Available online at www.jpsscientificpublications.com

Life Science Archives (LSA)

ISSN: 2454-1354

Volume – 3; Issue - 2; Year – 2017; Page: 1035 – 1049

DOI: 10.22192/lsa.2017.3.2.10

Research Article

EVALUATION OF THE PROTECTIVE ROLE OF SEEDS OIL OF *Linum usitatissimum* ON OXIDATIVE STRESS OF PHYSIOLOGICAL PARAMETERS AND SOME ASPECT OF HEART TISSUE IN HYPERTHYROIDISM FEMALE RABBITS

Muna H. AL - Saeed* and Abdullah Anas

Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Abstract

Oil seeds of *Linum usitatissimum* are exhibited numerous interesting pharmacologic activities, very potent antioxidant. Our study is to investigate the therapeutic effect of oil of seeds *Linum usitatissimum* against levothyroxin sodium –induced hyperthyroidism in female rabbits. The rabbit was used as a model to study the effects of hyperthyroidism induced with supra - physiologic doses of L - thyroxin sodium (L - T_4). Endocrine aspects of the thyroid in the pituitary - thyroidaxis have been studied extensively, but few controlled studies have been conducted on females with hyperthyroidism. Thirty two female rabbits were divided randomly into four groups. Group 1: Rabbits received orally administration of normal saline (3 ml) for 30 days (as served control group). Group 2: Rabbits received orally administration of L -thyroxin sodium at dose 50 µg/kg B.W/day dissolve in 3 ml normal saline for 10 days for induced hyperthyroidism. Group 3: Rabbits received orally administration of L -thyroxin sodium at dose 50 µg/kg B.W/day dissolve in 3 ml normal saline for 10 days for induced hyperthyroidism and treated with oil of *Linum usitatissimum* (1 ml/kg B.W) for 20 days. Group 4: Rabbits received orally administration of oil of *Linum usitatissimum* (1 ml/kg/B.W) only for 30 days. The experimental results revealed that hyperthyroid rabbits had significant decrease ($P<0.05$) body weight and body weight gain, TSH, haematological parameters such as RBCs, Hb, PCV, WBC, Neutrophils and total protein levels while significant increase ($P<0.05$) in serum level of T3, T4, Basophils %, Eosinophils, Monocyte %, lipid profile, glucose concentration and urea. Histological sections showed that the changes of thyroid, heart, liver and kidney was moderately depressed in hyperthyroid female rabbits. Oil of *Linum usitatissimum* treatment suppresses the hyperthyroidism - induced oxidative damage. These results suggest that experiment is accompanied with increased oxidative aggressions. A therapeutic effect of oil of *Linum usitatissimum* on oxidative stress in hyperthyroidism female rabbits induced by excessive administration of thyroid hormones were detected and for the first time antithyroid activity were observed.

Article History

Received : 10.04.2017; *Revised* : 26.04.2017

Accepted: 05.05.2017

Key words: Oil seeds, *Linum usitatissimum*, Hyperthyroidism and Rabbit.

1. Introduction

Endocrine system is the second key regulator of organ system functions after nervous system in animal body. Hormones are actual messengers in endocrine signaling. Thyroid is a part of the hypothalamus – pituitary thyroid axis

LSA ISSN: 2454-1354

(HPT axis). Thyroid - stimulating hormone (TSH) is secreted by the anterior pituitary. Thyrotropin - releasing hormone (TRH) from the hypothalamus binds to its receptors at the pituitary to control release of TSH. The TSH binds to the TSH receptor on thyroid epithelial cells to signal thyroid gland secrete triiodothyronine T_3 and thyroxin T_4.

Thyroid gland holds a critical place in controlling brain and somatic development in infants and metabolic activities in adults. Upon stimulation by thyroid stimulating hormone (TSH), thyroid gland secretes thyroid hormones: T_3 and T_4. Although, thyroid hormones have a central role in controlling basal metabolic rate, growth, as well as the development and differentiation of many cells in the body (Wagner *et al*., 2008). Thyroid gland activity may be affected by natural compound such as seed oil of *Linum usitatissimum* is one of the most important special tyoils, which contains high levels (51 – 55 %) of α-linolenic acid (n-3 and ω-3 fatty acid). Flaxseed, also known as linseed, is derived from the flax plant (*Linum usitatissimum*), of the family Linaceae, which was cultivated worldwide for its fiber and oil. Flaxseed contains 6 % mucilage or soluble fibers, insoluble fibers 18 %, 25 % proteins and 30 – 40 % oil, with alpha-linolenic acid (ALA) making up about 50 – 60 % of the total fatty acids. ALA is a precursor of omega-3 fatty acids, which makes flaxseed the leading source of plant - derived omega - 3. Several experimental and clinical studies have demonstrated that ALA reduces total cholesterol, coronary heart diseases and colon cancer (Barcelo Coblijn and Murphy, 2009).

Alpha - linolenic acid has been widely reported to have many beneficial effects on blood lipid profile, blood pressure, cancer, skin diseases and immune disorders such as renal failure, rheumatoid arthritis and multiple sclerosis (Prasad, 2000; Tzang *et al*., 2009). A number of investigations have demonstrated that the diet supplemented with flaxseed oil has profound beneficial health effects in various pathologies. Likhodii *et al*. (2000) reported that flaxseed oil has been shown to slow the rise in blood glucose levels. Flaxseed oil in rats fed high cholesterol diet resulted in significant decrease in serum total cholesterol, LDL, VLDL and triglyceride levels as compared to control rats. Rasmy (2007); EL-Sahar and Abde EL-Rahman (2014); Hussein *et al*. (2014); Rahman *et al*. (2014) and Rangrej *et al*. (2015) reported that replacement of shortening with flaxseed oil from 0 to 50 % level demonstrated an increase in weight, diameter, thickness, spread ratio and it operates directly on thyroid tissue through estrogen receptor and causes hypothyroidism. Flaxseed oil has very high content of alpha - linolenic acid (C18:3 n-3, omega-3 [n-3] fatty acid). Based on the usefulness of n-3 fatty acid in fish oil against cardiovascular diseases, oil of *Linum usitatissimum* is marketed as a health food. The n-3 fatty acid in oil of *Linum usitatissimum* is different than that of fish oil and causes inhibition the activity of thyroid peroxidase (Kandir and Keskin, 2016). Consumption of flaxseeds reduced progression of arterial wall thickening among patients with the highest degree of oxidative stress and the most severe disturbances in their lipid profiles (Gonzalex *et al*., 2007). Actually thyroid peroxidase is essential to normal thyroid function as it catalyze the reactions required for thyroid hormones synthesis. The aim of the study was: (i) To study the relationship between thyroid hormone level and cardiovascular system. Therefore, this study was carried out to investigate: The histological alteration in heartinduced by hyperthyroidism in female rabbits. (ii) A therapeutic effect of seed oil of *Linum usitatissimum* on oxidative stress in hyperthyroidism female rabbits.

