Biological Study to Evaluate the Effect of Intake Flaxseed Oil on Kidney Failure Rats

Ahmed S. M.[1] , Abo El-Naga N. I. [1], Hussein M. M. [2], Bedir A. M. [1]

ABSTRACT

The main aim of this research was to assess the effect of intake flaxseed oil on kidney failure rats by measured kidney functions .Twenty-four adult male albino rats, weighting 166g±10 were divided randomly into four groups, each group had six rats. All animals were given libitum standard diet. 6 rats were left as negative control (Group 1), the remain 18 rats were injected with diclofenac Sodium a dose of (100 mg/kg) for 3 days then divided randomly into equal 3 groups as following: Control positive group (C+) fed on standard diet only (Group 2), kidney failure rats fed on 2% flax seed oil from fat meal enteric injection (Group3), kidney failure rats fed on 10% flax seed oil from fat meal by enteric injection (Group4). During the experimental period for 5 weeks, the feed intake was recorded every day and body weight every week. At the end of the experiment, blood samples were collected and hemoglobin, platelets, relative weight of internal organs, kidney and liver functions, as well as histological tests of the kidneys and liver were done. The results showed a significant improvement in all experimental groups compared with the control group. Rats that fed on the flaxseed oil, noticed a significant decrease in the relative weight of the liver, kidney, heart, lungs, and brain as compared with the positive control group. The lowest level of urea, uric acid, creatinine was observed in the group of rats that fed on 10% flaxseed oil. Furthermore, a non-significant decrease in both ALT and AST in blood serum in the group that fed on 10% flaxseed oil compared with positive control group. Total protein and albumin levels were non-significantly higher in the group fed on 10% flaxseed oil. Finally, the results revealed that feeding flaxseed oil 10% improved kidney functions significantly, and we can recommend the using of flaxseed oil in food products because due to its health benefits.

Keywords: Flaxseed oil, Kidney failure rats, Diclofenac sodium, Omega3 fatty acid.

INTRODUCTION

Kidney disease (KD) is defined as a syndrome characterized by a progressive deterioration in kidney function over a period of months or years, the glomerular filtration rate decreases, the kidney's ability to remove waste products and maintain the internal balance of water and mineral salts in the body decreases, and kidney function decrease to less than 15 ml/minute, which results, the kidneys lose their full function (EL-Sayed, 2015).

Mortality has increased from 813 000 persons in year 2000 to 1.3 million in year 2018. The numbers of cases all-stage kidney disease 697.5 million were recorded, as a global prevalence of 9.1%. Regions with the highest incidence of end-stage kidney disease per million were Mexico 594, Taiwan 523 on, Hungary 508, the U.S. 395, and Aguascalientes Mexico 372, Thailand 365, Singapore 347, South Korea 340 and Japan 300 (USRDS, 2020). According to Egyptian Renal Data System (2018) the number of registered cases reached 50,000 patients in 2018 divided into 58% male and 42% female. The 50% of total patients was ages 45-64 years. The most common cause of the kidney faileure diseases were high bloodpressure 38%, followed by diabetes 18%.

Kidney failure occurs without clear symptoms at first, as the patient goes through several stages from a simple decline in the glomerular filtration rate until the occurrence of complete kidney failure and a significant decrease in the rate of filtration and kidney failure develops, causing cardiovascular diseases and high blood pressure as a result of water and salt retention. Also, arrhythmias were recorded in about 10-30% of patients (Mohamed and Ahmed, 2018).

Flax is an ancient crop that has been used as food and fiber, the Latin name of the flax is "*Linumusitatissimum*" (Hall et al., 2006). The total world production of linseed is approximately 8.7 million tones, in Canada (40%), the Russian Federation (15%), and China (13%). In Egypt production was 10,000 tones and is predicted to change by an average of 36.78% (FAO, 2019). West Asia and the Mediterranean are the native habitats of this plant. Flaxseed has a smooth, lustrous surface that ranges from dark brown to yellow

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[1] Applied Entomology and Zoology Department, Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt.

[2]Cotton Pesticides Evaluation Department, Plant Protection Research Institute, Agricultural Research Center, El-Sabhia, Alexandria, Egypt.

*Corresponding author: Sahar Elsayed Eldesouky, PhD

E-mail: elsayedsahar@gmail.com / Sahar_Eldesouky@yahoo.com

ID orcid: https://orcid.org/0000-0003-4823-9013

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in color. It has a crisp, chewy texture and a pleasant nutty flavor (Ganorkar and jain, 2013). Flaxseeds are rich in essential fatty acids, linoleic acid, and linolenic acid, as well as flaxseed oil contains antioxidants, low price more available compared to fish oil (Moghimian et al., 2019). Flaxseed oil contains 35.44% of alpha linolenic acid (ALA) per 100 grams, which concludes that flaxseeds contain a high percentage of Omega3 (Mostafa et al., 2012 and Ali et al., 2018).

