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The Beneficial Effects of Different Types of Olive Oil, Flaxseed Oil and Their Blend on CCl_4 – Induced Liver Hepatitis in Rats

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THIS study was conducted to evaluate the hepatoprotictive effects of olive, flaxseed oil and their blend on the hepatocytes liver of rat fed on basal diet supplemented with these oils. The effect of feeding rats on deferent levels of these oils on lipid profile was studied. The results indicated that the diet containing olive and flaxseed oils (at 100% from the oil content of the diet) led to significantly increase in the weight of rats compared to the control positive group. The results showed that the diet containing olive oil, flaxseed oil and blend oils 100% substitution caused significantly decrease of total cholesterol and recorded 79.71,76.18 and 76.66 mg/dl respectively, compared to the positive control group while there were no significantly differences between these three treatments regarding to the triglyceride. The Histopathological examination indicated that after CCl4 treatments, the livers of injured rats showed centrolobular coagulative necrosis, fatty change of the hepatocytes and dilatation with congestion in the central vein. The results revealed that feeding of rats on basal diet containing olive oil 100%, flaxseed oil 100% and mixed oil 100% lead to the best improvement in liver and significantly ameliorated the CCl4 induced necrosis and infiltration of lymphocytes.

Keywords: Olive oil, Flaxseed oil, Liver histopathology, Omega-3 oils, Hepatitis.

Introduction

In recent years, liver diseases became one of the very important worldwide health problems (Kavitha et al. 2011). Liver was found to be subjected to recurrent metabolic insults because of it is accomplish an indispensable assignment in detoxification, metabolism of a variety of drugs and xenobiotics and exposure to toxic chemicals including insecticides, pesticides and environmental pollutants (Sethuraman, 2003). CCl_4 was reported to be used in animal models to study the chemical toxin-induced liver damage (Elbakry et al., 2017). The toxicity of CCl_4 is attributed to its metabolized and producing a highly reactive free radicals and oxygen species which lead to lipid peroxidation of cell lipid and liver injury (Manickam et al., 2017). The hepatocyte injury of liver including that relating to CCl_4 was found to activate the Kupffer cells which promote tissue damage (Muriel and Escobar, 2003).

Omega-6 (ù-6) and Omega-3 (ù-3) fatty acids are essential, polyunsaturated fatty acids (PUFAs) and cannot synthesize in vivo. Omega-3- and omega-6 fatty acids are derive from á-linolenic and linoleic acids (LA), Considerable quantities of the polyunsaturated fatty acids are found naturally in fish oil, olive oil, flaxseed, sunflower and canola oils. Its regulate a wide variety of biological benefits including protective effects against cardiovascular diseases,anti-inflammatory effects, essential for the functioning of the brain and nervous system d retina, (Bassett et al., 2009). The ratio between omega-3 and omega-6 to fatty acids in the healthy diet has to be 1: 2 to 1: 4**.** Recently, the intake of ù-6 and ù-3 showed remarkable increase and recorded ratio of (15: 1) Patterson et al., (2012).

Olive oil became one of the pillars of the called Mediterranean diets. The importance of olive oil is due to its content of the bioactive components such as polyphenols and unsaturated fatty acids. These ingredients make olive oil used as a functional food. It has been reported also that olive oil is able to reduce the risk of Coronary Heart Disease (CHD) by decreasing levels of artery-clogging lipids in the blood (Kok and Kromhout, 2004).

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Virgin olive oil is known to be more resistant to oxidation comparing to the other edible oils due to its high level of natural antioxidants (mainly polyphenolic compounds) which was found to have protective effect on oxidative stress (Ocakoglu et al., 2009). Olive oil was found to reduce inflammation, lowering the total and low-density lipoprotein (LDL) cholesterol, reducing blood platelet clumping, prevent oxidation damage of tissues and provides protection against cancer risk (Kozic et al., 2015 and Engel and Tholstrup, 2015).

Flaxseed (*Linum usitatissimum* L.) found to contain the highest percentage of á-linolenic acid (ALA) (18:3 - omega-3 fatty acids) amongst the known oilseeds, ranged from 40: 60% of its oil. Flaxseed oil (FO) had high content of the essential polyunsaturated fatty acids (PUFA) such as olic, lenoliec and linolenic acid**s** which make it one of the most useful vegetable oils (Hamed and Abo-Elwafa, 2012). Flaxseed oil, because of its content of omega-3 fatty acids, was found to be unstable and is sensitive to heat, oxygen and light and therefore it is usually cold-pressed from the whole seeds (Daun, et al., 2003). This study aimed to evaluate the effect of different types of olive and flaxseed oil and its blended on lipid profile, antioxidant enzymes, total antioxidant capacity and the function of liver in rats, as well as, to investigate the hepatoprotective action of these oils in different concentrations on rat liver.

Materials and Methods

Materials

Olive Fruits (*Olea europea* L.) (*Koronaki* CV) were obtained from olive farm (25kg fruits) at Fayoum Governorate, Egypt. Flaxseeds (Sakha1) were obtained from oil Seeds Research Institute (13kg seeds), Agriculture Research Center, Egypt**.** Commercial Flaxseeds Oil, Commercial Virgin Olive oil and Corn oil were obtained from local market, Egypt.