2. Materials and Methods

Experimental Animals

In the present study, a total of thirty two adult female local rabbits were obtained from the local market. Rabbits initially weighing 1500-1700 g and seven-month-old were used. Animals were acclimated to holding facilities for two weeks prior to the experiment. The rabbits were housed in groups and kept in room under controlled temperature (24 °C), humidity (30-70 %) and light (12: 12 hr / light: dark). All animals were provided balanced diet throughout the experimental period. This formed of proteins,

fibers, wheat, clover, minerals and many vitamins. Animals were given food and water *ad libitum.*

Experimental Materials

Preparation of Oil seeds of *Linum usitatissimum*

A 50 gm of dried seeds powder was defatted with 500 ml n-hexane for 16 hrs by soxhlet. The combined n-hexane extract was concentrated below 50 °C under reduced pressure in a rotary evaporator to get 10 gm of yellow oil. This oil was dried at room temperature (Harborn, 1984).

Experimental Design

Thirty two adult female rabbits (8 each groups) divided into four groups - 8 each - and treated with oil of *Linum usitatissimum* oral administration for 20 successive days as follows. Group - 1: Rabbits received orally administration of normal saline (3 ml) for 30 days (as served control group), Group - 2: Rabbits received orally administration of L - thyroxin sodium at dose 50 µg/kg B.W/day dissolve in 3 ml normal saline for 10 days for induced hyperthyroidism, Group - 3: Rabbits received orally administration of L - thyroxin sodium at dose 50 µg/kg B.W/ day dissolve in 3 ml normal saline for 10 days for induced hyperthyroidism and treated with oil of *Linum usitatissimum* (1 ml/kg B.W) for 20 days, Group - 4: Rabbits received orally administration oil of *Linum usitatissimum* (1 ml/kg B.W) for 20 days.

Body weight & body weight gain measurement

The animals were weighed before and end period of the experiment.

Sampling

Blood samples

At the end of each experimental period, blood samples were collected from fasted female rabbits (control and treat animals) from the heart by cardiac puncture in heparinized tubes for studied haematological parameters such as RBCs count and RBCs Index ,WNC and differential leukocyte and in plain tubes, and allowed to be clotted at room temperature and put in centrifuge at 5000 rpm to obtain serum for hormonal assay and biochemical analysis such as (Lipid profile, total protein, glucose, Urea AST and ALT) (Reitman and Frankel, 1957).

Hormonal Assay

Serum samples and plasma semen were assayed for TSH, T4, T3, Stradiol and Progesterone using the Enzyme linked immunosorbent assay (ELISA) technique using the Fortress kit.

Histology examination

After removing the thyroid, heart, liver and kidney they were immediately fixed in Bouin's fluid for 12 hrs and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hrs, 95 % alcohol for 2 hrs, 100 % alcohol for 2 hrs, 100 % alcohol for 2 hrs and finally 100 % alcohol for 2 hrs. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hrs using xylene. The tissues were then filtrated in molten Paraffin wax for 2 hrs in an oven at 57 °C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5 µm). The satisfactory ribbons were picked up from a water bath (50 °C – 55 °C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 sec each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 sec and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 sec each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at 40 X, 100 X and 400 X magnifications (Luna, 1993).

Statistical Analysis

The data were analyzed by SPSS software using one way variance analysis ANOVA, Version16. In all tests, a P-value of <0.05 was considered statistically significant (SPSS, 2007).

3. Results

Effect of Oil of *Linum usitatissimum* on Body Weight and Body Weight Gain in Hyperthyroidism Female Rabbits

The results in Table - 1 showed that the effect of oil of seeds of *Linum usitatissimum* on body weight and body weight gain in hyperthyroidism female rabbits. The results were showed significant (p<0.05) decrease body weight and body weight gain in hyperthyroidism male rabbits compared with control group and another groups while that showed significant (p<0.05) increase body weight and body weight gain in hyperthyroidism male rabbits treated with Oil of *Linum usitatissimum* compared with hyperthyroidism female rabbits group and non – significant (p<0.05) increase compared with control group.

Effect of Oil seeds of *Linum usitatissimum* on TSH, T4 and T3 levels in serum Hyperthyroidism Female Rabbits

The results in Table - 2 showed that the effect of oil of *Linum usitatissimum* on TSH, T4 and T3 in serum hyperthyroidism female rabbits. The results were showed significant (P<0.05) increase of T_3 and T_4 in serum hyperthyroidism female rabbits compared with control group and other groups while the result was revealed significant (P<0.05) decrease TSH in serum hyperthyroidism female.

Table - 1: Effect of Oil seeds of *Linum usitatissimum* on Body weight and Body weight Gain in Hyperthyroidism female rabbits

Parameters / Groups	Initial Body weight (G)	Final Body weight (G)	Body weight gain (G)
Control (Normal Saline) 0.9 % NaCl	1500±10.37 A	1700±30.43 A	200 ± 9.36 B
L-Thyroxin sodium (50 µg/kg)	1550±47.39 A	1250±20.31 B	-300 ± 20.35 C
L-Thyroxin + Oil of *Linum usitatissimum* (1 ml/kg)	1655±43.62 A	1935±45.6 A	180 ± 10.42 B
Oil of *Linum usitatissimum* (1 ml/kg)	1670±56.82 A	2110±25.13 A	440 ± 18.51 A

N = Number of animals; A, B, C = differences between groups; P≤0.05 vs. control; Mean ± SD; N = 8

Table - 2: Effect of Oil seeds of *Linum usitatissimum* on TSH, T4 and T3 levels in serum Hyperthyroidism Female Rabbits

Parameters / Groups	TSH (µlU/ml)	T4 (µg/dl)	T3 (ng/ml)
Control (Normal Saline) 0.9 % NaCl	2.36 ± 0.02 A	11.85 ± 0.16 B	1.46 ± 0.07 B
L-Thyroxin sodium (50 µg/kg)	0.41 ± 0.009 B	19.41 ± 1.07 A	2.98 ± 0.032 A
L-Thyroxin + Oil of *Linum usitatissimum* (1 ml/kg)	1.89 ± 0.025 A	13.21 ± 0.43 B	1.61 ± 0.034 B
Oil of *Linum usitatissimum* (1 ml/kg)	2.79 ± 0.036 A	11.91 ± 0.14 B	1.49 ± 0.012 B

N = Number of animals; A, B, C = Differences between groups; P≤0.05 vs. control; Mean ± SDN = 8

Effect of Oil seeds on *Linum usitatissimum* on RBC Count and RBC Index in Hyperthyroidism Female Rabbits

The results in Table - 3 observed that the effect of oil seeds of *Linum usitatissimum* on RBCs count and RBC index in hyperthyroidism female rabbits. The results were showed significant (P<0.05) decrease of RBC count, Hb, PCV and MCV in hyperthyroidism female rabbits compared with control group and other groups while the result was revealed non- significant (P<0.05) change in MCH and MCHC in hyperthyroidism female rabbits compared with control group and other groups.

