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Research Article

Biochemical Effects of Flaxseed, Olive Oils and *Thymus vulgaris* on Kidney Function of Carbon Tetrachloride CCl4 Induced Toxicity of Male Albino Rats

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Abstract

This study aimed to investigate the biochemical effects of flaxseed, olive oils and/or Thymus vulgaris on carbon tetrachloride CCl4 induced toxicity in kidney function of male albino rat. The results showed that thyme and oil medicinal products have shown promising potential for the treatment of infected kidney. The positive control group (+) had a significant increase in serum urea, creatinine and uric acid as compared to negative control group (-) ($P \leq 0.05$). Feeding rats on different ratios of thyme, olive oil and flaxseed oil decreased serum urea, creatinine and uric acid compared to the positive control group (+).

It could be recommended from the above results that High doses of thyme, olive oil and flaxseed oil could ameliorate carbon tetrachloride (CCl4)-induced tissue injury in rats, suggesting that diet rich in flaxseed oil, olive oil and thyme might be a promising approach in the management of infected kidney/tissue.

Keywords: Thyme; Olive Oil; Flaxseed Oil; CCL4, Albino Rats; Kidney Functions

Introduction

For thousands of years, medicinal plants have been used to treat various diseases. Traditional medicine is still popular among people today and many people use the properties of medicinal plants to treat diseases [3]. In the modern era, interest has been shifted more towards the natural products as compared to the classical or synthetic products due to better affordability, acceptability and compatibility with the human physiology and minimal side effects [28]. Natural antioxidant/their derivatives products, especially phytochemicals, are widely used in the treatment and management of kidneys infection derivatives [49]. Olive oil is known for its health benefits. Diet patterns with higher intakes of olive oil are associated with a reduced risk of death from all causes [38]. Olive oil is a valid source of essential fatty acids: a-linolenic acid (ALA) (α-3) and linoleic acid (α-6) that human body requires and cannot synthetize [1]. It contains tocopherols and polyphenols [13]. Flaxseeds oil (FO) have nutritional characteristics and are rich source of ω3 ALA, short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogenic lignans secoisolariciresinoldiglycoside (SDG), proteins and an array of antioxidants [22,24]. It has anti-inflammatory activity with a promising functional food ingredient [44]. Thyme (*Thymus vulgaris* L.) is a well-known herbal medicine. It has many therapeutic functions including antioxidant [7]. Most pharmacological effects of thyme are the consequence of its high antioxidant activity that is mainly attributed to the presence of phenolic monoterpenes, thymol and carvacrol, as the major constituents of essential oil of thyme [25,31]. Mokrane., *et al.* 2020 demonstrate the therapeutic effects of the *T. vulgaris* L. in minimizing Aluminum liver and kidney toxicity. Furthermore, vitamin E is essential to human nutrition because it is a potent antioxidant and can prevent oxidative damage to cells through inactivation of free radicals and reactive oxygen species [5,29].

Materials and Methods

Materials

Thyme powder (*Thymus Vulgaris*) was obtained from local market in Cairo. Extra virgin olive oil produced by Wadi Food Indus-

tries Company. Flaxseed oil obtained from International Health Shop. Carbon tetrachloride CCl_4 {freshly diluted in paraffin oil (1:1, v/v) before use} were obtained from Sigma-Aldrich. Kits for biochemical analysis were purchased from Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Giza.

Analytical methods

Antioxidant activity measurement (DPPH assay)

The DPPH method **(14)** was used to determine free radical-scavenging potential of each sample.

Antioxidant activity (%) = $(A_{Control} - A_{sample})/A_{control}$ X 100

Where: $A_{control}$, was the absorbance of the control sample and A_{sample}, the absorbance in the presence of the sample.

Fatty acids analysis

Fatty acids (Omega 3,6) were quantitatively analyzed by gas chromatography (GC) as their methyl esters according to [21].

