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Effect of Flaxseed, Sesame Oil and their Mixture on Liver Dysfunction of Albino Rats

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Abstract

The present study aimed to evaluate the effect of flaxseed, sesame oil and their mixture on carbon tetrachloride-induced liver dysfunction in albino rats. Thirty (30) Sprague-Dawley white male albino rats, each weighing 140 ± 5 g, were housed in group cages under conditions and fed on basal diet for adaptation for at least seven days before experiments. The rats were divided into five main groups (6 rats each) as follows: Group (1) Negative or normal group (-Ve): Fed on basal diet only. The other rats were injected by Ccl_4 then divided into four groups as follow: Group (2) Positive group (+Ve): Fed on basal diet only. Group (3): Fed on basal diet plus 5% of flaxseed oil. Group (4): Fed on basal diet plus 5% of sesame oil. Group (5): Fed on basal diet plus 5% of equalized mixture of flaxseed and sesame oil. The results revealed that liver dysfunction reduced FI, BWG, FER and serum HDL-c. On the other hand raised serum TC, TG, VLDL-c, LDL-c, AI, glucose, AST, ALT, ALP, urea, creatinine, uric acid levels and kidney weight. Feeding on diets containing flaxseed oil, sesame oil and their mixture at 5% level reversed such changes, sometimes to better level as compared to control (+Ve) group, provided that the best treatment was found for the mix diet indicating the synergistic action and mostly sesame oil revealed relatively better improvement of liver and renal functions. Feeding on diets containing flaxseed oil, sesame oil and their mixture at 5% level revealed normal structure of liver section exactly as in case of control (-Ve) group.

Conclusion: In conclusion, feeding on diets containing flaxseed, sesame oil and their mixture improved the biological and

biochemical parameters of liver dysfunction rats, provided that best results obtained with their mixture diet.

Introduction

Liver is the largest most complex organ in the body which has a number of important functions such as detoxification of toxic chemicals including drugs, alcoholic and toxic substances from intestinal microbes. Liver is responsible for storage of sugar and regulation of blood sugar levels. It is involved in production and storage of proteins and protein metabolism. Liver is also responsible for production of bile which aids in the digestion of fats, production of blood proteins clotting factor and substances important to production of red blood cells, regulation of a number of hormones, neutralization of free radicals by antioxidants as well as storage of vitamins namely A, D and B₁₂(**Patton *et al.*, 2007**)and(**Zhou *et al.*, 2007**).

In recent years, many researchers have examined the effect of plants used traditionally to support function and treat diseases of the liver. Phytotherapy is the treatment and prevention of disease using plants, plant parts and preparations made from them. Plants traditionally used in phytotherapy are called medical plants (**Weiss and Fintelman, 2000**).Flaxseed oil is a particularly rich source of α -linolenic acid (ALA, C18:3n-3), a plant-derived n-3 PUFA. Recently, flaxseed oil has been reported to ameliorate impaired lipid homeostasis with concurrent modification of proinflammatory mediators and oxidative stress, which play a key role in the development of liver dysfunction. Thus, it is possible that supplementation of flaxseed oil could attenuate liver dysfunction (**Zhang *et al.*, 2017 and Xu *et al.*, 2017**).

Sesame, with several polyphenolic ligands and high oil content (50–60%) (**Latif and Anwar, 2011**), becomes a promising tool for treatment of cardiovascular diseases. Sesame oil is an excellent source of unsaturated fatty acids consist from oleic acid (37%) and linoleic fatty acid (46%). Sesame oil also contains an amount of bioactive components such as tocopherols, polyphenols, flavonoids, phenolic ligands, sesamin, and sesamol. All of them are considered to be protective (**Wanet *al.*, 2015**) acting as

antioxidants, antihypertensives, anti-inflammatory, and cardioprotective substances (Aslamet *al.*, 2017). By reducing 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity, sesamin could also potentially reduce LDL levels in a similar manner as statin drugs without side effects (Hsuand Parthasarathy, 2017). Similarly, different other polyphenols, also those including in the sesame, may improve vasoactivity, endothelial function, carbon tetrachloride and nitric oxide (NO) production.

