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ABSTRACT

Background: Hypothyroidism, also known as low thyroid hormone, is a disorder of the endocrine system in which the thyroid gland does not produce enough thyroid hormone. The aim of this study is to identify the protective effect of flaxseed powder and extract on the chemical and biological properties of rats with hypothyroidism.

Methods: 30 male (SPRD) rats were randomly divided into 5 groups of 6 rats each and one of them was kept as a negative (-ve) control group, the rest of rats were administered intraperitoneally with 6-n-propyl-2-thiouracil (PTU, 10 mg/kg) for 15 days to induce hypothyroidism. Then, the groups were divided as follows, group 2 positive control (+ve) fed on basal diet. Treated group (3) fed on basal diet and injection of L-thyroxin drug at a dose of 0.5 mg/kg, group 4 fed on basal diet and flax seed powder 5g /100g / diet daily and group 5 fed on basal diet and orally dosed of flax seed extract at dose 1ml/kg b.wt / rats daily by stomach tube.

Results: The results of the analysis of phenolic compounds showed the highest amount of flaxseed is catechein, gallic, pyrogallol and e-vanillic. The results of the biological evaluation at the end of the experiment showed that the treated groups fed flaxseed powder and extract with levels of thyroid hormones had a significant decrease in the level of (T3) in the blood plasma of the groups affected by thyroid gland. On the other hand, thyroxine (T4) was recorded and also an improvement in the group Treatment with L-thyroxin compared to the control group (+ve). The treated groups showed a significant decrease in serum total cholesterol, triglycerides, LDL cholesterol, ALT, AST, urea, creatinine, uric acid. While body weight gain (BWG), and HDL cholesterol were significantly increased compared to (+ve) group. It showed an increase in the level of thyroid stimulating hormone (TSH) in the blood plasma.

Conclusion: Flax seeds powder and extract are readily available supplier of polyphenols with major antioxidants that are functional food components as they influence physiological and biochemical processes, resulting in better health and improved level of thyroid hormone.

Key words: *Linum usitatissimum,* Thyroid hormones and functional food

التأثيرات المضادة لقصور الغدة الدرقية لمسحوق ومستخلص بذور الكتان في الفئران

ملخص البحث: قصور الغدة الدرقية المعروف أيضًا انخفاض هرمون الغدة الدرقية وهو اضطراب في نظام الغدد الصماء حيث لا تنتج الغدة الدرقية ما يكفي من هرمون الغدة الدرقية. والهدف من هذه الدراسة التعرف على التأثير الوقائي لمسحوق ومستخلص بذور الكتان على الخواص الكيميائية والبيولوجية للفئران المصابة بقصور الغدة الدرقية.

الطريقة: تم تقسيم ٣٠ فأر ذكور من سلالة سبراغ داولي (SPRD) عشوائياً إلى ٥ مجموعات، كل مجموعة ٦ فئران وتم الاحتفاظ بواحدة منهم كمجموعة ضابطة سالبة، وتم إعطاء بقية الفئران ٦-ن-بروبيل-٢ ثيوراسيل (10ملجم/كجم,PTU) لمدة ١٥ يومًا للحث على قصور الغدة الدرقية. بعد ذلك، تم تقسيم المجموعات على النحو التالي، المجموعة الضابطة الموجبه التي تغذت على النظام الغذائي الأساسي. المجموعة الثالثة التي تغذت على عليقة أساسية وحقن عقار الثيروكسين بجرعة ٠.٥ ملجم/كجم، المجموعة الرابعة تغذت على عليقة أساسية ومسحوق بذور الكتان ٥ جم/١٠٠ جم/عليقة يومياً والمجموعة الخامسة تغذت على عليقة أساسية مع إعطاء جرعات من مستخلص بذور الكتان عن طريق الفم بجرعة ١مل/كجم من وزن الجسم/الفئران يومياً بواسطة أنبوب المعدة.