Methods

Extraction of Olive Oil

The virgin olive oil was obtained According to Di Giovacchino et al., (1994) modified methods. The fruits were cleaned using de-leaver and washed. The clean fruits were crushed l and slowly mixed at 25ºC for 30 min. The oil was obtained by pressing the paste 300 bar/in2. Then the obtained (aqueous and oily) was separated by centrifugation at 5000 rpm for 15min. The obtained oil was transferred into a dark glass bottle and stored at -18°C

Extraction of Flaxseed Oil

Flaxseeds were cleaned by hand carefully and crushed. The flaxseed oil was obtained using a fresh cold press. The crude oil obtained was centrifuged at 5000 rpm for 15min without addition of water or chemicals. The obtained oil was transferred into a dark glass bottle and stored at -18°C.

Identification and determination of fatty acids by Gas Liquid Chromatograph (GLC)

Lipid samples were saponified over-night with ethanolic KOH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with hydrochloric acid (5 N), followed by extraction with pt. ether 40-60ºC. The ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off. Identification and determination of fatty acids in olive oil, flaxseed oil and their blends were carried out according to the method described by Farag et al., (1986). Identification and determination of fatty acids in olive oil, flaxseed oil and their blends were carried out according to the method described by Farag et al., (1986).

Biological Investigation:

Sixty Female albino rats weighting (180-200 ±5 g) used in this study were maintained in clean, sterile polypropylene cages. Animals were housed for one week before experiments under standard laboratory condition of constant temperature (24±3°C), relative humidity (60% ± 4%) and 12-hour light- dark cycle. The animals had free access to basal diet and water. The basal diet was prepared according to Reeves et al., 1993**.**

Experimental design

After the adaptation period on basal diet, the rats were divided into two main groups. The first group (5 rats) was fed on basal diet as a negative control (NC), corn oil used as a source of fat. The second group (55 rats) was treated with CCl4 in paraffin oil (50% v/v 2ml/kg) for two weeks (three times a week) by subcutaneous injection to induce chronic hepatic in the liver, according to the method described by (Jayasekhar et al., 1997). This group was divided into positive control group and ten treatment groups (5 rats each) as following: Positive control: was fed on basal diet containing 7% corn oil as a positive control group.

Treatment (1): received basal diet containing 7% commercial virgin olive oil.

Treatment (2): was fed on basal diet containing

3.5% extracted virgin olive oil and 3.5% corn oil.

Treatment (3): was fed on basal diet containing 7% extracted virgin olive oil.

Treatment (4): was fed on BD containing 7% commercial flaxseed oil.

Treatment (5): received BD containing 3.5% extracted flaxseed oil and 3.5% corn oil.

Treatment (6): received BD containing 7% extracted flaxseed oil.

Treatment (7): was fed on BD containing 3.5% extracted blended oil and 3.5% corn oil.

Treatment (8): was fed on BD containing 7% extracted blended oil.

Treatment (9): was fed on BD containing 7% corn oil supplemented with hepaticum 0.2 ml / rat daily.

Treatment (10): was fed on BD containing 7% corn oil supplemented with hepaticum 0.4 ml / rats daily.

During the experimental period (30 days), body weights were recorded three times a week.

Body weight gain (BWG) was determined according to Anitha et al., (2008).

Biochemical analysis of liver functions

At the end of experimental period (30 days), rats were sacrificed after overnight fasting. Blood samples were collected for each rat and placed in dry clean centrifuge tube, and then centrifuged for 10 minutes at 3000 (rpm). Serum was carefully separated and stored at -80°C until analysis. Liver, heart, kidney, lungs and spleen were removed, then washed in saline solution and weighted after drying with filter paper.

Enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, albumin and creatinine in serum were measured using the commercially available diagnostic kits supplied by Randox Laboratories (Ardmore, Northern Ireland, UK) according to (Henry, 1974; Balistreri, and Shaw, 1987; Koller, 1984 and Young, (1990).

Liver histopathology

Histopathology examination Specimens from liver tissues were taken immediately after sacrificing animals, and fixed in 10% buffered neutral formalin solution for twenty four hours. Washing by tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slide microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin stains (Banchroft et al*.,* 1996) for histo-pathological examination through the light microscope.

Statistical analysis

Data were expressed as the mean± SD for five rats in each group. The data were analyzed using SPSS software package version 20.0 and values were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range tests at 5% level of significance was used to compare between means.

Results and Desiccation

Fatty acid composition of virgin olive; flaxseed and blended oils:

Fatty acid composition, percent of saturated fatty acids, percent of unsaturated fatty acids and ratio of omega-3 to omega-6 fatty acids of virgin olive oil, cold pressed flaxseed oil and blend oil (virgin olive oil and cold pressed flaxseed oil 50:50 V/V) are summarized in Table (1). The fatty acid composition of virgin olive oil was 81.34% unsaturated fatty acid divided into mono unsaturated 68.5% and poly unsaturated 13.17%. These results are in agreement with that obtained by Torres and Maestri, (2006). and Gimeno et al*.,* (2002) reported that the major fatty acids in olive oil of *Arbequina* variety were oleic (67-75%), palmitic (13-15%), linoleic (9-11%), stearic (1.2-2%) and palmitoleic (1-1.5%). Our results are different from that obtained by Hashempour *et al.,* (2010) .The difference in fatty acid composition of oils could be due to the cultivars, origin and its corresponding environmental variation.