Flaxseed oil could protect the kidneys from deterioration due to its richness in omega3 fatty acid, while reducing oxidative damage in rats when taken orally for 14 days (Mokhtair et al., 2017). Over the past few decades, the effectiveness of Omega3 fatty acid has been proven in treating cardiovascular diseases, but there are some studies indicating its effectiveness on kidney function and its ability to reduce kidney failure disease. However, initial indications indicate that Omega3 fatty acid significantly reduces the severity of Kidney failure disease with low protein content in urea (Hu et al., 2017). Omega-3 fatty acid works to reduce the inflammatory symptoms compared to Omega6 and Omega9, and also has no obvious side effects, for this reason recommended to incorporate it into the food (Panahi et al., 2016).Studies have proven the ability of omega3 to reduce inflammatory factors associated with oxidative processes, as well as reduce the level of triglycerides, which reduces the risk of cardiovascular disease, one of the causes of chronic kidney disease that causes kidney failure (Hu et al., 2018).The effect of omega3 fatty acid includes reducing triglycerides and cholesterol levels, preventing platelet aggregation and vasodilation (El-Blooni, 2007 and Svensson & Carrero, 2017). Omega3 fatty acid was founded to be beneficial in preventing and delaying the decline in kidney function by controlling inflammation, oxidative stress, and reducing triglycerides (Lee et al., 2018).

The effects of flaxseed oil on the inflammatory state of 160 patients with chronic kidney failure, when received 1 g of flaxseed oil twice a day for 120 days compared with patients received mineral oil. The results found 33.3% of the FSO group changed to a non-inflamed, obverse 16.9% in the mineral oil group. That means flaxseed oil can decrease the levels of inflammatory (Lemos et al., 2012).

Diclofenac sodium is a phenylacetic acid molecule that is lipophilic, nonsteroidal, and widely used as an anti-inflammatory, analgesic, antirheumatic, and antipyretic. However, high levels of nonsteroidal anti-inflammatory drugs (NSAIDs) cause urea crystals to form in the kidneys, liver, heart, and spleen, which inhibit the activities of various enzymes, chondrocyte proteoglycan synthesis, the ionic exchange rate, and processes dependent on prostaglandins, resulting in toxicity. Diclofenac sodium toxicity resulted in mitochondrial malfunction and ATP depletion, as well as reduced glutathione (GSH), lipid peroxidation, and calcium concentration changes.in kidney cells (Ahmed et al., 2017). NSAIDs (non-steroidal anti-inflammatory drugs) are one of the most commonly prescribed drugs, and their nephrotoxic effects are well known. They are widely used as analgesics and anti-inflammatory drugs, but there have been reports of diclofenac sodium causing acute kidney failure, hypertension, and kidney function decline due to drug interaction (Dhanvijay et al., 2013).

MATERIALS AND METHODS

Experimental design and treatments

The experiment and biochemical determination were conducted at Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt. A total of 24 male Albino rats of wistar strain with average body weighting (166 g ±10) were used in this experiment. The animals were housed in 4 plastic cages and kept under standard healthy laboratory conditions (light period of 12 h per day and temperature 27± 2°C). All animals were given anlibitum standard diet which consisted of 44 % soybean, 12% barseem clover hay, 13.5 % fat, 9.8 % yellow maize, 13.2 % starch, 5 % minerals, 2% lime stone and 0.5 % vitamins mixture (Elnaga et al., 2016). 6 rats were left as negative control (Group 1),the remain 18 rats were injected with diclofenac Sodium a dose of (100 mg/kg) for 3 days then divided randomly into equal 3 groups as following, Control positive group (C+) fed on standard diet only. (Group 2), Kidney failure rats fed on 2% flax seed oil from fat meal enteric injection (FSO 2%). (Group 3), Kidney failure rats fed on 10% flax seed oil from fat meal by enteric injection (FSO 10 %). (Group 4)

Biochemical measurements

The blood samples were collected from vein plexus in dry clean tubes with EDTA (anti-coagulant). The non-coagulated blood was used to determine red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and packed cell volume (PCV) by using (Bayer Advia 120 hematology analyzer) according to Friedman and Young (1997). Urea concentration was measured according to method of Talkeand Schubert (1965), Gutmann and Bergmey (1974).Creatinine was measured according to method of Larsen (1972). Uric acid concentration was measured according to method of Barham and Trinder (1972)and Fossati et al. (1980). Serum total proteins (STP) were determined according to method described by Gornal et al. (1949). Serum Albumin (SAIb) was determined

according to the method described by Doumas et al. (1971). Serum Globulin (SGlob) was determined according to the method of Kingsley (1939). Serum Aspartatee amino transferase level (AST) and Alanine aminotransferase (ALT) were determined according to the method of Gella et al. (1985).