Biological experiment

Animal, housing and diets:

Sixty adult male albino rats (Sprague Dawley strain), weighing 130 ± 5g were purchased and housed in the animal house of the Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered, pathogen-free air and water and maintained at a temperature between 20-25ºC with a 12h light/dark cycle and light cycle (8-20 h) and relative humidity of 50%. The animals acclimatized for one week as an adaptation period. The animals were randomly divided into 12 groups (5 rats/group). The first group of rats [control (-)] was fed on Basal diet (Table 2) for 10 weeks (total period of experiment). The other 11 groups were injected under peritoneal with CCL_4 in paraffin oil (50% v/v, 2 ml/kg) twice per week for 2 weeks to induce chronic damage in the liver tissue [23] and fed on basal diet and after that each group was fed on its specific treatment as shown in table 1. The second group {control (+) group} was fed on basal diet.

At the end of the experiment period the animals were fasted for 12h, Blood samples were withdrawn from orbital plexus venous by using fine capillary glass tubes. Blood samples were collected into plain tubes without anticoagulant and allowed to clot, Blood samples were centrifuged at 3000 rpm for 10 min at 4°C, to obtain clear serum. Separation of serum samples were carefully aspired using a pastier pipette and transferred into dry clean test tubes. Serum was frozen at -18°C until analyzed. The animals were anesthetized with ether and sacrificed. They were quickly dissected to excise the kidney, then washed with saline solution and dried with filter paper. These organs were weighed and then kept in formalin until histological investigations.

Groups	Experimental diets
Frist	Basal diet (control (-) group)
Second	CCL_4 + Basal diet (control (+) group)
Third	(CCL_4 + Basal diet) + (Thyme (10 % in substitution of fiber).
Fourth	(CCL_4 + Basal diet) + (Thyme (15% in substitution of fiber).
Fifth	(CCL_4 + Basal diet) + (Olive oil (7 mg/kg/day).
Sixth	(CCL_4 + Basal diet + Olive oil (14 mg/kg/day).
Seventh	(CCL_4 + Basal diet + Flaxseed oil (7 mg/kg/day)
Eighth	(CCL_4 + Basal diet + Flaxseed oil (14 mg/rat/ daily)
Ninth	(CCL_4 + Basal diet + Mixture of Thyme (10 %/rat/day) and Olive oil (7mg/kg/day)
Tenth	(CCL_4 + Basal diet + Mixture of Thyme (15 %/rat/day) and olive oil (14mg/kg/day)
Eleventh	(CCL_4 + Basal diet + Mixture of Thyme (10 %/rat/day) and Flaxseed oil (7 mg/kg/day)
Twelfth	(CCL_4 + Basal diet + Mixture of Thyme (15 %/rat/day) and Flaxseed oil (14 mg/kg/day)

Table 1: Experimental diets.

Biological determination

Biological evaluation of the different tested diets was carried by determination of body weight gain% (BWG%) according to [15].

BWG% = [(Final weight - Initial weight)/(Initial weight)] X 100

Relative weight = (Organ weight/Final weight) X 100

Biochemical analysis

The separated serum/plasma samples were analyzed using Bio-diagnostic kits using spectrophotometer (model DU 4700) adjusted for suitable wave length for each analysis: Serum uric acid was determined according to [35]; Serum urea nitrogen was determined according to [42]; Serum creatininewas determined according [41]. The determination of the antioxidative capacity is measured according to [26-33].

Statistical Analysis

Statistical analyses were performed using computer program, the PROC ANOVA procedure of Statistical Analysis System (SAS, 2006) as all parameter are normally distributed. Duncan's post hoc multiple comparisonat 5% level of significance was used to compare between means.

Results and Discussion

General signs in the rats

No rats died during the experimental period (10 weeks) or exhibited abnormal signs from all groups throughout the experimental period.

Final body weight (FBW), body weight gain (BWG), and Relative Kidney weight (RKW) of rats fed on different ratios of thyme, olive and flaxseed oil

Results are represented in table 5. A significant difference ($P \leq 0.05$) was found in the final body weights of rats in the control (+) group (175.8 ± 22.0) on comparing with the remaining groups. G 1 and 12 had the lowest BWG (24.0 and 23.1 respectively). Groups 4-7, 10-11 had BWG range 34.05-36.31. Groups 2-3, 9 had BWG range 40.11-42.98 while G 8 had BWG 31.31.