When the olive oil was blended with flaxseed oil (50:50%) the ÓUF acids and ÓPUF acids of blended oil were higher than that of olive oil and lower than the flaxseed oil (Fig.1). The results also showed that ÓMUF acids in the blended oil were higher than flaxseed oil and lower than olive oil. Blended could enhance the stability of flaxseed oil because of the polyphenolic compounds in olive oil which had antioxidant activity and improve the nutritional value of the olive oil due to the higher content of ÓPUF acids in flaxseed oil.

TABLE 1. Fatty acid composition of olive, flaxseed and blended oils.

Relative percentage of fatty acid	Olive oil	Flaxseed oil	blended oil
Palmitic acid (C16:0)	14.76	5.94	10.13
Palmitoleic acid (C16:1)	1.59	0.17	0.83
Heptadecanoic acid (C17:0)	0.04	0.05	0.065
Heptadecenoic acid (C17:1)	0.11	0.04	8 0.0
Stearic acid (C18:0)	2.67	3.21	3.06
Oleic acid (C18:1)	61.38	18.38	41.34
Linoleic acid (C18:2)	12.09	17.77	15.21
Linolenic acid (C18:3)	1.08	53.54	28.32
Arachidic acid (C20:0)	0.26	0.37	0.39
Ecoseoic acid (C20:1)	5.42	0.55	0.31
Behenic acid (C22:0)	0.108	0	0.11
Lignoceric acid (C24:0)	0.49	0	0

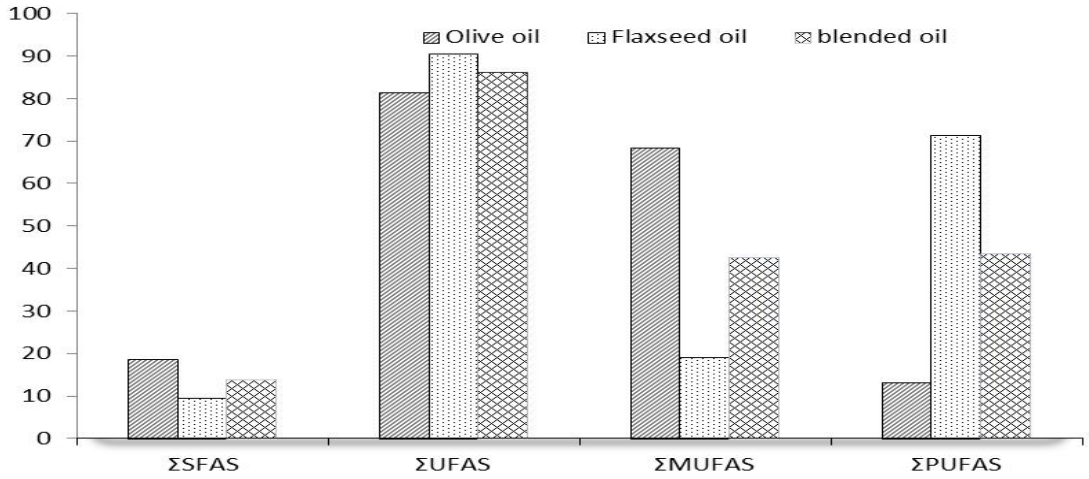

Fig.1. Saturated and unsaturated fatty acids % of olive, flaxseed and its blende oils .

Increase in oleic acid content is due to the triacylglycerols active biosynthesis which takes place throughout fruit ripening, involving a fall in the relative percentage of palmitic acid content. On the other hand, the increase in linoleic acid content is due to the transformation of oleic acid into linoleic acid by the oleate desaturase activity which is active during triacylglycerol biosynthesis **(**Bozan and Temelli, 2008). The results also showed that total unsaturated fatty acids in the flaxseed oil was 90.45% while poly unsaturated fatty acid content was 71.30%. Omega3: omega 6 fatty acids ratio was 3.08. The percentage of linoleic and linolenic acid was 17.77 and 53.54% respectively.

Biological Investigation

Body and organs weight%

All treated rats survived, and oils and Hepaticum administration did not provoke diarrhea in the rats. Data in Table 2 indicated that body weight of the rats significantly decreased ($p< . 5$) in CCl_4 treated rats, and significantly increased ($p< . 1$) in control negative group. On the other hand, all groups treated with CCl_4 and fed on BD containing different concentrations of olive, flaxseed and blended oils recorded a significant increase in BWG% compared to the positive control and Hepaticum 0.2ml and Hepatecom 0.4ml treated groups respectively.

TABLE 2. Effect of olive and flaxseed oil addition on rat's weight.