Histopathological investigation

Autopsy samples were taken from the kidney of rats in different groupand fixed in 10% formalin saline for 24 h. washing was done in tapwater; then, serial dilutions of alcohol (methyl, ethyl, and absoluteethyl) were used for dehydration. Specimens were cleared in xyleneand embedded in paraffin at 56◦ in hot air oven for 24 h. Paraffin beeswax tissue blocks were prepared for sectioning at 4 μm thickness bysledge microtome. The obtained tissue sections were collected on glassslides, deparaffinized, and then stained by hematoxylin and eosin stainfor routine examination through the light electric-microscopeaccording to methods mention by Bancroft et al. (1996).

Statistical analysis

Analysis of variance for a completely randomized design and t-test design were done according to Gomez and Gomez (1984) by using SPSS software program. The level of significant difference was determined at $p<0.05$.

Effect of dietary flaxseed oil on final weight and body weight gain (%)

As shown from Table (1) control group (C+) decreased body weight gain percentage when compared to negative control group (C-) by 17.85% with non-significantly variance at $P\leq0.05$. Meanwhile there was a significant difference between flaxseed oil 2% and the control group by 27.91%, and non-significant difference between flaxseed oil 10% and the control group by 26.71 %. Fawzy et al. (2020) found no significant differences of body weight gain between the negative control group of rats and of flaxseed oil group which disagree with this study. On the other hand, group treated with flaxseed oil (10%) significantly increase their body gain ($p<0.05$) when compared with the negative control group which agree with our study (El-Aziz et al., 2020).

Relative organs weight of kidney failure rats feed different levels of flaxseed oil

Data presented in Table(2)showed that the changes in relative weight of liver, kidney, lung, spleen, heart and testicles in all groups were perceived there were non-significant ($P<0.05$) differences. Treatment with flaxseed oil 10% had the best result. At the same time, rats feed with flaxseed oil decrease in the relative weight of kidney when compared with positive control rats (C+) and feed with flaxseed oil 2% was better than flaxseed oil 10%.When feed with flaxseed oil did not cause statistical differences in lung, spleen, heart and testicles relative weight of male rats, except in flaxseed oil 10% group in lung, when compared to the positive control rats (C+). Han et al. (2017) founds that the effects of flaxseed oil on liver weight for 12 weeks had decreased liver weight compared with rats untreated group ($P < 0.05$). Aly-Aldin et al. (2015) mentioned that liver and kidney weights to rats were fed by different replacement levels of flaxseed oil, data showed that positive control rats had higher ($P\leq0.05$) Liver and kidney weights than negative control rats and rats fed flaxseed oil diets. Flaxseed oil at different replacement levels resulted in significant ($P\leq0.05$) reduced in liver and kidney weights and that is the same at our study.

Table 1 .Effect of feed kidney failure rats with different levels of flaxseed Oil on final weight and body weight gain (%)

Experimental groups	Body weight			Body weight gain(%)
	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	
C-	175.7±1.54[a]	227.08 ±30.1[a]	51.38±28.92[a]	29.24±19.35[a]
C+	176.14±13.06[a]	218.45±23.01[b]	42.31±9.98[b]	24.02±8.69[a]
FSO 2%	164.78±12.76[b]	226.41±32.11[a]	61.63±19.37[a]	37.40±18.66[b]
FSO 10%	171.44±11.67[a]	234.97±18.41[a]	63.53±6.76[a]	37.05±5.56[a]

-Values are expressed as mean ± SD -FSO: Flax seedOil C-: Negative control group - C+: Positive control group
-Different letters are significant at p<0, 05 as column

Table 2. Relative organs weight of kidney failure rats treated with flaxseed oil

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
Liver	2.79±0.21[a]	3.00±0.21[a]	2.87±0.22[a]	2.60±0.25[a]
kindney	0.77±0.76[a]	1.06±0.14[a]	0.76±0.07[a]	0.83±0.11[a]
Lung	0.79±0.08[a]	0.73±0.13[a]	0.69±0.52[a]	0.75±0.11[a]
spleen	0.47±0.07[a]	0.44±0.07[a]	0.47±0.06[a]	0.43±0.09[a]
Heart	0.34±0.03[a]	0.34±0.04[a]	0.34±0.07[a]	0.33±0.05[a]
Testicles	1.34±0.16[a]	1.31±0.14[a]	1.31±0.17[a]	1.30±0.19[a]
Brain	0.68±0.04[a]	0.71±0.07[a]	0.65±0.122[a]	0.68±0.08[a]