النتائج: أظهرت نتائج تحليل المركبات الفينولية لبذور الكتان أن أعلى نسبة الكاتشين والجاليك والبيروجالول والفانيليك. واوضحت نتائج التقييم البيولوجي في نهاية التجربة أن المجموعات المعالجة التي تغذت على مسحوق ومستخلص بذور الكتان على مستويات هرمونات الغدة الدرقية كان لها انخفاض معنوي في (T3) في بلازما الدم للمجموعات المصابة بالغدة الدرقية .ومن ناحية أخرى سجل مستوي هرمون الغدة الدرقية (T4) تحسن مع المجموعة التى تناولت العلاج بدواء L-thyroxin مقارنة بالمجموعة الضابطة الموجبة وأظهرت أيضا المجموعات المعالجة انخفاضًا معنويًا لمستوي الكوليسترول الكلي في الدم ، والدهون الثلاثية ، وكوليسترول LDL ، و ALT ، و AST ، واليوريا ، والكرياتينين ، وحمض البوليك. بينما سجلت ارتفاع معنويا فى وزن الجسم واللبيوبروتينات مرتفعة الكثافة ومستوى هرمون الغدة الدرقية في بلازما الدم مقارنة بالمجموعة الضابطة الموجبة.

الخلاصة: أن مسحوق ومستخلص بذور الكتان يحتوي على العديد من البوليفينول تعمل كمضادات الأكسدة الرئيسية ومكونات غذائية وظيفية لأنها تؤثر على العمليات الفسيولوجية والكيميائية الحيوية ، مما يؤدي إلى تحسين الصحة وتحسين مستوى هرمونات الغدة الدرقية.

الكلمات المفتاحية: بذور الكتان ، هرمونات الغدة الدرقية ، الغذاء الوظيفي

INTRODUCTION

Hypothyroidism is a disease caused by a deficiency of the thyroid hormones thyroxine (T4 - thyroxine) and triiodothyronine (T3 - triiodothyronine) in the tissues of the body, perform it gradually and continuously. This hereditary disease sometimes affects more than one member of the same family. In people with Hashimoto's disease, there are a very large proportion of antigens that attack the main protein produced by thyroid cells, thyroglobulin, as well as the enzyme peroxidase (peroxidase) to produce these two hormones thyroxine. And triodothyronine. This affects women more than 4:1 by 8:1, as this stage appears in the third stage of life. Hypothyroidism presents with many different signs and symptoms. Signs and symptoms include: Fatigue Weakness General Drowsiness Sensitivity to cold, muscle and joint pain, forgetfulness and memory disorders, excessive hair loss.

Constipation of fertility problems, mood Swings, hoarseness, lack of appetite, and sweating in the palms of the hands. Older adults with hypothyroidism may develop additional symptoms such as decreased brain function, a condition very similar to dementia **Biondi and Cooper, (2019).**

Flax (*Linum usitatissimum*), is an annual plant of oil crops belonging to the flax family. The original home of flax extends from the eastern Mediterranean to India and it is believed that the beginning of its use took place in the Fertile Crescent. The parts used in the flax plant are oil and seeds. The flax plant reaches a height of about a meter and has slender stems and leaves. Its flowers are blue in color, and the seeds are brown. It is grown in the eastern Mediterranean to India and is also cultivated in Europe **(Muir *et al.*, 2003 and Ganorkar and Jain, 2013**). Flax is a source of nutrients 20g of flax provides 15-25% of the RDI for men and women of fiber, carbohydrates 29%, and the proportion of fiber 95% of carbohydrates is considered a low-carbohydrate food, and it is also a source of proteins rich in amino acids such as glutamine, and arginine, both of which are important for the health of the immune system and the heart, flaxseed contains omega-6 and omega-3 fatty acids and reduces symptoms of constipation Also, due to its insoluble fiber content **(Hassan *et al.*, 2012 and Elbostany *et al.*, 2016).** Rich in vitamins and minerals: examples of which are: phosphorous, thiamine, copper, magnesium, molybdenum and p-coumaric acid. Which is one of the antioxidants including polyphenols plant sterols (phytosterols). Ferulic acid is an antioxidant that may help prevent many chronic diseases, which contribute to lowering cholesterol. These compounds help fight the signs of aging and may reduce the risk of chronic diseases. A source of anti-inflammatory compounds because it contains alpha-linolenic acid, and

lignans may reduce inflammation associated with certain diseases such as asthma and Parkinson's disease (**Amiri *et al.*, 2021**).

MATERIALS AND METHODS

MATERIALS:

Flax seeds *(Linum usitatissimum*) was obtained from Agriculture Research Center, Giza, Egypt. Casein, cellulose , all vitamins and minerals were purchased from El-Gomhoryia Company, Tanta city, Egypt.

- Thiouracil drug© (4-Hydroxy-2-mercapto-6-methylpyrimidine (MTU) and L-thyroxin drug © Thiouracil were one of product of Sanofi-Synthelabo Company, Paris, France.
- The rats were obtained from Laboratory of Animal Colony, Helwan, Egypt.