Rats group	Initial body weight (g)	Final body weight (g)	Body weight gain (%)	Liver (% of FBW)	Kidney (% of FBW)	Spleen (% of FBW)
Control negative	143.00±4.02^{b}	156.29±2.29^{b}	7.26±1.35^{c}	2.28 ±0.08^{d}	0.43±0.2^{f}	0.19± .01^{f}
Control positive	148.10±5.13^{bc}	122.70±4.00^{f}	-17.15±3.6^{g}	3.36 ±0.1^{a}	0.77±0.01^{b}	0.29±0.01^{ab}
Treatment (1)	145.80±5.70^{b}	156.7±3.27^{bc}	7.48±1.41^{bc}	3.16± 0.2^{ab}	0.70±0.01^{c}	0.29±0.01^{ab}
Treatment (2)	146.20±4.34^{bc}	158.70±2.38^{b}	8.55±1.79^{b}	3.19 ±0.32^{ab}	0.71± 0.02^{c}	0.29±0.0$^{a\ b}$
Treatment (3)	145.50±3.59^{b}	157.8±4.39^{bc}	9.07±2.44^{ab}	2.58 ± 0.13^{bc}	0.65±0.01^{cd}	0.27±0.01^{bc}
Treatment (4)	150.00±3.08^{ab}	153.43±5.73^{c}	2.28±0.59^{e}	3.17 ±0.19^{ab}	0.86±006^{a}	0.33±0.02^{a}
Treatment (5)	146.50±3.66^{b}	159.9±3.19^{ab}	9.14±1.17^{a}	2.85 ±0.15^{b}	0.57±0.04^{gfe}	0.20±0.01^{efg}
Treatment (6)	145.00±5.58^{b}	159.0±1.91^{ab}	9.66±0.59^{a}	2.40 ±0.08^{bc}	0.64±0.01^{cde}	0.32±0.005^{a}
Treatment (7)	140.20±2.44^{b}	146.0±1.83^{d}	4.14±1.16^{d}	2.49 ±0.14^{bc}	0.6±0.05^{d}	0.26±0.01^{bc}
Treatment (8)	154.30±3.65^{a}	162.0±3.85^{a}	4.90±0.76^{d}	2.42 ±0.05^{bc}	0.55±0.02^{de}	0.22±0.05 ^{cd}
Treatment (9)	143.40±3.96^{b}	139.50±2.76^{e}	-2.70±0.59^{f}	2.79 ±0.02^{b}	0.67±0.02^{cd}	0.24±0.01^{c}
Treatment (10)	150.10±4.16^{ab}	152.67±5.15^{c}	1.68±0.38^{e}	2.40 ±0.11^{bc}	0.52±0.03^{e}	0.21±0.01^{de}

a,b...ect Means in the same column with different letters, differ significantly $p<0.05$

The body weight gain% of control negative was 7.26±1.35% and control positive was -17.15±3.6%. The results also showed that group treated with Hepaticum 0.2ml recorded decrease in body weight gain (-2.70±0.59%) while the group treated with Hepaticum 0.4 recorded a little increase in body weight gain about (1.68±0.38%). Similar results were reported by Ismail et al., 2009, and Kumar et al., 2013. The results also showed that there are significant increases ($p<0.05$) in liver weight and the liver /body weight% for the PC as compared to the NC group. Concerning to the other organs, there were no significant differences between the treated samples and the control negative and control positive in the most of the organs. These results are consistent with previous investigations that showed feeding animals a diet rich in ù-3 fatty acid caused liver weights to be significantly higher (Alaraj and Qiblawi (2015).

Change on serum lipids

The effects of replacement of BD fat with different dietary oils and different levels of Hepaticum on serum levels of total cholesterol (TC) and triglycerides after the treatment of rats with CCl_4 are summarized in Table (3). Total cholesterol (TC) and triglycerides were determined at zero time (after CCl_4 treatments), 15 days and 30 days of feeding rats with at BD containing different dietary oils. The results indicated that at zero time total cholesterol (TC) in all treated groups were significantly higher than that of control negative group. These results revealed that treated rats with CCl_4 caused hepatitis which lead to increase of TC. After 15 day of feeding the TC start to decrease in all groups except the control positive group while after 30 days of feeding there was significant different between the control positive and the other groups. Replace of BD fat with flaxseed oil (100%) recorded the lower TC content (76.18 mg/dL) followed by blended oil 76.66 mg/dL and olive oil (100%) 79.71 mg/dl while the control positive recorded 187.95 mg/dl and control negative (80.20 mg/dl).

TABLE 3. Effect of replacement of dietary oil be different concentration of olive oil, flaxseed oil and there blended on serum lipids of rats suffering from chronic hepatitis liver.