Table - 3:-Effect of Oil of *Linum usitatissimum* on RBCs and RBC Index in Hyperthyroidism Female Rabbits

Parameters / Treatment	RBC × 10^6/µL	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
Control (Normal saline)	5.51±0.10 A	12.3±0.14 A	47.0±7.80 A	78.6±6.05 A	20.29±4.06 A	32.13±1.25 A
L-Thyroxin sodium (50 µg/kg)	3.01±0.05 B	7.6±0.39 B	24.1±1.46 B	80.2± 7.69 B	16.11±1.38 A	31.15±4.62 A
L-Thyroxin + oil of *Linum usitatissimum* (1 ml/kg)	6.06±0.94 A	12.90±0.52 A	42.3±3.51 A	83.7± 6.74 A	17.25±1.24 A	30.20±3.11 A
Oil of *Linum usitatissimum* (1ml/kg)	6.36±0.13 A	13.7±0.69 A	49.6±7.38 A	79.3± 6.74 A	20.35±5.62 A	33.17±1.81 A

N = Number of animals; A, B, C = Differences between groups; P≤0.05 vs. control; Mean ± SD; N = 8

Effect of Oil seed of *Linum usitatissimum* on WBCs and Differential Leukocyte in Hyperthyroidism Female Rabbits

The results in Table - 4 showed that the effect of oil seeds of *Linum usitatissimum* on WBCs count and differential leukocyte in hyperthyroidism female rabbits. The results were showed significant (P<0.05) decrease of WBC count and Neutrophils % in hyperthyroidism female rabbits compared with control group and other groups while the result was revealed significant (P<0.05) increase in Eosinophils %, Basophils % and Monocytes % in hyperthyroidism female rabbits compared with control group and other groups but the result was revealed non-significant (P<0.05) change in Lymphocyte % in hyperthyroidism female rabbits compared with control group and other groups.

Table - 4: Effect of Oil of *Linum usitatissimum* on WBCs and Differential Leukocyte in Hyperthyroidism Female Rabbits

Parameters / Treatment	WBC× 10^3/µL	Neutro %	Eosino %	Baso %	Lymph %	Monocyte %
Control (Normal saline)	8.50±0.34 A	56.7±7.8 A	0.33±0.31 B	0.12±0.03 C	40.5±2.59 A	2.4±0.09 C
L-Thyroxin sodium (50 µg/kg)	2.7 ±0.01 C	45.1±6.2 B	3.0±0.49 A	0.36±0.02 A	44.0± 2.81 A	7.7±1.03 A
L-Thyroxin+ Oil of *Linum usitatissimum* (1 ml/kg)	6.1±0.02 B	51.5±4. 3 A	0. 30±0.41 B	0.26±0.03 B	43.05±10.6 A	5.5±0.52 B
Oil of *Linum usitatissimum* (1 ml/kg)	8.9±0.26 A	57.0±9.03 A	0. 20±0.53 B	0.13±0.01 C	40.7±10.6 A	2.0±0.80 C

N = Number of animals; A, B, C = Differences between groups, P≤0.05 vs. control; Mean ± SD; N = 8

Effect of Oil of *Linum usitatissimum* on Biochemical Analysis in serum Hyperthyroidism Female Rabbits

The results in Table - 5 showed that the effect of oil seeds of *Linum usitatissimum* on biochemical analysis in hyperthyroidism female rabbits. The results were showed significant (P<0.05) increase of glucose concentration and urea concentration, AST and ALT level while the results of total protein levels revealed significant (P< 0.05) decrease in serum hyperthyroidism female rabbits. Also the results were showed significant (P<0.05) decrease of glucose concentration and urea concentration in serum hyperthyroidism female rabbits treated with oil of *Linum usitatissimum*.

Effect of oil of *Linum usitatissimum* on Lipid profile in serum Hyperthyroidism Female Rabbits

The results in Table - 6 showed that the effect of oil of *Linum usitatissimum* on lipid profile in hyperthyroidism female rabbits. The results were showed significant (P<0.05) increase of total cholesterol, triglyceride, LDL and VLDL in serum hyperthyroidism male rabbits while revealed significant (P< 0.05) decrease HDL in serum hyperthyroidism female rabbits compared with control group and other groups.

Table - 5: Effect of Oil of *Linum usitatissimum* on Biochemical Analysis in Serum Hyperthyroidism Female Rabbits

Parameters / Groups	Total protein mg/dl	Glucose mg/dl	Urea mg/dl	AST U/L	ALT U/L
Control (Normal Saline) 0.9 % NaCl	65.13 ± 7.11 A	98.5 ± 5.63 B	33.57±2.08 B	17.4±3.8 C	16.25±8.47B
L - Thyroxin sodium (50 µg/kg)	41.27 ± 8.69 B	259.57±15.4 *A*	60.35± 9.2 A	65.42±9.86 A	48.21±10.8 A
L – Thyroxin + Oil of *Linum usitatissimum* (1 ml/kg)	60.49 ±9.42 A	140.63± 9.12 B	38.53± 8.19 B	30.43±7.52 B	25.47±9.31B
Oil of *Linum usitatissimum* (1 ml/kg)	67.16 ±6.34 A	89.75 ± 7.13 B	32.11± 2.83 B	18.97±4.83 C	17.67±6.93B

N = Number of animals; A, B, C = Differences between groups, P≤0.05 vs. control; Mean ± SD; N = 8

Table - 6: Effect of Oil of *Linum usitatissimum* on Lipid profile in serum Hyperthyroidism Female Rabbits