These results agree with the results of [46] who showed that flaxseed oil decrease body weight in normal animals without increasing food intake. [17], showed that olive oil causes weight reduction hence it is indirectly related to control the NAFLD. It also agrees with [37] where they stated that flaxseed oil has the ability to lubricate the colon and works as a natural laxative and soothes constipation too. Smoother outflow of toxins and waste facilitates weight loss. Vafania., *et al.* 2019, found that the thyme powder can lose weight in rats due to thymoloil, which promotes the process of dissolving fat,prevent oxidation and accumulation of fat, presence of antioxidanr vitamins and dietary fibre,saves the body from water retention [16,43].

The relative kidney weight of rats is shown in table 2. In injected groups with CCl4 (control +) and group 7-8, there was almost significant difference (decrease) compared with control (-) group. The remaining groups showed increasein RKW compared with control (+) (0.7-079 vs 0.64 respectively).

Data in Colums with different superscript letters are statistically different ($P \leq 0.05$)

Treatments	Mean body weight gain %		
	FBW	BWG	RKW
	g	g	g
Control (-)	155.0 ± 21.7 ^{c}	24.0 ± 6^{c}	0.79 ± 0.25^{a}
Control (+)	175.80 ± 22^{a}	42.92 ± 9.4^{a}	0.64 ± 0.15^{d}
Thyme groups			
Group 3	168.30 ± 5.3^{b}	42.98 ± 4.2^{a}	0.71 ± 0.08^{bc}
Group 4	159.10 ± 7.1^{bc}	34.05 ± 4.2^{b}	0.76 ± 0.08^{b}
Olive oil groups			
Group 5	162.30 ± 20.7 ^{b}	36.31 ± 3. 6^{ab}	0.79 ± 0.26^{a}
Group 6	159.00 ± 7.8^{bc}	34.92 ± 5.8^{b}	0.78 ± 0.25^{ab}
Flaxseed oil groups			
Group 7	164.90 ± 7.49^{b}	35.72 ± 4.85^{ab}	0.64 ± 0.15 ^{d}
Group 8	160.30 ± 20.7 ^{b}	31.31 ± 3.2^{bc}	0.65 ± 0.08cd
Thyme and Olive oil groups			
Group 9	175.01 ± 0.8^{a}	40.11 ± 9.8^{ab}	0.70 ± 0.07 ^{c}
Group 10	165.30 ± 20.7 ^{b}	36.31 ± 3. 6^{ab}	0.79 ± 0.26^{a}
Thyme and flaxseed oil groups			
Group 11	158.40 ± 7.1^{bc}	34.92 ± 5.8^{b}	0.72 ± 0.06^{bc}
Group 12	151.0 ± 11.2 ^{c}	23.10 ± 2^{bc}	0.79 ± 0.26^{a}

Table 2: Final body weight (FBW), body weight gain (BWG), and Relative Kidney weight (RKW) of rats fed on different ratios of thyme, olive and flaxseed oil.

*Data are presented as means ± SDM (n = 5).

Antioxidant activity (DPPH assay)

α, α-diphenyl-β-picrylhydrazyl (DPPH)radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. The results are given in table 3. The highest antioxidant activity was in thyme 96.31% followed by flaxseed oil 12.02% then olive oil 7.23%.

The highest percentage of DPPH of thyme compared to olive and flaxseed oils could be attributed to its content of vitamins and antioxidant compounds as phenolic compounds as thymol and carvacrol [19,47,48]. Thymol concentration was much higher than carvacrol concentrations in thyme. Plants have been used for treatment of many diseases; phenolic compounds are the most widely occurring chemicals, which have strong antioxidant properties [18]. Aleksandar., *et al.,* 2015 found that total antioxidant activity of olive oil can be improved by extracting oil from destoned olives. Antioxidant activity of flaxseed oil is due to presence of the main phenolic compounds as p-hydroxybenzoic acid, ellagic acid, p-coumaric acid, ferulic acid and ascorbic acid, respectively.