Rats group	Cholesterol mg/dl			Triglyceride mg/dl		
	Zero time	15 days	30 days	Zero time	15 days	30 days
Controlnegative	87.84± 5.09^{e}	85.21±8.60^{e}	80.20±7.53^{d}	134.80±9.95^{c}	129.20±6.61^{e}	125.01±5.07^{d}
Control positive	167.68±5.31^{bc}	177.38±2.37^{a}	187.95±5.95^{a}	251.98±9.21^{a}	278.99±9.25^{a}	290.13±5.53^{a}
Treatment (1)	166.38±2.45^{bc}	155.07±1.17^{b}	135.92±3.49^{b}	199.20±9.72^{b}	174.18±12.2^{bc}	148.57±6.35^{c}
Treatment (2)	170.72±5.36^{b}	132.02±2.49^{cd}	90.27±5.64^{c}	202.65±11.21^{b}	174.68±8.01^{bc}	101.94±4.92^{g}
Treatment (3)	184.35±3.75^{a}	86.37± 9.28^{e}	79.71±4.98^{d}	198.43±11.22^{b}	153.03±7.70^{cd}	90.57±5.100^{f}
Treatment (4)	167.25±3.86^{bc}	142.64±3.51^{c}	132.40±8.86^{b}	197.25±13.74^{b}	181.56±6.82^{b}	169.75±6.43^{b}
Treatment (5)	166.67±4.90^{bc}	125.97±9.39^{d}	80.95±5.98^{d}	196.87±7.08^{b}	161.70±9.60^{c}	97.48±4.17^{f}
Treatment (6)	163.80±5.37^{c}	98.43±8.71^{e}	76.18±1.02^{d}	199.49±10.27^{b}	152.36±10.80^{d}	89.56±1.26^{f}
Treatment (7)	166.09±1.60^{bc}	130.72±3.53^{cd}	78.55±6.14^{d}	197.30± 10.22^{b}	174.67±6.91^{bc}	114.39±4.21^{e}
Treatment (8)	171.01±6.23^{b}	119.71± 6.66^{d}	76.66±5.94^{d}	198.43±10.28^{a}	151.76±9.52^{cd}	90.72±3.93^{f}
Treatment (9)	145.49±5.41^{d}	137.24±3.73^{cd}	130.28±6.98^{b}	197.44±6.98^{b}	188.21±7.50^{b}	159.29±3.94^{c}
Treatment (10)	168.67±5.94^{bc}	125.79±6.29^{d}	81.02±9.05^{d}	199.30± 6.28^{ab}	165.67±11.19^{c}	130.74±4.64^{d}

,a,b. ect Means in the same column with different letters, differ significantly $p<0.05$

Concerning to the triglycerides, similar trends were observed. Rats fed diets containing flaxseed oil (100%) had significantly lower level of TG followed by rats fed diets containing olive oil (100%) and mixed oil (100%) and recorded TG levels of 89.56 mg/dl, 90.57 mg/dl, and 90.72 mg/dl, respectively while the positive control recorded 290.13 mg/dl. These results are agreed with that obtained by (Cintra et al., 2006; Vijaimohan et al., 2006, and Ismail et al., 2009)**.** The ù-3 fatty acids are reported to lower serum cholesterol and triglyceride levels. In addition, beneficial effects of ALA on plasma lipid and lipoproteins is more controversial; it has been indicated to decrease in total cholesterol (TC), LDL-cholesterol (LDL-C), LDL-C/HDL-C. Similar findings were reported by Cho et al., (2009). They found that flaxseed oil significantly lowered plasma total cholesterol and free fatty acid whereas it significantly increased HDL-C concentration.

Biochemical indicator of liver functions

Total protein, Albumin, Creatinine and T. bilirubin of rats suffering from chronic hepatic liver

The results in Table 4 revealed that the treated rats with CCl_4 significantly decreased the TP level in rats compared to the negative control. After 30 days of feeding the treated groups on BD containing olive oil, flaxseed oil and mixed oil at different levels and 2 levels of Hepaticum, the results showed no significant differeces between the negative control and the other groups. On the other hand, there was significant differeces between the positive control and the other groups. This finding indicating that olive oil and flaxseed oil which are rich in omega 6 and omega 3 fatty acids could be used to the treatment of hepatitis. Results in the same table showed that the treated rats with CCl_4 decreased the albumin level in the positive control which recorded 2.0±0.089g/dL. While, feeding the rats on BD containing the

tested oils increased the albumin content to the normal range. These results are consistent with that reported by Wiesenfeld et al., (2003) and Fukumitsu et al., (2010) for flaxseed oil and that obtained by Kasdallah-Grissa et al., (2008) and Nakbi et al., (2010) for olive oil.

The results showed that after the hepatic liver was induced by CCl_4, the T. bilirubin level was significantly increased in all groups compared to the control negative. This increase in the total bilirubin could be returning to blockage of bile ductules the regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids (Ahmed, 2001). On the other hand, at the end of feeding experimented the T. bilirubin levels were significantly decreased in the treated groups compared to the positive control and was much closed to the normal level recorded for the negative control. The highest T. bilirubin level was recorded for the positive control group (1.46mg/dL.) followed by group fed on BD with commercial olive oil 100% and hepaticum .2ml and recorded 1.28 and 1.21mg/dL respectively. Similar results was reported by Chavan et el., 2013 and Singh et al., 2014)

Concerning to the creatinine level, the results showed that the creatinine level was increased in all groups compared to the control negative at zero time of feeding experiment. After 30 days of feeding, the creatinine levels showed no significant differeces between the treated groups and the control negative while the control positive group showed significant higher creatinine level compared to the other groups and recorded 1.24mg/dL. while the control negative recorded 0.62mg/dL.