METHODS:

- **Preparation of flax seeds powder and extracts:** Flax seeds material were milled in a mixer to give a powder and kept in dusky stoppered glass bottles in a dark dry location till use, according to **Russo, (2001)** who reported that herb is best kept in a dry and dark location to reduce oxidation of their contents. Seeds and herbs were extracted seven times with 10 volumes of distilled water at 40^{o}C for 8 h followed by cooling. The aqueous extract was combined, and then concentrated twice under vacuum at 40 ^{0}C.
- **Chemical constituents of flax seeds powder:** Moisture, protein, crude fibers, fat content and ash contents were determined according to the method described in the **A.O.A.C. (2000).** Total carbohydrates were calculated by difference.
- **High-performance liquid chromatography analysis of polyphenols and flavonoids:** High-performance liquid chromatography (HPLC) analysis of extracts was performed using an Agilent 1200 chromatograph it was selected for detection of polyphenol and flavonoids as described by the method of (**Goupy *et al.*, 1999**).
- **Biological Experiment:**

Standard Diet: Standard diet was prepared according to **(NRC, 1995).**

Biological assay:

Animals and experiential design: The study was performed on 30 male albino rats of Sprague Dawley strain weight 110± 5g was used for the experiments. The rats were obtained from Laboratory of Animal Colony, Helwan, Egypt. The animals were allocated in plastic cages with metallic stainless covers. They were kept under constant laboratory conditions room temperature 25±2°c and lighting (12L:12d). Rats were fed the basal diet for 7 days before the beginning of the experiment for adaptation. The

standard diet prepared according to, **Reeves *et al.*, (1993)** diet and water were provided *ad libitum*

Experimental design: After adaptation period the animals were randomly divided into 5 groups of 6 rats each and one of them was kept as a negative (-ve) control group. The rest of rats were administered intraperitoneally with 6-n-propyl-2- thiouracil (PTU, 10 mg/kg) for 15 days to induce hypothyroidism by the method used by **Davidson *et al.*, (1978)**. The groups were divided follows:

Group (1): Negative control (-ve): Fed on basal diet

Group (2): Positive control (+ve): Thiouracil group fed on basal diet

Group (3): Fed on basal diet and injection of L-thyroxin drug at a dose of 0.5 mg/kg according to the previous established method **Saxena *et al.*, (2012)**.

Group (4): Fed on basal diet and flax seed powder 5g /100g / diet daily.

Group (5): Fed on basal diet and orally dosed of flax seed extract at dose 1m/1kg b.wt /rats daily by stomach tube.

Body weight was recorded to calculated body weight gain at the end of experiment. Food Efficiency Ratio (FER) was calculated at the end of experiment as following: FER= Body weight gain (g) / Food intake (g) according to **Chapman *et al.*, (1959).**

Blood samples were collected at the end of experiment from the eye plexuses by fin capillary glass tubes into a dry clean centrifuged glass tube to prepare the serum. Blood samples were left 15 min at room temperature then the tubes were centrifuged for 10 min at 3000 rpm and supernatant was kept frozen at -20 ^{0}C. All experimental animals in this study were managed according to the guidelines for the Behavioral Research and were approved by the Research Ethics Committee, Home Economics Department, nutrition and food science, Zagazig University, Egypt, under animal protocol (ZU/FSE/2024/4/No 1).

Biochemical Analysis:

Serum levels of tri-iodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to (**Larsen, 1972), (schuurs and van weeman, 1977) and (Bhowmich *et al.*, 2007)**, respectively. Serum aspartate aminotransferase (AST) and alanine amino transferase (ALT) concentrations were determined according to (**Reitman and frankel 1957**). Serum total cholesterol, LDL- cholesterol, HDL- cholesterol and trigycerides content were determined according to the methods of (**Roeeschlau *et al.*, 1974), (Wieland and seidel, 1983), (Assman, 1979) and (Trinder, 1969),** respectively. Serum creatinine, uric acid and urea levels were determined according to (**Bartles *et al.*, 1972),**

(Caraway, 1955) and (Fawcett and Scott, 1983), respectively. Serum total protein was measured according to the method of (**Watanabe *et al.*, 1986**).