Materials And Methods

Materials:

Basal diet, flaxseed and sesame oil have been purchased from local market and have been bought from grocer from Menoufia governorate, Egypt. Carbon tetrachloride CCl₄, chemical kits for biochemical analysis was obtained from EL-Gomhoryia pharmaceutical company, Cairo, Egypt. Thirty (30) albino rats, Sprague Dawley strain, weighing 140 ±5 g, were obtained from Serum and Vaccine Center-Cairo.

Experimental design:

Thirty (30) Sprague-Dawley white male albino rats, each weighing 140±5 g, were housed in group cages under conditions and fed on basal diet for adaptationfor at least seven days before experiments. The rats were divided into five main groups (6 rats each) as follows: Group (1) Negative or normal group (-Ve): Fed on basal diet only. The other rats were injected by CCl₄ then divided into four groups as follow: Group (2) Positive group (+Ve): Fed on basal diet only. Group (3): Fed on basal diet plus 5% of flaxseed oil. Group (4): Fed on basal diet plus 5% of sesame oil. Group (5): Fed on basal diet plus 5% of equalized mixture of flaxseed and sesame oil. Twenty four (24) rats were treated subcutaneous injection with carbon tetrachloride CCl₄ mixed with an equal volume of paraffin oil and injected subcutaneously in a volume of 2ml/kg BW twice weekly to induce liver dysfunction (Kanter *et al.*, 2003).

Biological evaluation:

During the experimental period (28 days), body weight was recorded every week. Biological evaluations of the different diets were carried out by determination of body weight gain (BWG), feed

intake (FI) and feed efficiency ratio (FER) calculated according to **Chapman et al., 1959**, using the following formulas:

$$\text{Body weight gain (BWG) (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Feed efficiency ratio (FER)} = \text{BWG} / \text{FI}$$

Biochemical analysis:

Blood samples were used for determination of the following parameters by commercially available (BioMerieux) kits: Determination of Glucose (**Kaplan, 1984**) and (**Trinder, 1969**), total cholesterol (**Thomas, 1992**) and TG (**Fassati and Principe, 1986**), HDL (**Lopez, 1977**), (LDL-c = TC - [HDL-c + VLDL-c]) (**Castelli, 1977**); (VLDL-c = Triglycerides /5) (**Srivastava et al., 2002**). Atherogenic index (AI) (**Kikuchi et al., 1998**), urea, uric acid (**Patton and Crouch, 1977**), creatinine (**Henry, 2001**), AST and ALT (**Reitman and Frankel, 1957**) and ALP (**Roy, 1970**).

Organs weight:

Liver and kidney of rats in each group were collected, cleaned and weighed. Liver sections were prepared and investigated by light microscope according to **Allain, (1974)**.

Statistical analysis:

Statistical analysis was performed by using computer program statistical package for social science **SPSS, 1998** and compared with each other using the suitable tests. The mean \pm SD were indicated. A paired T. test was used to evaluate differences between the groups of rats and its respective control. For statistical analysis of time course experiments, multiple measurements ANOVA were performed.

Results

The presented results in table (1) showed that the BWG of the liver dysfunction rats as influenced by diets containing 5% of flaxseed and sesame oil and their mixture. It is clear that control (+) revealed pronounced reduction when compared with those of the normal control group (-) rats, percent increase of control (-) rats weight showed 120% increase as compared to control (+) group. Meanwhile feeding on diets containing 5% of flaxseed and sesame oil and especially that of 5% mixture indicated as increases in BWG reaching 100% of control (+) group for the mixture diet. Also, Feed

intake reduced considerably with hepatointoxication, where control (-) group indicated 68.9% increase as compared to control (+) rats. Some improvement occurred when rats fed on flaxseed oil diet (+4%), while appreciable increases (45.5 and 45.2%) of FI comparing to control (+) group reduced for sesame oil and oil mix diets respectively, with nonsignificant differences between them. Data in the same table indicated that feed efficiency ratio (FER) of control (-) rats was 30.3% higher than that found for control (+) rats, values were 0.142 and 0.109 respectively. When feeding on flaxseed diet FER showed 27.5% increase as compared to control (+) rats which was raised to 30.3% for 5% sesame oil diet. Nevertheless, maximum improvement was found for the oil mixture diet revealing 34.9% increase when compared to control (+) group which indicated a synergistic action. This was true also for both FI and BWG results.