TABLE 4. Effect of replacement of dietary oil be different concentration of olive oil, flaxseed oil and there blended on liver functions of rats suffering from chronic hepatitis liver.

Rats group	Total protein g/dL	Albumin g / dL	T. bilirubin mg/dL	Creatinine mg/dL
Control egative	7.59±0.34^{a}	3.97±0.035^{e}	0.45±0.05^{g}	0.62±0.06^{f}
Control positive	2.10±0.25^{e}	2.0±0.089^{f}	1.46±0.02^{a}	1.24±0.04^{a}
Treatment (1)	5.0±0.17^{c}	3.26±0.127^{d}	1.28±0.01^{b}	0.80± .05^{cd}
Treatment (2)	6.65±0.53^{ab}	3.87±0.39^{cd}	0.93±0.05^{c}	0.75±0.03^{e}
Treatment (3)	7.03±0.31^{a}	4.13±0.29^{b}	0.66±0.017^{e}	0.63±0.07^{g}
Treatment (4)	4.37±0.14^{d}	3.08±0.051^{e}	1.19 ±0.03^{b}	0.94±0.01^{b}
Treatment (5)	0.69^{b}±6.18	4.07±0.07^{bc}	0.72 ±0.05^{d}	0.68±0.03^{ef}
Treatment (6)	6.69±0.17^{ab}	4.5±0.138^{a}	0.62 ±0.01^{e}	0.65±0.01^{e}
Treatment (7)	0.36^{b}±6.08	4.58±0.051^{a}	0.66±0.02^{e}	0.79±0.03^{cd}
Treatment (8)	6.33±0.43^{c}	4.84±0.13^{a}	0.56 ±0.01^{f}	0.72±0.0^{e}
Treatment (9)	6.60±0.30^{ab}	3.11±0.063^{e}	1.21 ±0.02^{b}	0.89±0.04^{bc}
Treatment (10)	6.53±0.16^{ab}	4.06±0.11^{bc}	0.73±0.01^{d}	0.78±0.02^{c}

a,b...ect Means in the same column with different letters, differ significantly $p<0.05$,

AST, ALT and ALP

Liver injury is determined by measuring the levels of hepatocellular enzymes in liver (AST, ALT and ALP). When the levels of these enzymes increased, this proved mitochondrial and cell membrane damage. The levels of serum asparate amino transaminase (AST), alanine amino transaminase (ALT) and alkaline phosphate (ALP) were taken as an index for hepatotoxicity induced by CCl_4. The levels of AST, ALT and ALP were analyzed in serum samples after treatment with CCl_4 and after feeding on BD containing different levels of investigated oils. Data in table (5) showed that serum levels of ALP, ALT and AST were significantly ($P<0.05$) increased in CCl_4-treated rats and recorded 219.66, 77.33 and 72.99 for ALP, ALT and AST respectively. The results indicated that treated rats with CCl_4 for two weeks induced an increase in the levels of serum ALT, AST and ALP in rats due to membrane damage which releases these enzymes into circulation this results agreed with the previously reported results (Al-Atar et al., 2017). However, treated rats with the olive, flaxseed and there blende oils after CCL_4 intoxication decreased the rates of AST, ALT, ALP towards the normal level found in the control group. These results are agreed with that reported by Ismail et al., (2009) and Kumar et al.,

(2013). In addition to flaxseed oil which is the rich sources of omega 3-fatty acids and is consequently potent antioxidants, olive oil has been found to be rich source of poly phenolic compounds which had antioxidant activity. Dietary supplementation of olive and flaxseed oil are known to ameliorate hepatotoxicity. These oils have been shown to protect liver against toxicity (Fadlalla et al., 2013).

Nagaaju and Lokesh (2008) reported that blended and interesterified oils of coconut with groundnut oil or olive oil enhanced hepatic antioxidant enzymes, decreased lipid peroxidation in liver and reduced the susceptibility of LDL to oxidation.

Histopathological of rats' liver

The liver tissues of the rats were microscopically examined before any treatments (control negative) and after CCl_4 treatment for to induce chronic hepatic in liver (positive control). After feeding the CCl_4 injured rats on BD containing different levels of olive, flaxseed, and blended oil and two levels of epaticum . ml and . ml rat dayfor 30 days. The results (Fig.2) indicated that the control negative (A) showed normal histopathological structure with a well-preserved cytoplasm and legible nucleus and a well-defined sinusoidal line. On the other hand, the livers of CCl_4 injured rats showed centrolobular coagulative necrosis, fatty change (amw) of the hepatocytes and dilatation with congestion in the central vein, disarray in the hepatic cods and increase of inflammatory cells (B). These results are agreed with that reported by Fang et al., (2008).