Statistical Analysis: The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967).**

RESULTS AND DISCUSSION

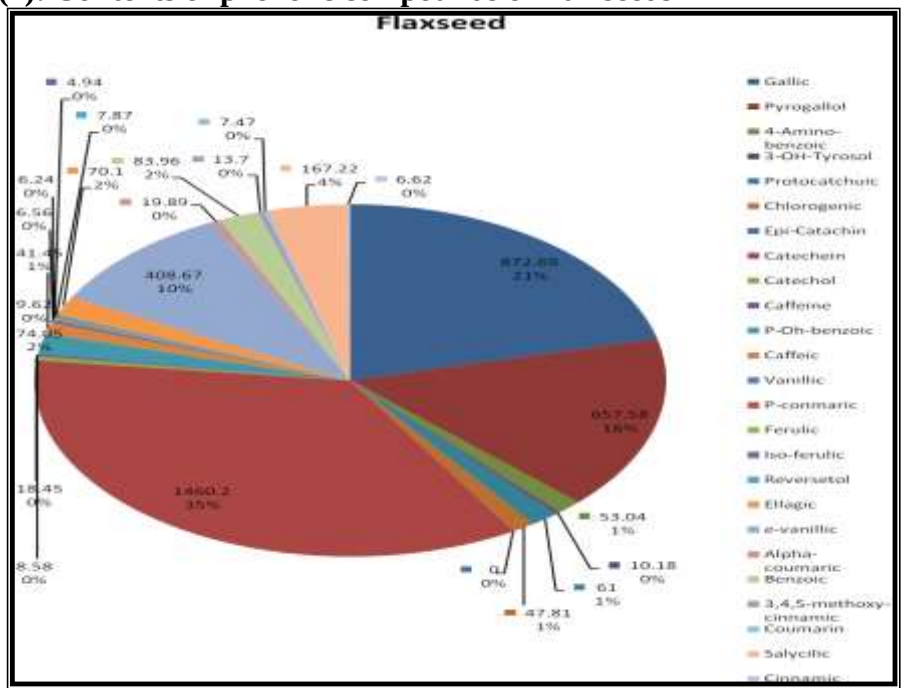
The chemical composition of flax seeds are illustrated in Table (1). The main constituents of raw flax seeds were moisture which was (4.93 g/100g) and the value of protein (22.84 g/100g) but the value of fat was (38.59 g/100g) and ash was (3.97 g/100g), while the value of fiber was (11.02 g/100g) and carbohydrate (18.65 g/100g). The results of elementary chemical composition of flax seed agreed with those reported by **Morris, (2017) and Wang *et al.*, (2019)** asserted that, the unique and diverse properties of flax seed. It is containing proteins (26.9– 31.6 %), fat (31.9–37.8 %,) and dietary fiber (10.7–13.8 %).

Table (1): Chemical composition of flax seeds powder (100g D/W)

Parameters / Samples	Moisture %	C. protein %	T. fat %	Ash %	C. fiber %	T.carbohydrates %
flax seeds powder	4.93	22.84	38.59	3.97	11.02	18.65

Data advanced in **(**fig.1**)** showed a summary of results of flaxseed employed in this study contained considerable amount of phenolic compounds with an average from 4.94 to 1460.20 ppm. The highest amount of flaxseed is catechein, gallic, pyrogallol and e-vanillic which their content was 1460.20, 872.69, 657.58 and 408.67 ppm respectively. While the lowest amount of phenolic compound in flaxseed were: iso-ferulic, ferulic, p-conmaric and cinnamic their content were 4.94, 6.24, 6.56 and 6.62 ppm respectively. These results agreed with the results of **Westcott *et al.*, (2016), Eliasson *et al.*, (2018) and Siger *et al.*, (2018)** who demonstrated that, the major flavonoids in flaxseed powder are p-coumaric, ferulic, caffeic, gallic acid, traces of 4-hydroxybenzoic acid and o- glycosides. **Stagos *et al.*, (2016)** showed that, some phenolic acids (caffeic acid, ferulic acid, gallic acid) contribute to their activity against various types of cancer such as breast, lung, and gastric cancer.

Fig. (1): Contents of phenolic compounds of flax seeds



The body weights of rats during the 4 weeks housing period in each groups are shown in Table (2). The results indicated that weight gain of the hypothyroidism group (+ve) was significantly lower than group (-ve) (26.64±8.11 < 93.76±8.11 g). While when this group treated with L--thyroxin drug, this value improved and increased to (92.13±9.13 g). Where weight gain reached (91.44±9.17 g) in (group5) which feed on flax seeds extract (group5). There were no differences (P>0.05) in food intake between experimental groups and (-ve) except in (+ve) which recorded the lowest score (13.94±2.20 g/d).On the other hand the same trend was observed in feed efficiency ratio, where it was (0.187±0.03) in (-ve) control) group and (0.068±0.02) in (+ve) control group. The present result was confirmed by **Elbostany *et al.*, (2016)** who indicated that, there was significant higher in body weight gain when treated with flax seed when compared to hypothyroidism group. These results may be due to nutritional values of flax seeds powder because of an excellent source of vitamin A, vitamin K, vitamin C, magnesium, iron, potassium, and calcium. Flax seeds are rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms. These results are in harmony with those obtained by (**Caligiuri *et al.*, 2014**).