- Values are expressed as mean ± SD -FSO:FlaxseedOil-C-:Negativecontrolgroup- C+: Positive control group
-Different letters are significant at p<0, 05 as row

Effect of feed different levels of flaxseed oil on liver enzymes ALT and AST of kidney failure rats

Data exposed in Table (3) significant differences at (p≤0.05) with high level of ALT liver enzyme inthe positive group compared to the negative groupwith an average of 153.57 and 45.94 U/L respectively. While the flaxseed oil groups, exposed that the variances were non-significant (p≤0.05) when, the percentage of addition increasing from 2% to 10% of FSO, and that led to a development in ALT liver enzyme level from 151.76 to 139.08 U/L respectively. In case of AST liver enzyme were the high level in the positive group (C+) compared to the negative group (C-) were significant (p≤0.05) with an average of 70.49 and 51.03 U/L for each of them. Whereas differences were non-significant (p≤0.05) at the flaxseed oil groups, the results revealed that increasing the percentage of addition from 2% to 10% caused to a progress in AST liver enzyme level from 45.13 to 41.37 U/L successively. EL-Sayeda and Abor (2014) presented that significant increase in AST and ALT compared with control positive, these results was different with the current our study and (Shun et al., 2009) whose found that significantly decreased the AST and ALT in the FSO group.

Effect of feed different levels of flaxseed oil on Glucose, Total protein, Albumen, Urea, Uric acid and Creatinne of kidney failure rats

Table (4) noticed that significantly differences at (p≤0.05) with high level of glucose in C+ compared to the C- with an average of 52.03 and 38.26 Mg/dl for each of them consecutively. With regard to the flaxseed oil groups had no-significant (p≤0.05) difference with increasing the percentage of addition from 2% to 10% at FSO groups headed to a non-improvement in glucose level from 49.50 to 50.32 Mg/dl respectively. The outcomes showed that positive control group had low level of total protein when compared to the negative control group, and the variances was significantly with an average of 4.33 and 7.26 g/d (p≤0.05) for each of them successively. As the flaxseed oil groups, the results showed that no significant increasing the percentage of addition from 2% to 10% led to an improvement in total protein from 4.98 to 5.91 g/dl (p≤0.05) respectively. Albumin register low level with the variances was significant (p≤0.05) in C+ compared to the C- with an average of 2.56 and 4.29 g/ dl. While the flaxseed oil groups, the results showed that increasing the percentage of addition from 2% to 10% led to an improvement in albumin level from 2.98 to 3.57 g/dl respectively and the differences were notsignificant (p≤0.05). Urea level scored high level in C+ compared to C- with an average ability of 48.46 and 25.22 Mg/dl for each of them with the significantly differences, were (p≤0.05) . Increasing the percentage of addition from from 2% to 10% in FSO groups headed to an improvement in urea level from 39.61 to 29.28 Mg/dl consecutively.

Table 3. Effect of feed different levels of flaxseed oil on liver enzymes ALT and AST of kidney failure rats

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
ALT U/L	45.94±3.98[a]	153.57±32.65[b]	151.76±1.51[b]	139.08±1.77[b]
AST U/L	51.03±5.31[a]	70.49±8.82[b]	45.13±7.91[b]	41.37±5.42[b]

- Values are expressed as mean ±SD -FSO: Flax seedOil -C-: Negative controlgroup -C+: Positive control group
- ALT: Serum Alanine amino transferase level - AST: Serum Aspartate amino transferase level
- Different letters are significant at p<0, 05 using as row.

Table 4. Effect of feed different levels of flaxseed oil on Glucose, Total protein, Albumen, Urea, Uric acid and Creatinne of kidney failure rats

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
GLUCOUSE Mg/dl	38.26±4.30[a]	52.03±4.78[b]	49.50±3.62[b]	50.32±3.45[b]
TOTAL PROTEIN g/dl	7.26±0.46[d]	4.33±0.81[a]	4.98±0.47[ab]	5.91±0.29[bc]
ALBUMIN g/dl	4.29±0.32[d]	2.56±0.57[a]	2.98±0.33[ab]	3.57±0.17[bc]
UREA Mg/dl	25.22±2.35[a]	48.46±6.78[c]	39.61±3.41[b]	29.28±2.02[a]
URIC ACID Mg/dl	1.12±0.32[c]	3.34±1.28[c]	2.06±0.17[a]	1.48±0.27[ab]
CREAT Mg/dl	1.27±0.08[a]	2.70±0.33[d]	1.98±0.17[c]	1.59±0.17[ab]