Antioxidant	Different samples (%)		
	Thyme	Flaxseed oil	Olive oil
DPPH	96.31	12.02	7.23

Table 3: The free radical scavenging (DPPH%) of thyme, olive and flaxseed oils.

Total antioxidant of rats fed on different ratios of thyme, olive and flaxseed oils with different ratio

An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules and can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, Substances which protect biomolecules from free radical-mediated damage both *in vivo* and *in vitro* fall under this category [12]. The results in table 4 illustrated that treating rats with different ratios of thyme, olive and flaxseed oil showed a significant decrease TACin control (+) group as compared to the control (-) group (0.75 vs 1.75 respectively). Other groups (G 3-12) exhibited a significant increase of TAC as compared to the control (+) group, while G 3-8 and 10; 12 showed a significant decrease of TAC as compared to the control (-) group. Group 9, 11 showed no significant change.

Treatment	Total antioxidant (mmol/L)
Control (-)	1.75 + 0.17 [a]
Control (+)	0.75 + 0.06 [f]
Thyme groups	
Group 3	1.45 + 0.11 [d]
Group 4	1.43 + 0.20 [d]
Olive oil groups	
Group 5	1.43 + 0.18 [d]
Group 6	1.53 + 0.38 [c]
Flaxseed oil groups	
Group 7	1.57 + 0.10 [c]
Group 8	1.51 + 0.17 [c]
Thyme and Olive oil groups	
Group 9	1.79 + 0.2[a]
Group 10	1.37 + 0.48[e]
Thyme and flaxseed oil groups	
Group 11	1.70 + 0.05 [a]
Group 12	1.64 + 0.10 [b]

Table 4: Total antioxidant (mmol/L) of experimental rats treated by different ratios of thyme, olive and flaxseed oil.

Fatty acids (Omega 3, 6)

The ω6 eicosanoids without ω3 fatty acids are pro-inflammatory. Therefore, their balance is very important in our daily intake [30]. All lipids and ω3 of Flaxseed may help protect against certain infections, inflammation and increase health benefits [6]. Omega 3 and 6 were higher in flaxseed oil than olive oil.

Fatty acid relative %	Olive Oil	Flaxseed oil
Omega 3	1.08	54.95
Omega 6	15.08	18.73

Table 5: Fatty acids (Omega 3, 6).

Kidney function of rats fed on different ratios of thyme and olive and flaxseed oil

It is well recognized that urea is a major uremic toxin and plays an important role in human disease. It is usually accepted that levels above 50 mmol/L induce anorexia. Furthermore, the level of blood urea nitrogen is used for the clinical evaluation of renal function, and it considered as an indicator for the accumulation of all nitrogen waste products producing from the degradation of protein [7].

Results in table 6 and figure 1-3 showed that kidney function (uric acid, creatinine and urea was statistically different from control (+) group (2.25 ± 0.4, 3.4 ± 0.82, and 65.25 ± 0.06 mg/dl) respectively. Amounts of uric acid, creatinine and urea were statistically different from control (-) (1.76 ± 0.06, 1.22 ± 0.14 and 25.91 ± 0.93 mg/dl) respectively. It was also noticed that there was a decrease in the mean values of uric acid, urea and creatinine in all tested groups, compared to the control (+) group. There was a significant decrease ($P < 0.05$) in uric acid, creatinine and urea of group10 and 12 when compared with group 9 and 11 due to high concentration of thyme with olive oil in group 10 and thyme with flaxseed oil in group 12 decrease the uric acid, creatinine and urea compared with low concentration in group 9 and 11. Results of groups 6 and 8 (high doses of olive and flaxseed oil) showed significant ($p < .001$) decrease in Serum urea, uric acid and creatinine levels compared with 5 and 7groups.