Table (2): Effect of flax seeds powder and extract on nutritional parameters of rats suffering from hypothyroidism

Parameters / Groups	Weight gain (g)	Daily food intake (g/d)	Feed efficiency ratio (FER)%
Group (1): -ve	93.76±8.11 [a]	17.94± 2.20 [a]	0. 187±0.03[b]
Group (2): +ve	26.64±8.11[c]	13.94± 2.20 [b]	0.68±0.02 [c]

Group (3)	92.13±9.13 [a]	16.66±2.32 [a]	0.186±0.04 [a]
Group (4)	84.77±9.17 [b]	16.65±2.21 [a]	0.183±0.03 [b]
Group (5)	91.44±9.17 [a]	16.66±2.32 [a]	0.196±0.04 [a]

Values are expressed as mean ± SD; n = 6. Mean values in each column having different superscript (a, b) are significant. Means with the same letter are insignificantly different.

The level of thyroid stimulating hormone (TSH) triiodothyronine (T3) and thyroxine (T4) and in the serum rats fed on different treated with flax seed powder and extract comparing drug groups was summarized in Table (3). The level of thyroid stimulating hormone (TSH) in the serum increased significantly in groups treated with flax seed powder and extract and L-thyroxin drug compared with (+ve group). The previous results reported that flax seed powder and extract helped to improve thyroid hormone level. The maximum rate of increase of triiodothyronine (T3) in the serum was observed at (+ve group) (5.22±2.01 µg/dl) and markedly decreased levels in all treatment hypothyroidism groups. While thyroxine (T4) had the least value of (9.07±4.71 µg/dl) in (-ve group) control and recorded the highest value of (12.33±5.66 µg/dI) in (+ve group). Meanwhile, it relatively decreased in L-thyroxin drug group and groups fed on different drug, flax seed powder and extract treated groups. Flaxseed contains linolenic acid, which has anti-inflammatory effects and works to balance disturbances in thyroid hormones. Flax seeds also contain magnesium and vitamin B-6, which have a role in regulating the work of the thyroid gland (**Ross *et al.*, 2020 and Amiri *et al.*, 2021**).

Table (3): Effect of flax seeds powder and extract on serum thyroid hormones in rats suffering from hypothyroidism

Parameter / **Groups**	Thyroid stimulating hormone **(TSH) (µIU/ml)**	Triiodothyronine **(T3) (µg/dl)**	Thyroxine **(T4) (µg/dl)**
Group (1): -ve	5.13±0.32 [a]	2.12±1.7 [d]	9.07±4.71 [d]
Group (2): +ve	1.84±0.16 [c]	5.22±2.01 [a]	12.33±5.66 [a]
Group (3)	4.97±0.37 [ab]	2.51±0.10 [c]	10.02±2.09 [c]
Group (4)	4.26±0.21 [b]	3.71± 0.12 [b]	11.69±2.70 [b]
Group (5)	5.02±0.23 [a]	2.30± 0.12 [d]	10.13±2.70 [c]

Values are expressed as mean ± SD; n = 6. Mean values in each column having different superscript (a, b) are significant. Means with the same letter are insignificantly different.

The level different of flax seeds powder and extract on serum liver and kidney function thyroid hormones in rats suffering from hypothyroidism in Table (4). The results in Table (4) indicated that (+ve group) recorded highest significantly in AST and ALT levels in comparing to (-ve group). Animals treated with flax seeds powder, extract

and drug groups showed significantly lower of these parameters in compared with (+ve group). The decrease in serum ALT and AST in all rats fed on flax seed me be contains soluble and insoluble dietary fiber, antioxidants, and plant estrogens called lignans, and flaxseeds have many benefits for the liver as they reduce hepatitis, are useful in treating hepatitis B, treat fatty liver, and reduce liver enzymes (**Balić *et al.*, 2020 and Troesch *et al.*, 2020**).