- Values are expressed as mean ± SD - FSO: Flax seed Oil - C-: Negative control group -C+: Positive control group

- Different letters are significant at p<0, 05 using as row

Effect of feed flaxseed oil on red blood cells of kidney failure rats

The results presented in Table (5) the C+ group had low level of Hb level , with an average ability of 5.44 and 13.24 g/dl , Rbcs level an average ability of 2.90 and 7.02 10^6/mm3, and Hct level with an average ability of 18.52 and 44.45 g/dl for each of them separately when compared to the C- group, and the variances were insignificant ($p\leq0.05$), and differences were no significant at ($p\leq0.05$) with low level of Mcv with average of 63.23 and 67.40 μm^3,also, Mchc level with a rate of 29.54 and 30.54 g/dl. In the flaxseed oil groups, the results repoted that non-significantly differences between FSO groups, and increasing the percentage of addition from 2% to 10% was improving in Hb level from 9.53 to 10.80 g/dl, and Rbcs level from 4.85 to 5.47 10^6/mm3, and Mchc level slightily enhancement from 30.35 to 30.42 g/dl and Mcv level from 60.95 to 61.67 μm^3 ,while significantly snowballing on the percentage of addition from 2% to 10% in Hct level from 31.98 to 36.08 g/dl separately ($p\leq0.05$). The marks of Mch level exposed that slightly non-significantly ($p\leq0.05$) low level in the positive control group compared to the negative control group with an average ability of 18.74 and 18.94 pg/cell for each of them. While the flaxseed oil groups, the results showed that increasing the percentage of addition from 2% to 10% led to a slightly improvement in Mch level from 19.59 to 19.78 pg/cell separately and the variances were not significant ($p\leq0.05$).

The consequences was significant ($p\leq0.05$) and high level of Plt level in the C+ compared to C- with an rate 886.00 and 348.86 K/mcl respectively, and non-significant ($p\leq0.05$) slightly high of Pv level with an average of 8.97 and 8.89 Fl. When the flaxseed oil groups, the results showed that increasing the percentage of addition from 2% to 10% led to an improvement in plt level from 536.67 to 479.00 K/mcl respectively and the differences were non-significant ($p\leq0.05$), and an slightly upgrading in Mchc level from 9.15 to 8.75 Fl. Tabibi et al. (2017) reported that the effect of flaxseed oil on Hb, Hct, MCV, MCH and MCHC increased significantly when compared with the positive control group. Also, agreed with (Kaddam et al., 2015) and our current study too.

Effect of feed flaxseed oil on white blood cells of kidney failure rats

The effect of fed groups on flaxseed oil on white blood cells of kidney failure of rats clarified in Table(6) level of Wbcs value was high with significantly differences ($p\leq0.05$) in the positive control group compared to the negative control group with an average of 13.66 and 9.04 $x10^3$/ ul for each of them. With regard to the flaxseed oil groups, the increasing in the percentage of addition from 2% to 10% headed to an non-improvement in Wbcs level from 6.62 to 8.28 $x10^3$/ul successively and the variances were between groups non-significant ($p\leq0.05$), and improvement when compared to the positive control group from 8.28 to 13.66 $x10^3$/ ul consecutive, and the variances between groups were significant ($p\leq0.05$). About values of lymphocytes level in the positive control group registered low level, and significantly variances ($p\leq0.05$) compared to the negative control group with an average of 65.00 and 77.57% successively. As to the flaxseed oil groups when the percentage of addition

increased from 2% to 10%, but when compared to the positive control group we noticed that improvement from 67.83 to 65% respectively and the differences between groups were not significant ($p \leq 0.05$). The results reported that a high value of the neutrophils level in the positive control group compared to the negative control group with an average of 18.29 and 10.86 % for each of them, and the variances between groups were not significant ($p \leq 0.05$). While the flaxseed oil groups, the results showed that increasing the percentage of addition from 2% to 10% led to an non-improvement in neutrophils level from 10.14 to 14.50 % successively with not significantly variances ($p \leq 0.05$),and improvement when compared to the positive control group from 14.50 to 18.29 respectively and the differences were non-significant ($p \leq 0.05$). Level of monocytes level in the positive control group was high and were not significantly differences ($p \leq 0.05$) compared to the negative control group with an average of 10.57and 7.57% for each of them. We observed the outcomes in the the flaxseed oil groups, increasing the percentage of addition from 2% to 10% led to an non-improvement in monocytes level from 9.85 to 10.50 % and the differences were non-significant ($p \leq 0.05$), and slightly improvement when compared to the positive control group from 10.50 to 10.57 respectively and the differences were non-significant ($p \leq 0.05$). The results showed high level of eosinophils level in the positive control group compared to the negative control group with an average ability of 6.14and 4.29 % for each of them, and the differences between groups were not significant ($p \leq 0.05$).