Serum urea nitrogen is a substance that is formed in the liver when the body breaks down protein. In healthy people, most urea nitrogen is filtered out by the kidneys and leaves the body in the urine. If the patient's kidneys are not functioning properly or if the body is using large amounts of protein, the serum urea nitrogen level will rise**.** Increased serum creatinine above normal levels may reflect destroy of 50% of renal nephrons [11]. Rubió., *et al.,* 2014 noticed that, virgin olive oil treatment markedly reduced elevated serum creatinine, urea and uric acid levels and contracted the deleterious effects of CCl_4 on oxidative stress markers changes caused by CCL_4 in kidney, virgin olive oil could have a beneficial role against CCL_4 induced oxidative and renal stress in rat. Abdel-Azeem., *et al.,* 2017 evaluate the effect of *Thymus vulgaris* powder as a natural antioxidant on liver and kidney functions and antioxidant status of growing rats. Serum urea, uric acid and creatinine levels were significantly ($p < 0.05$) decreased in treated groups compared with control group, thyme powder to growing rats showed no adverse effects on liver and kidney function parameters and improved antioxidant status.

Treatments	Kidney function (mg/dl)		
	Urea	Creatinine	Uric Acid
Control (-)	25.91 ± 0.93^{cd}	1.22 ± 0.14^{ab}	1.76 ± 0.06^{bc}
Control (+)	65.25 ± 0.06^{a}	3.4 ± 0.82^{a}	2.25 ± 0.41^{ab}
Thyme groups			
Group 3	35.6 ± 6.54^{b}	1.02 ± 0.06^{b}	2.53 ± 0.26^{a}
Group 4	32.70 ± 5.66^{ab}	0.57 ± 0.03^{cd}	2.01 ± 0.25^{bc}
Olive oil groups			
Group 5	29.50 ± 4.51^{bc}	0.98 ± 0.01^{bc}	2.02 ± 0.18^{bc}
Group 6	26.90 ± 6.42^{cd}	0.50 ± 0.09^{d}	1.82 ± 0.18^{bc}
Flaxseed oil groups			
Group 7	26.00 ± 5.00^{cd}	0.80 ± 0.01^{bc}	1.02 ± 0.12^{c}
Group 8	25.70 ± 4.88^{d}	0.60 ± 0.07^{cd}	0.99 ± 0.25^{d}
Thyme and Olive oil groups			
Group 9	33.4 ± 4.93^{bc}	0.59 ± 0.05^{cd}	2.33 ± 0.25^{ab}
Group 10	31.80 ± 6.76^{bc}	0.58 ± 0.03^{cd}	2.21 ± 0.23^{b}
Thyme and flaxseed oil groups			
Group 11	36.60 ± 4.98^{ab}	0.77 ± 0.07^{c}	2.41 ± 0.26^{ab}
Group 12	29.40 ± 5.41^{c}	0.58 ± 0.09^{cd}	1.82 ± 0.17^{bc}

Table 6: Kidney functions of experimental rats treated by different ratios of thyme & olive oil and flaxseed oil.

Data are presented as means ± SDM(*n = 5*).

Data in Colums with different superscript letters are statistically different ($P \leq 0.05$)

a, b, c: Means with different letter among treatments in the same colume are significantly different.

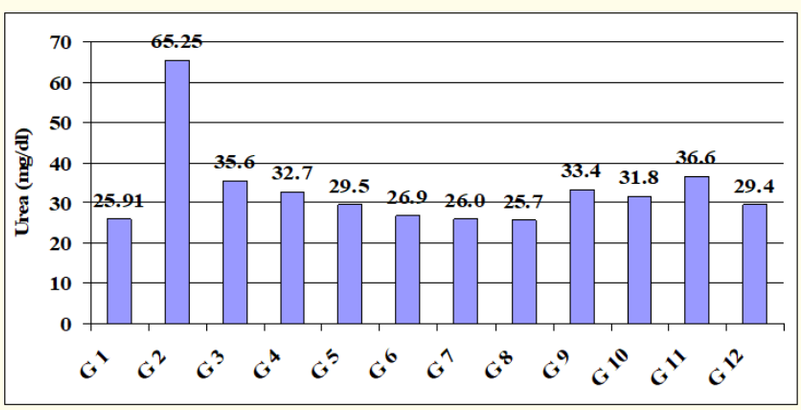

Figure 1: Effect of different ratios of thyme, olive and flaxseed oil on serum urea of experimental rats.