Table (1): Effect of flaxseed, sesame oil and their mixture on body weight gain, feed intake and feed efficiency ratio

Groups	FI (g/day)	BWG (g)	FER
	Mean±SD	Mean±SD	Mean±SD
Control (-)	15.5 ^a ±1.5	2.2 ^a ±0.055	0.142 ^a ±0.02
Control (+)	9.18 ^b ±0.82	1.0 ^c ±0.02	0.109 ^b ±0.04
Flaxseed oil	9.55 ^b ±0.22	1.75 ^{ab} ±0.14	0.139 ^a ±0.02
Sesame oil	13.36 ^a ±1.41	1.89 ^a ±0.13	0.142 ^a ±0.02
Mixture	13.6 ^a ±1.15	2.0 ^a ±0.13	0.147 ^a ±0.01
L.S.D* (P≤0.05)	2.33	0.22	0.29

*L.S.D:Least significant difference

Data presented in table (2) showed the effect of feeding on diets containing flaxseed oil, sesame oil and their mixture on liver and kidney weight. It is clear that due to CCl₄ injection both liver and kidney weights increased. Meanwhile, feeding oils diets reversed such changes. Maximum improvement recorded for the oil's mix diets where nearly the original weights regained, showing appreciable percent reductions compared to control (+) group; being 40 % and 50 % respectively for liver and kidney.

Table (2): Effect of flaxseed, sesame oil and their mixture on Liver and kidney weight

Groups	Kidney (g)	Liver (g)
	Mean±SD	Mean±SD
Control (-)	0.4 ^d ±0.2	2.6 ^c ±0.4
Control (+)	0.8 ^a ±0.2	3.5 ^a ±0.3
Flaxseeds oil	0.7 ^b ±0.1	2.9 ^b ±0.2
Sesame oil	0.5 ^d ±0.2	2.6 ^c ±0.4
Mix	0.4 ^d ±0.2	2.5 ^c ±0.2
L.S.D (P≤ 0.05)	0.09	0.252

The results of table (3) shows the AST, ALT and ALP enzymes activities of liver as affected by feeding on diets containing flaxseed oil, sesame oil and the oils mixture. Liver dysfunction raised the activities of all these mentioned enzymes indicating damage of liver cells and escape of enzymes in blood circle. Nevertheless, such changes ameliorated considerably the liver enzymes activities, in particular for the oils mix diet. In this case percent decrease of AST, ALT and ALP activities were 37.8 %, 45.1 % and 32.6% respectively, indicating that the best treatment was that of the oil mixture diet.

Table (3): Effect of flaxseed, sesame oil and their mixture on AST, ALT and ALP in albino rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
	Mean±SD	Mean±SD	Mean±SD
Control (-)	20.0 ^d ±3.0	19.83 ^d ±2.25	123.96 ^d ±1.95
Control (+)	55.0 ^a ±1.0	59.00 ^a ±2.00	160.33 ^a ±2.02
Flaxseed oil	56.0 ^b ±1.0	56.00 ^b ±4.00	154.66 ^b ±2.51
Sesame oil	57.0 ^b ±1.5	56.00 ^b ±2.00	150.00 ^b ±2.00
Mix	46.0 ^c ±1.5	43.96 ^c ±2.05	147.93 ^c ±2.05
L.S.D (P≤ 0.05)	3.46	5.22	4.13

According to data from table (4) demonstrate the effect of flaxseed oil, sesame oil and oils mixture on serum end product of protein metabolism (urea, creatinine and uric acid) of liver dysfunction rats. It could be observed that injection with CCl₄ raised the serum levels of urea, creatinine and uric acid, when control (-) rats revealed lower values at 32.3%, 27.6% and 19.7% compared to control (+) group. Meanwhile, flaxseed oil, sesame oil and in

particular the oil mixture reversed such changes. In the last treatment rats showed nearly same characteristic for the healthy control (-) group.