CCl_4 is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon-centered trichloromethyl radical, and leading to lipid peroxidation. Kupffer cells have been shown to be involved in many types of chemical-induced liver damage, including that relating to CCl_4 (Weber et al., 2003). Kupffer cells are activated in response to CCl_4 administration and promote tissue damage by releasing biologically active mediators, such as reactive oxygen species (ROS) and cytokines. It is considered that Kupffer cells are needed for initializing CCl4-induced fibrosis (Luckey and Petersen, 2001 and Muriel and Escobar, 2003).

The results revealed that feeding of rats on BD containing olive oil 100%,flaxseed oil 100% and blend oil 100% replacement lead to the best improvement in liver and significantly ameliorated the CCl_4 induced necrosis and infiltration of lymphocytes (Figure 2 C, D and E). The protective effect of flaxseed oil in liver is mainly assigned to the poly unsaturated fatty acids which shown to have antioxidant properties and lad to induce hypolipidemi, hypoglycemic and hypocholesterolemic effects. These results are in agreement with the results obtained by Ismail et al., (2009). In this concern, Cohen et al., (2005) found that n-3 PUFA as found in FO has anti-inflammatory properties that are mediated by the production of anti-inflammatory eicosanoids.

TABLE 5. Effect of different dietary oils on ALP, ALT and AST of rats suffering from chronic hepatic liver.

Rats group	P (U)	T (U)	T (U)
Control negative	124.33±2.01^{d}	28.2±1.59^{d}	47.00±2.1^{e}
Control positive	219.66±2.1^{a}	77.33±0.78^{a}	72.99±2.67^{a}
Treatment (1)	156.67±3.76^{c}	57.67±0.78^{b}	57.33±0.96^{c}
Treatment (2)	110.66±5.57^{f}	25.3±0.78^{bc}	63.43±2.42^{bc}
Treatment (3)	123.36±1.96^{d}	29.0±0.59^{d}	53.52±1.75^{cd}
Treatment (4)	166.3±1.89^{b}	57.2±1.59^{b}	66.39±2.33^{b}
Treatment (5)	110.2 ± 3.12^{f}	20.3±0.96^{e}	63.55±3.67^{bc}
Treatment (6)	117.0± 1.8^{e}	22.12±1.3^{e}	59.0±2.72^{c}
Treatment (7)	120.01±3.14^{d}	20.67±2.2e	59.67±8.41^{c}
Treatment (8)	114.53±1.93^{e}	21.7±0.78^{e}	51.81±1.09^{d}
Treatment (9)	155.77±2.89^{c}	56.66±1.29^{b}	65.67±1.25^{b}
Treatment (10)	114.24±4.03^{e}	50.01±1.2^{c}	55.67±1.50^{cd}

,a,b...ect Means in the same column with different letters, differ significantly $p<0.05$