Table 5. Effect of feed flaxseed oil on red blood cells of kidney failure rats

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
Hb g/dl	13.24±1.06^{C}	5.44±1.0^{a}	9.53 ± 1.70^{b}	10.80±1.3^{b}
Rbcs (10^6 /mm3)	7.02 ± 0.58^{d}	2.90±0.57^{a}	4.85 ± 0.82^{bc}	5.47±0.67^{d}
Hct g/dl	44.45±3.33^{d}	18.52±3.70^{a}	31.98 ± 5.47^{b}	36.08 ± 4.06^{c}
Mcv µm3	67.40±1.76^{a}	63.23±3.90^{a}	60.95 ± 4.33^{a}	61.67 ± 3.54^{a}
Mch pg/cell	18.94±0.39^{a}	18.74±0.77^{a}	19.59 ± 0.58^{a}	19.78 ± 0.47^{a}
Mchc g/dl	30.54±0.86^{a}	29.54±0.75^{a}	30.35 ± 0.42^{a}	30.42 ± 0.66^{a}
Plt K/mcL	348.86±26,36^{a}	886.00 ± 80.89^{c}	536.67±58.03^{b}	479.00 ± 48.42^{b}
Pv FL	8.89 ± 1.27^{a}	8.97±0.45^{a}	9.15± 0.37^{a}	8.75±0.85^{a}

-Values are expressed as mean ± SD - FSO: Flax seed Oil - C-: Negative control group-C+: Positive control group-Hb: hemoglobin- Rbcs: red blood cells -HCT: hematocrit - MCV: mean cell volumeMCH: mean corpuscular hemoglobin
-MCHC: mean corpuscular hemoglobin concentration -PLT: platelets count-PV: platelets volume-Different letters are significant at p<0, 05 as row

Table 6. Effect of feed flaxseed oil on white blood cells of kidney failure rats

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
Wbcs ($x10^3$/ ul)	9.04 ± 3.15^{a}	13.66 ± 8.90^{b}	6.62±1.08^{a}	8.28±0.901^{a}
Lymph %	77.57 ± 5.17^{a}	65.00 ± 7.09^{b}	72.28±7.51^{b}	67.83±5.23^{b}
Neutro%	10.86 ± 4.95^{a}	18.29 ± 6.63^{a}	10.14±3.02^{a}	14.50±6.25^{a}
Mono %	7.57 ± 3.15^{a}	10.57 ± 4.08^{a}	9.85±3.14^{a}	10.50±3.14^{a}
Eosino%	4.29 ± 1.80^{a}	6.14 ± 2.67^{a}	7.71±2.80^{a}	7.17±3.31^{a}

-Values are expressed as mean ± SD - FSO: Flax seed Oil - C-: Negative control group - C+: Positive control group
- Wbcs: white blood cells -Different letters are significant at p<0, 05 as row

In groups fed on flaxseed oil, the consequences reported that the variances between groups were not significant ($p \leq 0.05$) and increased the percentage of addition from 2% to 10% headed to perfection in eosinophils level from 7.71 to 7.17 % successively and the differences. Hendawi et al. (2016) showed that a significant ($p < 0.05$) improved in total Wbcs , neutrophilia and lymphocytosis at groups treatmented with FSO when compared with positive control group, these results are consistent with (Farag et al., 2007) and also our study.

Effect of feed flaxseed oil on Na, K, Ca of kidney failure of rats.

The effect of flaxseed oil on Na, K, Ca of kidney failure of rats have shown in Table (7) for Ca consecutively, but K level had highest value of the C+ compared to C- with an average of 8.67 and 3.85 mg/l respectively, and the variances were significantly ($p \leq 0.05$). While the flaxseed oil groups, the results showed that significantly variances ($p \leq 0.05$) when increased the percentage of addition from 2% to 10% led to an improvement in Na level from 129.27 to 134.37 mg/l, in Ca level from 13.58 to 13.90 mg/l, and in between kidney failure group and healthy group found that Na and Ca decrease at kidney failure group but K increase at kidney failuregroup and that agree with our current study. Bahr et al. (2014)showed that positive control group decrease Na increase k treatment with FSO reverse the effect that proved oral administration of FSO protect against kidney failure and that agree with this investigation.