Table (4): Effect of flaxseed, sesame oil and their mixture on urea, creatinine and uric acid in albino rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
	Mean±SD	Mean±SD	Mean±SD
Control (-)	21.66 ^b ±2.02	0.63 ^b ±0.02	3.30 ^b ±0.19
Control (+)	29.0 ^a ±2.00	0.67 ^a ±0.10	3.60 ^a ±0.22
Flaxseed oil	25.0 ^b ±1.50	0.65 ^b ±0.02	3.50 ^b ±0.20
Sesame oil	22.0 ^b ±2.00	0.69 ^b ±0.10	3.70 ^b ±0.20
Mix	20.16 ^{bc} ±2.05	0.62 ^b ±0.02	3.40 ^b ±0.27
L.S.D (P≤ 0.05)	3.99	0.136	0.40

From results of table (5) it is clear that liver dysfunction raised both T.C and T.G in serum. Accordingly, control (-) group had 32.9% and 48.3% levels as compared to control (+) group respectively; values were 106 and 91 mg/dl respectively. Feeding with flaxseed oil, sesame oil and their mix reversed such changes, especially for the mixture diet which showed percent decrease of 27.2 % and 41.5% respectively and this indicated a synergistic action.

Table (5): Effect of flaxseed, sesame oil and their mixture on total cholesterol and triglycerides in albino rats

Groups	T.C (mg/dl)	T.G (mg/dl)
	Mean±SD	Mean±SD
Control (-)	106 ^d ±1.5	91 ^c ±4.0
Control (+)	158 ^a ±2.0	176 ^a ±4.0
Flaxseed oil	127 ^b ±2.0	134 ^b ±2.0
Sesame oil	118 ^c ±4.0	115 ^c ±2.0
Mix	115 ^d ±3.0	103 ^d ±2.5
L.S.D (P≤ 0.05)	5.48	6.35

Data obtained from table (6) shows the levels of HDL-c, LDL-c and VLDL-c in plasma of liver dysfunction rats. Injection with CCl₄ raised the levels of HDL-c, LDL-c and VLDL-c while lowered the HDL-c, percent changes of control (-) group as compared to control (+) rats were 47.6, 47.9 and 50 % respectively. Nevertheless, reversed changes were found when liver dysfunction

rats fed on flaxseed oil diet, sesame oil diet and in particular their oil mixture which revealed the best treatment, showing near values in comparison with that of the control (-) rats and appreciable percent of changes as compared to control (+) group, being 50.0 %, 40.9 % and 41.0 % for HDL-c, LDL-c and VLDL-c respectively.

Table (6): Effect of flaxseed, sesame oil and their mixture on HDL-c, LDL-c and VLDL-c in albino rats

Groups	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
	Mean±SD	Mean±SD	Mean±SD
Control (-)	36 ^a ±5	51.8 ^d ±0.4	18.20 ^c ±0.2
Control (+)	34 ^b ±5	63.8 ^a ±1.2	35.20 ^a ±1.6
Flaxseed oil	33 ^a ±2	67.2 ^b ±0.2	26.80 ^b ±0.4
Sesame oil	35 ^a ±2	60.0 ^c ±2.5	23.00 ^c ±2.0
Mix	36 ^a ±2	58.4 ^b ±1.6	20.60 ^d ±1.0
L.S.D (P≤ 0.05)	6.93	2.27	2.18

Discussion

Similar results to that obtained in tables (1– 6) were reported by **Asmaa,(2012)** when liver dysfunction rats fed on flaxseed, sesame oil and their mixture diet due to unsaturated fatty acids and strong antioxidant properties. As mentioned by **Guimaraes et al.,(2013)** both sesame oil and flaxseed oil had important desirable medical effects on serum glucose, lipids profile, renal and liver functions as well as the biological parameters of rats. As reported in tables (1&2) hepatopathy lowered BWG, FI and FER, while raised the weight of internal organs, due to inflammations and abnormalities in the metabolism and stores of macro and micro nutrients. Actually, hepatopathic parameters are ill. Meanwhile; due to feeding on sesame oil and flaxseed oil, rich in phenolic compounds the mentioned changes were reversed.

