت الزيتون (بمفردهم أو معاً) يحسن المضاعفات أو الأعراض الجانبية التي تنتج من الحقن برابع كلوريد الكربون.

الكلمات المفتاحية: إصابة مزمنة بالكبد , إنزيمات الكبد , صورة دهون الدم , وظائف الكلى , الجلوكوز , سوبر أكسيد ديسميوتيز , أكسيد النيتريك.