Parameters / Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	DL mg/dl
Control (Normal Saline) 0.9 % NaCl	198.70 ± 28.1 B	115.60 ±24.32 B	89.42 ±56.29 A	58.7±2.5 B	45.6 ±7.11 B
L-Thyroxin sodium (50 µg/kg)	338.96 ±70.8 A	198.32 ±32.19 A	67.28 ± 43.27 B	89.1±6.3 A	67.2±8.21 A
L-Thyroxin + Oil of *Linum usitatissimum* (1 ml/kg)	219.37±52.90 B	120±17. 32 B	93.16 ± 24.97 A	64.74± 3.1 B	52.92±11.45 B
Oil of *Linum usitatissimum* (1 ml/kg)	150.19±27.86 C	95.20±15.84 B	100.96±5 8.21 A	59.35 ± 7.24 B	48.39±15.57 B

N = Number of animals; A, B, C = Differences between groups; P≤0.05 vs. control; Mean ± SD; N = 8

Histopathological changes

Thyroid gland

Thyroid gland of control female rabbit showed normal architecture, thyroid follicles, filled with colloid lined by cuboidal thyrocytes parafollicular cells while thyroid gland of rabbit treated with L-T_4 showing hyperatrophied follicular cells and depletion of parafollicular cells, almost microfollicles hyperplasia, some follicle present a variety in size of thyroid follicles but thyroid gland of female rabbit treated with L-T4 + Oil seeds of *Linum usitatissimum*. Showing colloid - rich uniform thyroid follicles are lined by a layer of cuboidal epithelial cells (thyrocyte) and parafollicular cells can be distinguished.

Heart

Heart of control female rabbits show normal architecture, normal cardiac fiber as shown in Figure - 5. While, the rabbits treated with L-T4 alone revealed histopathological changes in heart as shown in Figure - 6. The changes included edema, RBCs and few neutrophils infiltration between cardiac muscle bundle, in addition to vacuolar degeneration of muscle cells. But, heart of female rabbits treated with oil of *Linum usitatissimum* alone and L-T4 + Oil of *Linum usitatissimum* showed normal architecture cardiac muscle (Figure - 7 and 8). In addition to the heart of rabbits treated with L-T4 + Oil revealed amelioration in the hearts compared to treated with L-T4 alone.

Liver

Liver of control female rabbit showing normal hepatocyte, normal portal vein, sinusoid while liver of rabbit treated with L-T_4 showing irregular arrangement of hepatocyte, enlarged

spaces of sinusoid, occasional foci of inflammatory cells, minimal diffuse vacuolation of hepatocytes but Liver of female rabbit treated with L -T_4 + Oil seed of *Linum usitatissimum* and Oil alone showing normal hepatocyte normal central hepatic vein and sinusoid.

Kidney

As shown in Figure – 13, kidneys of control female rabbits show normal cardiac muscle, normal renal cortical tubules and normal epithelial cells lining of the renal tubules. While, the rabbits treated with L-T4 alone revealed histopathological changes in kidneys as shown in Figure - 14. The changes included infiltrations of inflammatory cells, vascular congestion and narrowed Bowman's space, glomeruli with high cellularity, cystic renal cortical tubules. But, kidneys of female rabbits treated with Oil of *Linum usitatissimum* alone and L-T4 + Oil of *Linum usitatissimum* showed normal architecture (Figure - 15 and 16). In addition to the kidneys of rabbits treated with L-T4 + Oil revealed amelioration in the kidneys compared to treated with L-T4 alone.

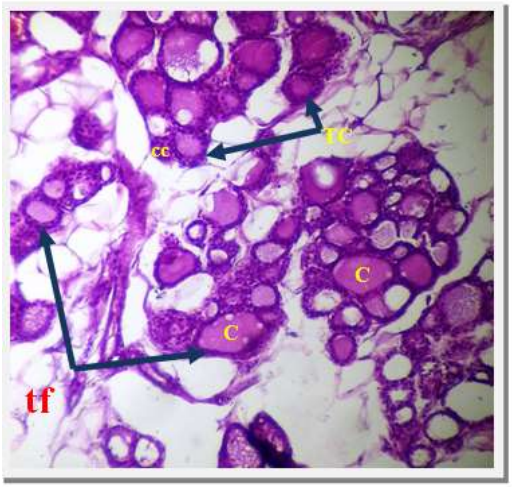


Figure - 1: Thyroid gland of control female rabbit. Showing normal architecture, thyroid follicles (tf), filled with colloid (C) lined by cuboidal thyrocytes (TC) (arrow), parafollicular cells (CC) stain (H & E). 200 X

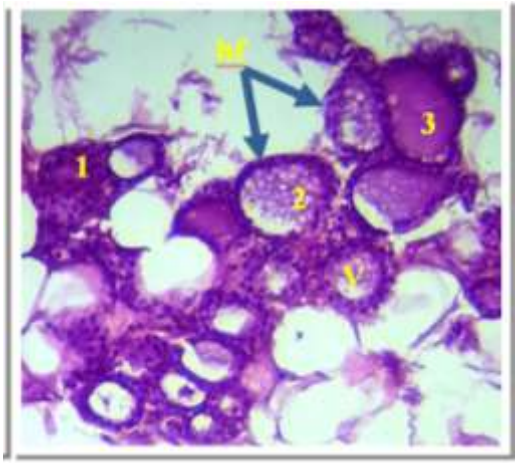


Figure - 2: Thyroid gland of female rabbit treated with L-T4. Showing hyperatrophied follicular cells (hf) and vacuolation colloid (V) of some follicle, depletion of parafollicular cells, almost microfollicles hyperplasia (mf), some follicle present a variety in size of thyroid follicles (tf) (1, 2, 3), stain (H & E) 200 X.

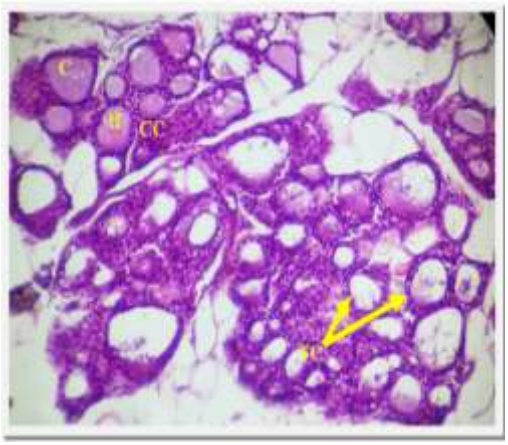


Figure - 3: Thyroid gland of female rabbit treated with L-T4 + Oil of *Linum usitatissimum*. Showing colloid – rich (C) uniform thyroid follicles (tf) are lined by a layer of cuboidal epithelial cells (thyrocyte) (TC) and parafollicular cells (CC) can be distinguished, stain (H & E) 200 X.