Ccl₄ injection induced hepatocellular injury, while sesame oil and flaxseed oil and their mixture alleviated the liver injury as indicated by lowering AST, ALT and ALP activities, which was also found in present work. Alleviation of hepatopathy could be ascribed to the antioxidation effect of plants, presence of phenolic compounds and essential fatty acids. According to **Bakr,(2009)** plant form locations (Sesame oil and flaxseed oil and the mixture of

all) when used in diets of hepatointoxicated rats lowered TC, TG, VLDL-c, LDL-c, while raised the HDL-c levels in serum. This was also found in present work using diets containing flaxseed and sesame oil, while may be also due to antioxidation effect of diets. As reported by **Samia,(2009)** feeding of liver dysfunction rats on diets containing sesame oil and flaxseed oil lowered urea. Uric acid and creatinine in serum of hepatic rats due to antioxidation effect. This was also reported in present work using flaxseed and sesame seeds oil diets.

Chinese medicine suggests that ancient food sesame oil may relieve the epidemic of liver damage form modern drugs. Sesame oil may be important. Sesame oil is rich in anti-inflammatory lignin known as a “sesamin”, which is as polyphenol. Sesamin health benefits include: lowering blood sugar and antioxidation power besides lowering blood pressure, anticancer potential, anti-aging effects, regulating the immune system and antioxidant effects. Sesamin helps combat liver damage by maintaining intercellular level of glutathione, anti-oxidants reduce levels of free radicals and inhibits oxidation of fats. Sesame oil contains poly-unsaturated fats and is a source of omega-3 and fatty acids (**Chandrasekaran et al., 2008**).

Upon analyzing its nutritional content, the flaxseed contains three distinct healthful constituents' lignins, omega-3 essential fatty acids and fiber. Each of these flaxseed components contributes to a healthful liver. Lignin from flaxseed primarily secoisolariciresinol diglucoside (SDG). High triglyceride levels and obesity can cause elevated liver enzymes a sign that inflammation and cell damage in the liver, while SDG had lowered such changes. It was reported that flaxseed is rich source of alpha-linolenic acid (ALA), a precursor of the omega-3 fatty acids that help address hyperlipidemia (High level of fat in the blood) by improving cholesterol ratio, which was found in present work (Tables 5&6). Since inflammation in the liver can damage liver cells, flaxseed's high dose of ALA can help treat the liver from cellular injury. Also, insoluble flaxseed fiber is a key dietary strategy for maintaining liver health. Effects of flaxseed oil, based on properties of its lignans, fibers and ALA content is an ideal addition to diets prioritizing liver chronic health which reduces liver

inflammation, improving cholesterol ratio (3, 5&6). In present work both flaxseed oil and sesame oil lowered serum total cholesterol (Table 6). It was reported that flaxseed oil had 39.9% of alpha linolenic acid and 12.25% of linolenic acid; sesame oil has 28.4% of linolenic acid. Sesame oil is characterized by potent oxidative stability due to high content of mono-unsaturated fatty acid oleic being 28.6%; flaxseed oil had also marked oleic acid 12.25%.