Control Control (-) (G 1); Control (+) (G 2); Thyme (G 3, LD); Thyme (G 4, HD), Olive (G 5, LD); Olive (G 6, HD) Flaxseed (G 7, LD) Flaxseed (G8, HD); Thyme and Olive (G 9, LD); Thyme and Olive (G 10, HD); Thyme and Flaxseed (G 11, LD); Thyme and Flaxseed (G 12, HD).

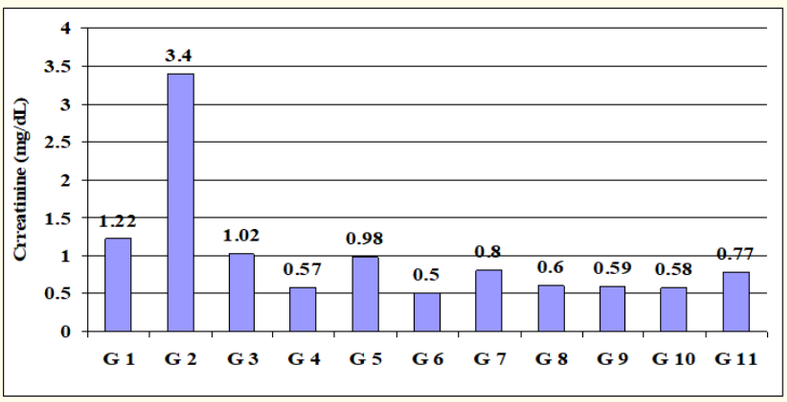

Figure 2: Effect of different ratios of thyme, olive and flaxseed oil on serum creatinine of experimental rats.

Control Control (-) (G 1); Control (+) (G 2); Thyme (G 3, LD); Thyme (G 4, HD), Olive (G 5, LD); Olive (G 6, HD) Flaxseed (G 7, LD) Flaxseed (G8, HD); Thyme and Olive (G 9, LD); Thyme and Olive (G 10, HD); Thyme and Flaxseed (G 11, LD); Thyme and Flaxseed (G 12, HD).

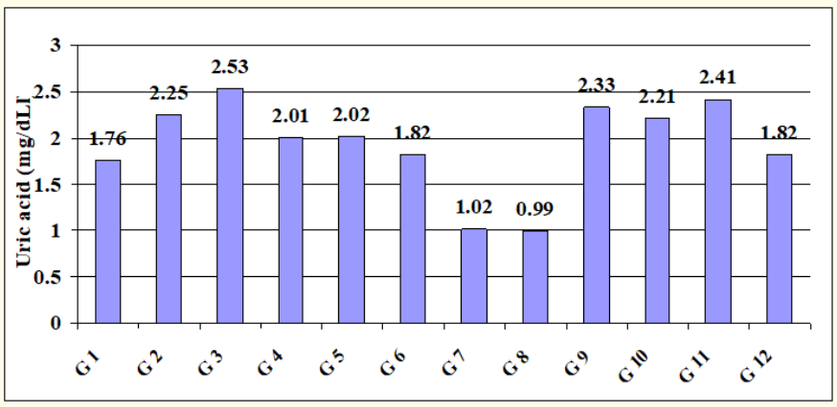

Figure 3: Effect of different ratios of thyme, olive and flaxseed oil on uric acid of experimental rats.

Control Control (-) (G 1); Control (+) (G 2); Thyme (G 3, LD); Thyme (G 4, HD), Olive (G 5, LD); Olive (G 6, HD) Flaxseed (G 7, LD) Flaxseed (G8, HD); Thyme and Olive (G 9, LD); Thyme and Olive (G 10, HD); Thyme and Flaxseed (G 11, LD); Thyme and Flaxseed (G 12, HD).

Conclusion

High doses of thyme, olive and flaxseed oils could ameliorate carbon tetrachloride (CCl_4)-induced toxicity in rats, suggesting that diet rich in flaxseed oil, olive oil and thyme might be a promising approach in the management of kidney diseases.

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