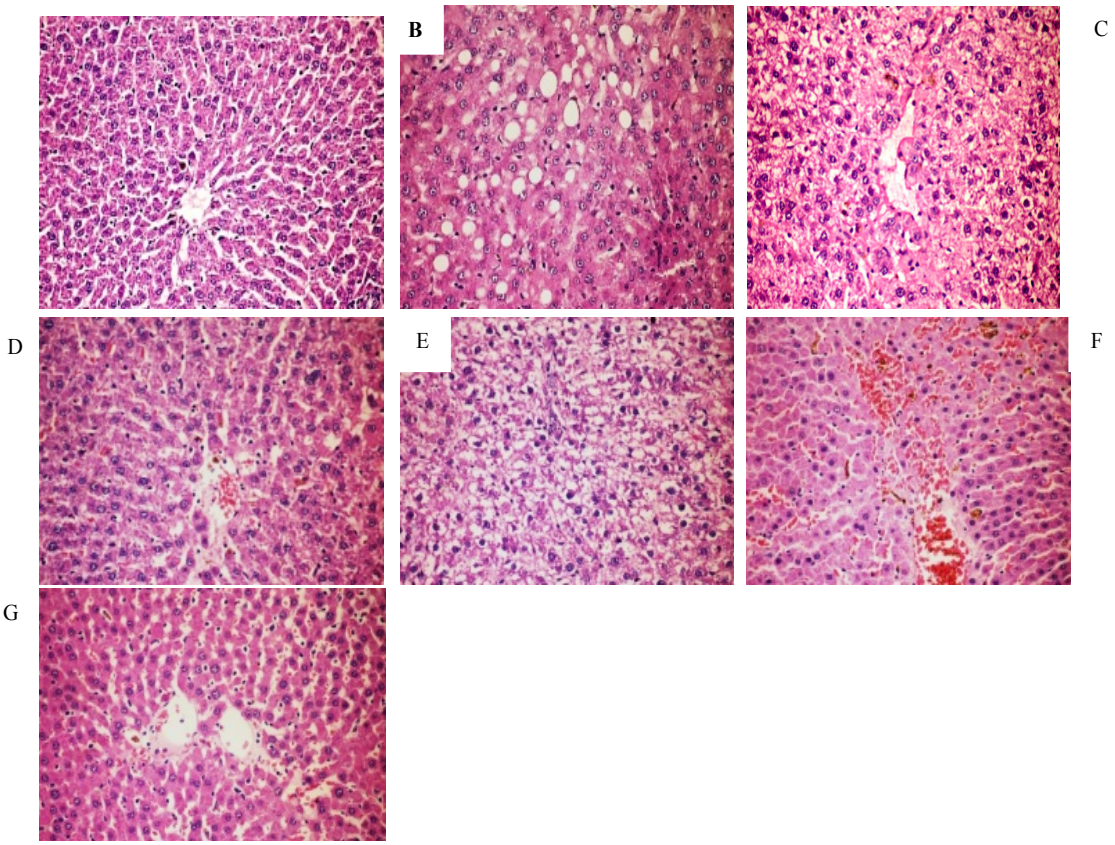

Fig.2. Effect of olive, falaxseed and their blanded oils on CCl4-induced hepatotoxicity in rats after one month. (A) negative control group;

(B) positive control group; (B) positive control group; (C) group feed on BD with 100% replace of oil diet with olive oil; (D) group feed on BD with 100% replace of oil diet with flaxseed oil; (E) group feed on BD with 100% replace of oil diet with blend oil; (F) group treated with hipeticam (2mg/kg/day) (G) group treated with hipeticam (4mg/kg/day). Liver sections were stained with haematoxylin and eosin (200X)

On the other hand, Perona et al., (2006) reported that the biological effects of olive oil is more than a simple mixture of fatty acids, and that it contains other biologically active substances such as tocopherols, polyphenols and phytosterol some of which have antioxidant and anti-inflammatory activities. Beside, Fang et al., *(*2008) found that olive oil treatment decreased the hepatic malondialdehyde and hydroxyproline levels.

Histological evaluations showed that olive oil could attenuate the liver fibrosis, necrosis, and expression of smooth muscle alpha-actin that are induced by CCl_4. When the CCl_4 induced rats was feed on BD and injected with hepaticum (0.2ml /rat/day), the liver histopathological results showed focal hepatic and kupffer cell activation. Even when the hepaticum dos was increased to 0.4ml/rat/day) for 30days, the liver also showed kupffer cell activation.

Conclusion

The present work investigated the protective effect of different types of olive, flaxseed oil and its blended on hepatic injury caused by CCl_4 (2ml/kg) for two weeks (three times a week) by subcutaneous injection to induce chronic hepatic in rats liver. The protective effects of flaxseed oil could retarind to contain omega-3 and omega 6 fatty acids (linolenic and linoleic acids) which could be metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in human intestine and shown antioxidant activity. In addition to the polyunsaturated fatty acids olive oil contains a considerable quantity of polyphenolic compounds which gave it more antioxidant power. The results revealed that feeding of rats on BD containing flaxseed oil, olive oil and blend oil 100% substitution lead to the best improvement in liver and CCl_4 induced necrosis and infiltration of lymphocytes**.**

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اجريت هذه الدراسة لتقييم التأثيرات المضادة للالتهاب الكبدي لكل من زيت الزيتون وزيت بذور الكتان ومخلوطهما على خلايا الكبد فى فئران التجارب المصابة بالالتهاب الكبدى والتى تمت تغذيتها على نظام غذائي قياسى مدعم بهذه الزيوت بنسب استبدال مختلفة من الدهن فى الوجبات الغذائية. كما تمت دراسة تأثير تغذية الفئران على وجبات غذائية تحتوى على مستويات مختلفة من هذه الزيوت على دهون الدم. أشارت النتائج إلى أن النظام الغذائي الذي يحتوي على زيوت الزيتون أو زيت بذور الكتان (بنسبة استبدال 100 ٪ من محتوى الزيت في النظام الغذائي) أدى إلى زيادة كبيرة في وزن الفئران مقارنة بالمجموعة الضابطة الإيجابية.أظهرت النتائج ايضا أن التغذية على وجبات تحتوي على زيت الزيتون او زيت بذور الكتان ومخلوطهما أدت الى انخفاض كبير في الكوليسترول الكلي وسجلت 79.71و76.18و 76.66 ملجم / ديسيلتر على التوالي مقارنة بمجموعة الكنترول الإيجابية في حين لم تكن هناك اختلافات كبيرة بين هذه الزيوت فيما بخص الدهون الثلاثية الكلية .اظهرت نتائج الفحص الهستوبولوجي لكبد فئران التجارب محل الدراسة أن المجموعة المعاملة برابع كلوريد الكربون اصيبت بالتهاب كبدى أدى الى تغير فى خلايا الكبد وحدوث تجمعات دهنية وكذلك حدوث احتقان فى الوريد المركزى والخلايا اللمفاوية. أظهرت النتائج أن تغذية الفئران المصابة على النظام الغذائي القياسى الذي يحتوي على زيت الزيتون ٪100 أوزيت بذور الكتان ٪100 أو الزيت المختلط ٪100 يؤدي إلى تحسن أفضل في الكبد ويخفف إلى حد كبير الالتهاب الذى حدث فى الوريد المركزى والخلايا اللمفاوية بسبب المعاملة برابع كلوريد الكربون