Table (4) showed significantly higher in uric acid, creatinine and urea nitrogen in compared with (-ve group) but illustrated that (+ve group) recorded highest significantly in uric acid, creatinine and urea nitrogen in comparison of (-ve group). While rats treated with flax seeds powder, extract and drug groups showed significantly lower of these parameters in compared with (+ve group). The flaxseed oil has also shown preventive effect against kidney dysfunction which is a chemotherapy agent (**Al-Okbi *et al.*, 2014**). Further, **Troesch *et al.*, (2020)** have suggested that the omega-3 fatty acids in these oils protect against liver and kidneys toxicity induced by acetaminophen overdose.

Table (4): Effect of flax seeds powder and extract on serum liver and kidney function in rats suffering from hypothyroidism

Parameters / Groups	AST (U/L)	ALT (U/L)	Uric acid mg/dl	Creatinine mg/dl	Urea Nitrogen mg/dl
Group (1): -ve	43.82±2.11 c	22. 51±1. 78 d	1.41±0. 4 d	1.34±0. 54 d	35.99± 2.91 d
Group (2): +ve	64.07±5.58 a	46.36±3. 27 a	2.99±0. 62 a	3.91±0.47 a	62.96±3.57 a
Group (3)	45.08±4.28 bc	35. 99±3.43 b	2.58±0.16 b	2.47±0.17 b	56.39±4. 48 b
Group (4)	49.82±3.02 b	26. 73±2.14 c	1.81±0. 80 bc	2.00±0.25 b	40.25±3.38 c
Group (5)	45.08±4.13 bc	25. 13±3.43 c	1.79±0. 70 c	1.85±0.25 c	37.77±3.38 d

Values are expressed as mean ± SD; n = 6. Mean values in each column having different superscript (a, b) are significant. Means with the same letter are insignificantly different.

The obtained results in table (5) illustrated that (+ve group) significantly increase in TC, TG, LDL-c, and VLDL-c , but showed significant decrease in HDL-c compared with (-ve group). The groups treated with treated with flax seeds powder, extract and drug groups showed significant decrease in these previous parameters while, significant increase in HDL-c compared with the (+ve group).

The results were agreed with those obtained by **García-Lafuente *et al.*, (2012)** indicating that, the nutritional intervention could produce considerable benefits in terms of the health of menopausal women and

has an effect in reduction of TC, HDLc and LDLc. Because they are using antioxidant compounds such as (e-vanillic and catechein).

Assmann and Gotto, (2014) concluded that, HDL particles exhibit anti-oxidant, anti-inflammatory, anti-thrombotic and vasodilatory activities. **Kim and Choi, (2015)** reported that, flaxseed is rich in omega 3 fatty acid that is responsible for its ability to improvement in blood lipids, reduce bad cholesterol (LDL), triglycerides (TG) and blood pressure. It also fights inflammatory reactions in the body. **Vijaimohan *et al.*, (2016)** showed that, flax seed reduce triacylglycerol levels, blood cholesterol and decreases total cholesterol and low-density lipoprotein (LDL) cholesterol.

Table (5): Effect of flax seeds powder and extract on serum lipid profile in rats suffering from hypothyroidism

Parameters / Groups	TC	TG	LDL-c	HDL-c	VLDL-c
	(mg/dl)				
Group (1): -ve	75.488 ^{e} ±3.856	45.136 ^{e} ±2.288	18.152 ^{e} ±0.718	48.309 ^{a} ±2.825	9.027 ^{e} ±0.457
Group (2): +ve	125.462 ^{a} ±3.884	75.424 ^{a} ±4.151	84.177 ^{a} ±1.182	26.200 ^{e} ±2.831	15.085 ^{a} ±0.830
Group (3)	105.477 ^{b} ±4.028	64.324 ^{b} ±3.083	59.584 ^{b} ±1.766	33.028 ^{d} ±2.767	12.864 ^{bc} ±0.617
Group (4)	89.269 ^{c} ±4.054	51.194 ^{c} ±3.456	42.067 ^{c} ±0.937	36.963 ^{c} ±2.755	11.239 ^{c} ±0.692
Group (5)	81.114 ^{d} ±3.644	47.188 ^{d} ±2.795	35.076 ^{d} ±2.386	40.601 ^{b} ±1.748	10.438 ^{d} ±0.559

Values are expressed as mean ± SD; n = 6. Mean values in each column having different superscript (a, b) are significant. Means with the same letter are insignificantly different

In conclusion, the results of this study increased the current knowledge of the bioactive components of flaxseed powder and extract. Therefore, dietary intake of flaxseed powder and its extract may be beneficial for the patients suffering from thyroid hormones.

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