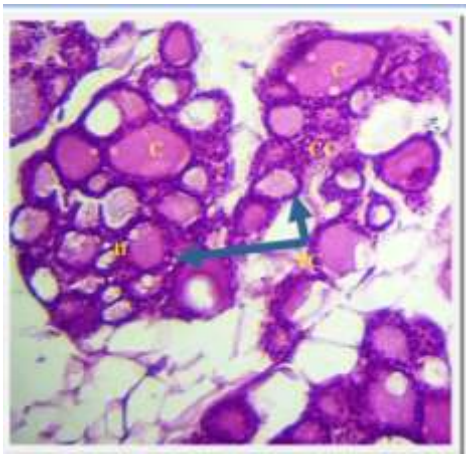


Figure - 4: Thyroid gland of female rabbit treated with Oil of *Linum usitatissimum*. Showing colloid – rich (C) uniform thyroid follicles (tf) are lined by a layer of cuboidal epithelial cells (thyrocyte) (TC) and parafollicular cells (CC) can be distinguished, stain (H & E) 200 X

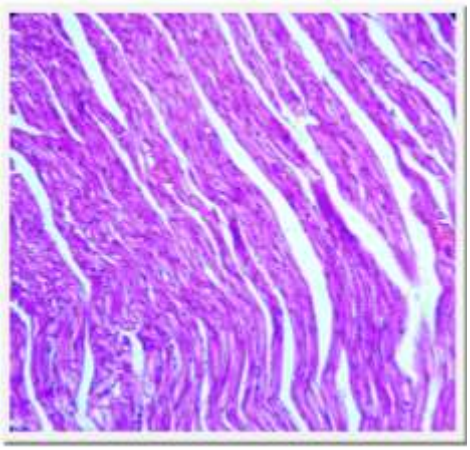


Figure - 5: Cardiac muscle of female rabbits of control group. Showing no clear lesions (H & E) stain 200 X

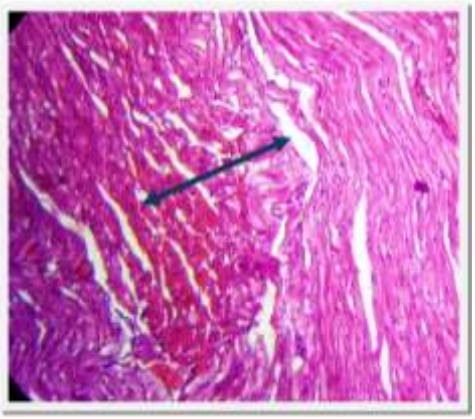


Figure - 6: Cardiac muscle of female rabbit treated with L-T4. Showing edema, RBCs and few neutrophils infiltration between cardiac muscle bundle in addition to vacuolar degeneration of muscle cells (H & E stain 200X

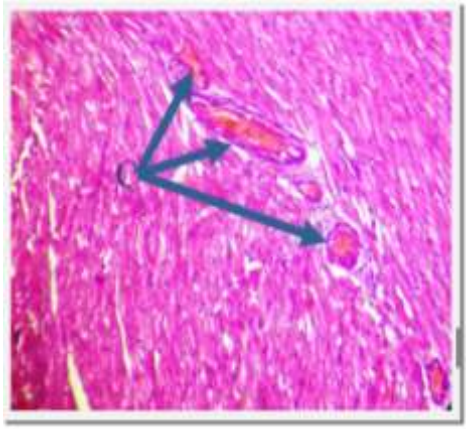


Figure - 7: Cardiac muscle of female rabbit treated with L4 + Oil of *Linum usitatissimum*. Note: congestion (C) and no clear lesions (H & E stain 200 X)

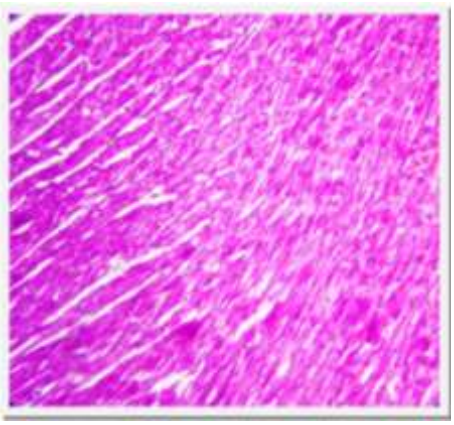


Figure - 8: Cardiac muscle of female rabbits of treated with of *Linum usitatissimum*. Showing no clear lesions (H & E) stain 200 X

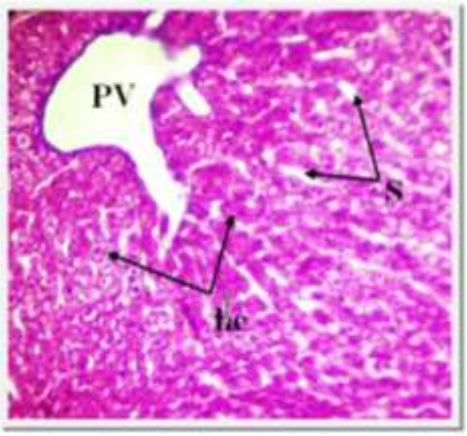
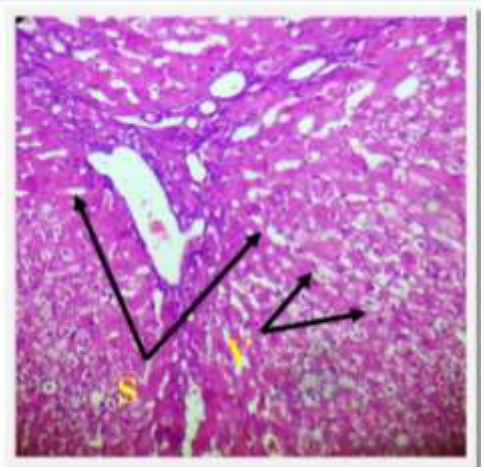


Figure - 9: Liver of control female rabbit. Showing normal hepatocyte (hc), normal portal vein (PV), siunsiod (S), stain (H & E) 200 X



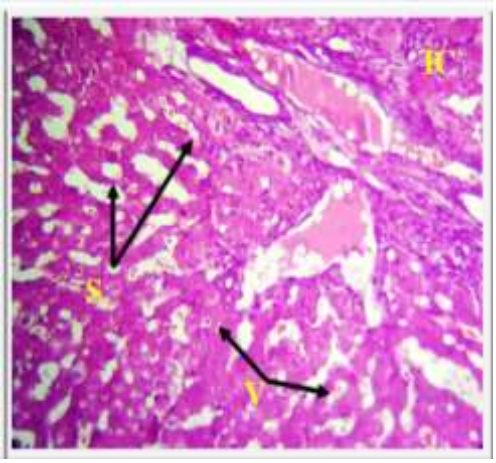


Figure - 10: Liver of female rabbit treated with L - T4. Showing irregular arrangement of hepatocyte, enlarged spaces of sinusoid (S), necrosis of hepatocyte, fibrosis and occasional foci of inflammatory cells (IC), minimal diffuse vacuolation of hepatocytes (V), stain (H & E) 200 X