Kidneys histopathological examination

Kidneys histopathological examination in the negative control group (C-) exposed normal glomerulus with the capillary flocculates, glomerular room and the vascular pole and the collecting ducts as shown in Figure (A-1). Kidney of positive control group (C+) displayed dilatation of the proximal and distal convoluted tubules with obvious cytoplasmic degenerations in their cells and widening of their lumens as shown in Figure (B-1). Kidney of flax seed oil 2% group revealed healthy glomerulus with abundant capsular space, the lumen of kidney tubules shows infiltration with edema exudate as shown in Figure (E-1). Kidney of flax seed oil 10% group revealed normal lumens as shown in Figure (F-1). Abdel-Moneim et al. (2011) study showed that feed with flaxseed oil resulted in a significant improvement in the histological of the kidney as well as the kidney function. Therefore, this oil may play a protective role against kidney injury, these results are consistent with (Moghimian et al., 2019), (Diab et al., 2020) and our study too.

Liverhistopathological examination

Figures (A-2) & (B-2) exposed the variance between the negative control group (C-) and the positive control group (C+), the last contained cytoplasmic vacuolization in between hepatocytes strands of blood sinusoids and dilated portal vein associated with formation of new bile ductules, some hepatocytes were free from nuclei and others contained pyknotic nuclei. flax seed oil 2% Figure (E-2) and flax seed oil 10% Figure (F-2) were not different from the tissues of negative control group. All these sections were essentially normal without any evidence of active inflammation, no fibrosis, and the central vein lies at the center of the obule surrounded by the hepatocytes with well-preserved cytoplasm, and distinct nuclei. Diab et al. (2020) exhibited a significant loss of tissue integrity, increased necrosis formation at positive control group (C+) ,when given oral supplementation with FSO exposed normal tissue appearance in liver tissues, these results were consistent with (Vijaimohan et al., 2006), (Alarifi et al., 2012) and our study too.

Table 7. Effect of feed flaxseed oil on Na, K, Ca of kidney failure of rats

Parameter	Experimental groups			
	C-	C+	2% FSO	10% FSO
Na mg/l	140.14 ± 2.67^{d}	118.03 ± 2.15^{a}	129.27 ± 0.99^{b}	134.37 ± 1.54^{c}
K mg/l	3.85 ± 0.52^{a}	8.67 ± 0.19^{c}	5.73 ± 0.64^{b}	4.17 ± 0.49^{a}
Ca mg/l	12.29 ± 0.43^{a}	11.32 ± 0.55^{c}	13.58 ± 0.31^{b}	13.90 ± 0.16^{a}

Values are expressed as mean ±SD -FSO:FlaxseedOil-C-:Negativecontrolgroup-C+:Positivecontrolgroup
Different letters are significant at p<0, 05 using as row

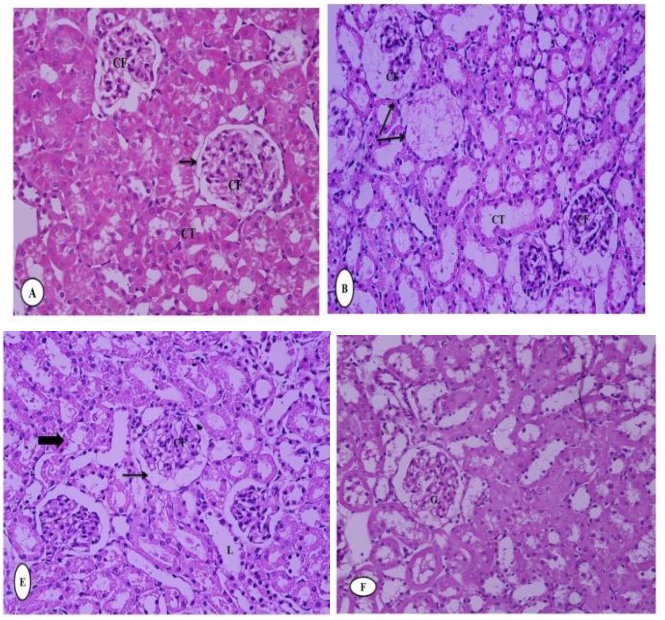

Figure 1.Light photomicrograph of kidney sections: A-1: Negative control group, B-1: Positive control group, E-1: Flaxseed oil 2% group, F-1: Flaxseed oil 10% group