As published by **Galvao, (2008)** ingestion of polyunsaturated fatty acids (PUFA) present in flaxseed and sesame oils is inversely related to heart disease by decreasing cholesterol and plasma triglycerides levels. Arteriosclerotic vascular disease (ASVD) is associated to hyper-tension and dyslipidemia (**Raposo, 2010**) this was recorded for CCl₄ injected rats raising (AI) values but was converted by consuming flaxseed and sesame oils. **Raposo, (2010)** found that PUFA fat gained importance as function food reducing arteriosclerotic vascular disease. Both flaxseed and sesame oils are nutritional supplement, deprecating excellent source of PUFA (**Chung et al., 2005**). As reported by mentioned authors oil of both seeds had marked effects on the lipid and glucose profile of rats and even sesame oil describe as one of the most powerful healing food on the planet which may also help relieve the epidemic of liver damage from modern drugs in Chinese medicine. Also, flaxseed described as god source of essential fatty acids and antioxidants, their consumption is associated with health benefits including the safeguarding of liver health. Anyhow, previous research as well as results of present study (Tables 1- 6) suggest using flaxseed and sesame oils for health in general and liver health particularly.

Conclusion

In conclusion, feeding on diets containing flaxseed, sesame oil and their mixture improved the biological and biochemical parameters of hepatopathic rats, provided that best results obtained with their mixture diet.

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تأثير زيت الكتان والسمسم وخليطهما على الفئران المصابة باختلال الكبد الوظيفي

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الملخص العربي

تهدف الدراسة الحالية إلى تقييم تأثير زيت بذور الكتان وزيت السمسم وخليطهما على اختلال الكبد الناجم عن رباعي كلوريد الكربون في الفئران البيضاء. استخدم ثلاثون (٣٠) من ذكور الفئران البيضاء، وزن كل منها 140 ± 5 جم، تم وضع الفئران في مجموعات في أقفاص مُتَحَكَم فيها وُغذيت على الوجبة القياسية الأساسية لِتتكيف على البيئة الجديدة وذلك لمدة أسبوع على الأقل قبل إجراء التجارب. بعد ذلك تم تقسيم الفئران إلى خمس مجموعات رئيسية (٦ فئران لكل منها) على النحو التالي: المجموعة (١) المجموعة الضابطة السالبة أو العادية: (-Ve) تغذت على النظام الغذائي الأساسي فقط. تم حقن الفئران الأخرى برابع كلوريد الكربون ثم قُسمت إلى أربع مجموعات على النحو التالي: المجموعة (٢) مجموعة ضابطة موجبة: (+Ve) تغذت على النظام الغذائي الأساسي فقط. المجموعة (٣): تغذت على النظام الغذائي الأساسي بالإضافة إلى ٥٪ من زيت بذور الكتان. المجموعة (٤): تغذت على النظام الغذائي الأساسي بالإضافة إلى ٥٪ من زيت السمسم. المجموعة (٥): تغذت على النظام الغذائي الأساسي بالإضافة إلى ٥٪ من خليط متساوٍ من زيت بذور الكتان وزيت السمسم. أظهرت النتائج أن اختلال الكبد يخفص FI و BWG و FER و HDL-c من ناحية أخرى ارتفع TC المصل، TG، VLDL-c، AI، LDL-c، الجلوكوز، AST، ALT، ALP، اليوريا، الكرياتينين، ومستويات حمض اليوريك ووزن الكلى. تناول وجبات غذائية تحتوي على زيت الكتان وزيت السمسم وخليطهما بنسبة ٥٪ عكس هذه التغيرات، وأحياناً إلى مستوى أفضل مقارنة مع المجموعة الضابطة الموجبة. أظهرت النتائج أن تناول أنظمة غذائية تحتوي على خليط من زيت الكتان وزيت السمسم بنسب متساوية (٢.٥٪ : ٢.٥٪) يؤدي إلى تحسين الخلل في وظائف الكبد، ودل على ذلك وجود بنية طبيعية لقطاع من الكبد كما هو الحال في المجموعة الضابطة السالبة.

الخلاصة: في الختام، فإن التغذية على وجبات غذائية تحتوي على زيت بذور الكتان وزيت السمسم وخليطهما قد حسّن المعلمات البيولوجية والكيميائية الحيوية للفئران المصابة باختلال الكبد وكانت أفضل النتائج هي المجموعة التي تُغذت على خليط زيت الكتان وزيت السمسم.