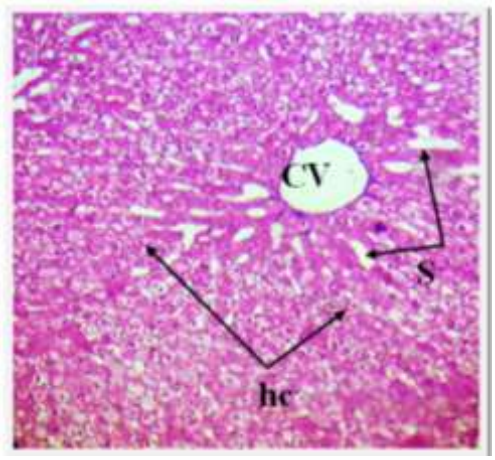


Figure - 11: Liver of female rabbit treated with L-T4 + Oil of *Linum usitatissimum*. Showing normal hepatocyte (hc) normal central hepatic vein (CV), siunsiod (S), stain (H & E) 200 X

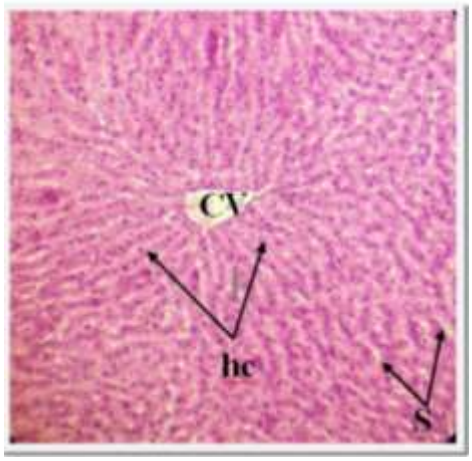


Figure - 12: Liver of female rabbit treated with Oil of *Linum usitatissimum*. Showing normal hepatocyte (hc) normal central hepatic vein (CV), siunsiod (S), stain (H & E) 200 X

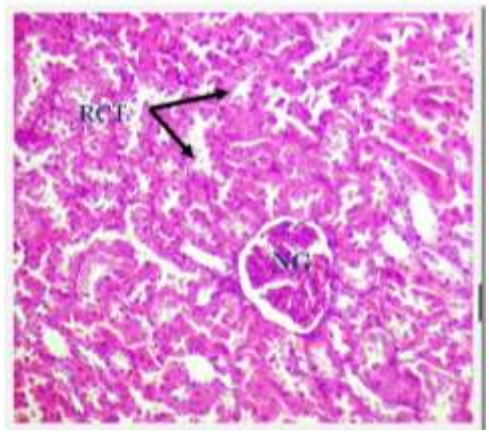


Figure - 13: Kidney of control female rabbit. Showing normal glomeruli (NG) and normal renal cortical tubules (RCT), stain (H & E) 200 X

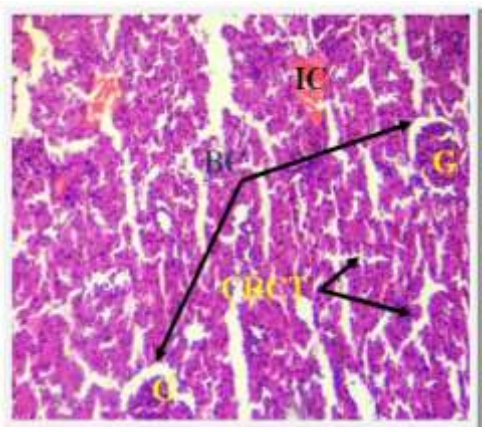


Figure - 14: Kidney of female rabbit treated with L-T4. Showing infiltrations of inflammatory cells (IC), vascular congestion and narrowed Bowman's space between Bowman's Capsule (BC)and glomeruli , glomeruli (G) with high cellularity ,cystic renal cortical tubules(CRCT), stain(H&E) 200 X

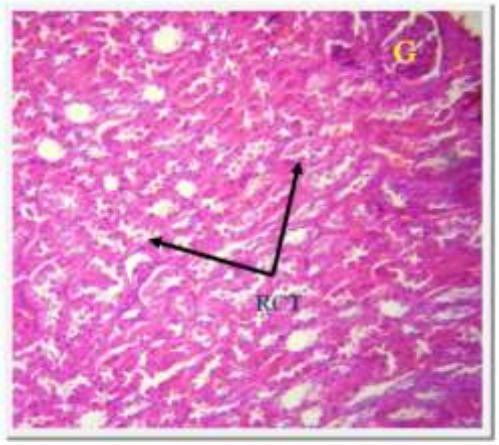


Figure - 15: Kidney of female rabbit treated with L-T4 + Oil of *Linum usitatissimum*. Showing normal glomeruli (G) and normal renal cortical tubules (RCT), stain (H & E) 200 X

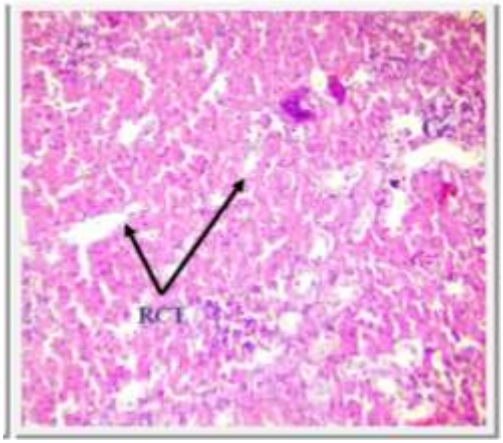


Figure - 16: Kidney of female rabbit treated with Oil of *Linum usitatissimum*. Showing normal glomeruli (G) and normal renal cortical tubules (RCT), stain (H & E) 200 X

4. Discussion

In the present study, an increased serum T_3 and T_4 levels and decreased in TSH levels were observed in the hyperthyroid animals induced by thyroxine. In this respect, the results of our study appear to be consistent with the findings of others (Halani *et al*., 2007; Solati *et al*., 2007; Suchetha *et al*., 2011; Salwa *et al*., 2011). Also, the TSH level was significantly lower in the hyperthyroid group compared to the control group and the histological changes of thyroid glands indicate this result. The mechanisms behind the oil seeds of *Linum usitatissimum* induced reduction in thyroid hormone are not clear. Possibilities include oil seeds of *Linum usitatissimum* induced modulation in deiodination system, which affects deiodinase activity through its antioxidant properties. Based on the results obtained, it can be concluded that the hyperthyroid group, which received oil seeds of *Linum usitatissimum*, shows a significantly different decrease of plasma T_3 and T_4 levels and significantly different increase of TSH levels. Pharmacological antioxidants may have an effect on the peripheral conversion of thyroid hormones byway of deiodination or mechanism of cell membrane defence, the integrity of which may have an effect on the activity of deiodinases (Fernandex and Videla, 1995; Mogulkoc *et al*., 2005; Subudhi *et al*., 2008).