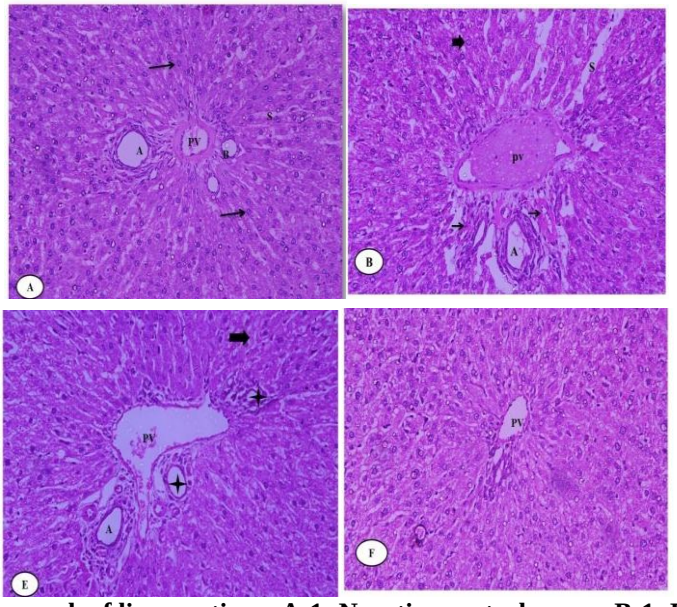

Figure 2. Light photomicrograph of liver sections: A-1: Negative control group, B-1: Positive control group, E-1: Flaxseed oil 2% group, F-1: Flaxseed oil 10% group

CONCLUSIONS

In conclusion, according to biological study, statistical analyzes, blood analysis and histopthological tests, this study found that feeding on flaxseed oil 10% (FSO 10%) improved kidney failure significantly,so we recommend that these substance can be used in food products such as baking products because of their health benefits.

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الملخص العربي

دراسة بيولوجية لتقييم تأثير تناول زيت بذور الكتان على الفئران المحدث لها الفشل الكلوى

سمير محمد أحمد، ناصر إبراهيم ابو النجا ، مروة محمد عصام الدين حسين، أشرقت محمد بدير

الهدف الرئيسي من هذا البحث هو تقييم تأثير زيت بذور الكتان على الفئران المصابة بالفشل الكلوي من خلال قياس وظائف الكلى ، حيث تم تقسيم ٢٤ من ذكور الفئران البالغة وزنها ١٦٦ جم ± ١٠ عشوائياً إلى أربع مجموعات ، كل مجموعة تضم ستة فئران . تم إعطاء جميع الحيوانات نظامًا غذائيًا قياسيًا. بعد عزل ٦ فئران كمجموعة ضابطة سالبة (المجموعة ١) ، تم حقن ١٨فأر متبقية بديكلوفيناك الصوديوم بجرعة (١٠٠ مجم / كجم) لمدة ٣ أيام ثم قسمت عشوائياً إلى ٣ مجموعات متساوية على النحو التالي: مجموعة ضابطة موجبة (+ C) تتغذى على نظام غذائي قياسي فقط (المجموعة ٢) ، فئران تعاني من الفشل الكلوي تتغذى على ٢٪ زيت بذور الكتان عن طريق الحقن المعوى (المجموعة ٥) ، فئران مصابة بالفشل الكلوي تتغذى على ١٠٪ زيت بذور الكتان عن طريق الحقن المعوي (المجموعة ٦). خلال فترة التجربة لمدة ٥ أسابيع ، تم تسجيل كمية الأكل المستهلكة كل يوم وتم تسجيل وزن الجسم كل أسبوع. في نهاية التجربة تم جمع عينات الدم الصفائح الدموية و الوزن النسبي للأعضاء الداخلية و وظائف الكلى و الكبد و كذلك الفحوصات النسيجية للكلى و الكبد. أظهرت النتائج تحسن معنوي في جميع المجموعات التجريبية مقارنة مع مجموعة الضابطة. لوحظ أن الفئران التي تغذت على زيت بذور الكتان حث لها انخفاضًا معنويًا في الوزن النسبي للكبد والكلى والقلب والرئتين والدماغ مقارنة بالمجموعة الضابطة الموجبة. لوحظ أقل مستوى من اليوريا وحمض البوليك والكرياتينين في مجموعة الفئران التي تتغذى على ١٠٪ زيت بذور الكتان. علاوة على ذلك ، هناك انخفاض غير معنوي في كل من ALT و AST في مصل الدم في المجموعة التي تغذت على زيت بذور الكتان بنسبة ١٠٪ مقارنة مع مجموعة الكونترول الإيجابية. كما اتضح المستويات الكلية للبروتين والألبومين أعلى معنوياً في مجموعة الفئران التي تغذت على زيت بذور الكتان بنسبة ١٠٪. وعموما أظهرت النتائج أن التغذية بزيت بذور الكتان ١٠٪ حسنت وظائف الكلى بشكل ملحوظ ، وبذلك ممكن أن ننصح باستخدام زيت بذور الكتان في المنتجات الغذائية لما لهذه الفوائد الصحية.