The present study revealed a decrease in body weight and body weight gain in hyperthyroidism male rabbits compared control. This result agrees with Soukup *et al*. (2001) the likelihood of weight loss occurring was related to the severity of the overactive thyroid. Thus, if the thyroid is extremely overactive, the individual's BMR increases which leads to increased caloric requirements to maintain that weight. If the person does not increase the calories consumed to match the excess calories burned, then weight loss will ensue. As indicated earlier, the factors that control our appetite, metabolism, and activity are very complex and thyroid hormone is only one factor in this complex system. Nevertheless, on average the more severe the hyperthyroidism, the greater the weight loss observed. Weight loss is also observed in other conditions where thyroid hormones are elevated, such as in the toxic phase of thyroiditis and if one is on too high a dose of thyroid hormone pills. Morris (1999) who showed that the rats treated with LT4 were lost body weight and attributed that to catabolic effect of L-T4. Al-Bishri (2013) who also noticed that the L-T4induce reductions in bone mineral mass and reduce of growth because increase metabolic catabolism while significant effect administration of oil and genestin extract of soybean seeds increase in body weight and body weight gain due to increases metabolic processes in body (Cunnane *et al*., 1993).

The present study revealed that the hyperthyroidism affect on haematological parameters such as decrease RBC, Hb, PCV, MCV, WBC, Neutrophils %, while lead to

increase Basophils %, Eosinophils % and Monocytes % attributed to elongation of the treatment duration with L-T4 could lead to changes on some hematological parameters [11]. The oil exhibited significant elevation of haematological parameters such as RBC, Hb, PCV, MCV, WBC, Neutrophils (Al – Bishri, 2013). The present study revealed that the hyperthyroidism affect on biochemical parameters such as increase in levels of glucose, cholesterol, triglyceride, low density lipoprotein (LDL-), ALT, AST and decrease (HDL-) high density lipoprotein (Table – 5 and 6). The oil exhibited significant reduction of serum cholesterol level in hyperthyroidism female rabbits. The abnormal high concentration of serum lipids in the hyperthyroid rabbits is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots (Zhao *et al*., 2004). Maintenance of serum cholesterol profile indicates that oil of *Linum usitatissimum* may exert their role in maintenance (Prasad, 2000). Oil seeds of *Linum usitatissimum* treatment decreased the elevated glucose concentration significantly ($P<0.05$) in treated hyperthyroid rabbits; however, their glucose concentrations were still significantly higher ($P<0.05$) than those of the control group. A reduction in the serum glucose levels of the groups treated with oil *Linum usitatissimum* were observed in this work because of oil seeds of *Linum usitatissimum* lead to stimulate insulin secretion when insulin is increased it leads to decrease glucose concentration. This result is in agreement with Kristensen *et al*. (2013), who found that the high flaxseeds diet increases insulin secretion. This result is attributed often to estrogen receptor agonism by isoflavones thus used to regulate glucose absorption and elevation in diabetes (Ahmad *et al*., 2012).

The effects of oil seeds of *Linum usitatissimum* on serum lipids and hypercholesterolemic atherosclerosis are not known. An investigation, therefore, was made of flaxseed oil on high - cholesterol diet - induced atherosclerosis, serum lipids (triglycerides, total cholesterol, high - density lipoprotein cholesterol, low - density lipoprotein cholesterol, risk ratio of total cholesterol to high - density lipoprotein cholesterol), serum and aortic malondialdehyde, an index of levels of reactive oxygen species, aortic chemiluminescence (a measure of antioxidant reserve), and reactive oxygen species - producing activity of white blood cell chemiluminescence in rabbits. Previous studies have suggested that hyperthyroidism increased free radical production and lipid peroxidation levels (Fernandex and Videla, 1995; Mogulkoc *et al*., 2005; Subudhi *et al*., 2008). Hyperthyroidism accelerates ROS generation and produces changes in the antioxidant systems of various tissues (Lemay *et al*., 2002). The cellular GSH plays an important role as biological antioxidant defense systems, which act as protective mechanisms against oxidative damage, therefore, the decreased level of GSH may be due to overproduction of free radicals and increased lipid peroxidation in hyperthyroidism (Suchetha *et al*., 2011). In our study, serum GSH levels were decreased in hyperthyroid animals as compared to control animals, possibly secondary to increased ROS generation.

Previous reports showed that both hyper- and hypothyrodisim are associated with increased oxidative stress (Mohamed *et al*., 2009). In cases of thyrotoxicosis, part of sustained injury to various body tissues is attributed to oxidative damage therefore can be inhibited effect of excess of oxidative stress by treatment of oil of *Linum usitatissimum*. Recently, there is great evidence that oil of *Linum usitatissimum* prevents oxidative injury by modulating the expression of antioxidant enzyme systems (Ahmad *et al*., 2012). The present study referred that the effect of oilseeds of *Linum usitatissimum* on hormones shows the decreased levels in T3, T4 and increase TSH. This changes in levels of hormones could be attributed to oil of *Linum usitatissimum* antioxidant and free radical scavengers (Babu *et al*., 2000; Clark *et al*., 2001; Resch *et al*., 2002). And may be oil of *Linum usitatissimum* play a regulatory role in antioxidant. Because oil seeds of *Linum usitatissimum* exhibit a wide range of biological effects, including antioxidant and enzyme - modulating action and anti-allergic, antiatherosclertic, antithrombotic,

antiviral, anticarcinogenic, antispasmodic, and diuretic effect.

4. References

1) Ahmad, N., Rahman, Z. U., Akhtar, N and Ali, S. 2012. Effects of aqueous methanolic extract of Flax seeds (*Linum usitatissimum*) on serum estradiol, progesterone, kidney and liver functions and some serum biochemical metabolites in immature female rats. *Pakistan Veterinary Journal*, 32 (2): 211 - 215.
2) Al-Bishri, W. M. 2013. Favorable effects of flaxseed supplemented diet on liver and kidney functions in hypertensive Wistar rats. *Journal of Oleogical Science*, 62(9): 709 - 715.
3) Babu, U. S., Mitchell, G. V., Wiesenfeld, P., Jenkins, M. Y and Gowda, H. 2000. Nutritional and hematological impact of dietary flaxseed and defatted flaxseed meal in rats. *International Journal of Food Science and Nutrition*, 51 (2): 109 - 117.
4) Barcelo Coblijn, G and Murphy, E. J. 2009. Alphalinolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Progress in Lipid Research*, 48: 355 - 374.
5) Clark, W., Kortas, C., Heidenheim, A., Garland, J., Spanner, E and Parbtani, A. 2001. Flaxseed in lupus nephritis: a two year non-placebo controlled cross over study. *Journal of American Colloidal Nutrition*, 20: 143 - 148.
6) Cunnane, S. C., Ganguli, S. and Menard, C. 1993. High – linolenicacid flaxseed (*Linum* usitatissimum): some nutritional propertiesin humans. *Brazilian Journal of Nutrition*, 49: 443 - 453.
7) EL - Sahar, E. G. E and Abed EL-Rahman, A. M. M. 2014. Study on the biological effect of use flaxseed oil as a source of fat on the Biomarkers of experimental rats. *Journal of American Science*, 10: 116 - 123.
8) Fernandez, V and Videla, L. A. 1995. Influence of hyperthyroidism on superoxide radical and hydrogen peroxide production by rat liver submitochondrial particles. *Free Radicals Research Community*, 18: 329 – 335.
9) Gonzalez, S., Jayagopal, V., Kilpatrick, E. S., Chapman, T and Atkin, S. L. 2007. Effects of isoflavone dietary supplementation on cardiovascular risk factors in type 2 diabetes. *Diabetes Care*, 30: 1871 – 1873.
10) Halani, C. G., Dewani, A. P., Kale, K., Umathe, S. N and Bhusar, I. M. 2007. Antioxidant and antithyroid in oxidative stress in diverse thyroid states. *International Journal of Pharmacology and Biological Sciences*, 1 (3): 5 - 8.
11) Harborn, J. B. 1984. Phytochemical methods. 2nd Ed., Chapman and Hall, New York, USA.
12) Hussein, S. A., El-Senosi, Y. A., Ragab, M. R and Hammad, M. M. F. 2014. Beneficial effect of flaxseed oil on lipid metabolism in high cholesterol diet fed rats. *Banha Veterinary Medical Journal*, 27: 290 - 301.
13) Kandir, S and Keskin, E. 2016. Effects of hypothyroidism and hyperthyroidism on hematological parameters in rats. *Journal of American Science*, 63: 371 - 376.
14) Kristensen, M., Savorani, F., Christensen, S., Engelsen, S. B., Bugel, S and Toubro, S. 2013. Flaxseed dietary fibers suppress postprandial lipemia and appetite sensation in young men. *Nutrition Metabolism and Cardiovascular Diseases*, 23 (2): 136 - 143.
15) Lemay, A., Dodin, S., Kadri, N., Jacques, H and Forest, J .C. 2002. Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterol emicmenopausal women. *Obstet Gynecology*, 100: 495 – 504.
16) Likhodii, S. S., Musa, K., Mendonca, A., Dell, C., Burnham, W. M and Cunnane, S. C. 2000. Dietary Fat, Ketosis and Seizure Resistance in Rats on the Ketogenic Diet. *Epilepsia*, 41: 1400 - 1410.

17) Luna, L. G. 1993. Manual of histology staining methods of the Armed Forces Institute of Pathology. 3rd ed., New York, Mcgraw Hill.
18) Mogulkoc, R., Baltaci, A. K., Oztekin, E., Aydin, L and Tuncer, L. 2005. Hyperthyroidism causes lipid peroxidation in kidney and testis tissues of rats: protective role of melatonin. *Neuro Endocrinology Letters*, 26: 806 – 810.
19) Mohamed, R. A., Ramadan, R. S and Ahmed, L. A. 2009. Effect of substituting pumpkin seed protein isolate for casein on serum liver enzymes, lipid profile and antioxidant enzymes in CCL4 - intoxicated rats. *Advanced Biology Research,* 3(1-2): 09-15.
20) Morris, J. C. 1999. Hyperthyroidism from toxic nodules and other causes. In alts of clinical endocrinology, Vol. 1: Thyroid diseases Surks, M. I. Philadelphia, P.A. Current Medicine.
21) Petrulea, M. S., Duncea, A and Resan, M. 2009. Thyroid hormones in excess induce oxidative stress in rats. *Acta Endocrinologica*, 2: 155 – 164.
22) Prasad, K. 2000. Flaxseed: a source of hypocholesterolemic and antiatherogenic agents. *Drug News Perspective*, 13: 99 - 102.
23) Prasad, K. 2000. Oxidative stress as a mechanisms of diabetes in diabetic BBd prone rats: Effect of secoisolariciresinol diglucoside (SDG) isolated from flaxseed. *Molecular Cell Biochemistry*, 209: 89 - 96.
24) Rahman, M. M., Pharm, B., Pharm, M., Mazid, M. A and Islam, S. N. 2014. Hypercholesterolemic effects of fish and vegetable oils on the serum lipid profile of experimentally induced hypercholesterolemic rats. *European Science Journal*, 10: 476 - 482.
25) Rangrej, V., Shah, V., Patel, J and Ganorkar, P. M. 2015. Effect of shortening replacement with flaxseed oil on physical, sensory, fatty acid and storage characteristics of cookies. *Journal of Food Science and Technology*, 52: 3694 - 3700.
26) Rasmy, G. E. 2007. Protective Effect of Linseed Oil on Hyperlipidemia in Experimental Animals. *Journal of Genetic Engineering and Biotechnology*, 5: 9 - 17.
27) Reitman, S and Frankel, S. 1957. Determination, of alanine aminotrans - pherase and aspartate aminotranspherase. *American Journal of Clinical Pathology*, 28: 57 - 63.
28) Resch, U., Helsel, G., Tatzber, F and Sinzinger, H. 2002. Antioxidant status in thyroid dysfunction. *Clinical Chemistry and Laboratory Medicine,* 40: 1132 - 1134.
29) Salwa, H. N., Al-Rubae, I and Abass, K. A. 2011. An evaluation of antioxidants and oxidative stress in Iraqi patients with thyroid gland dysfunction. *African Journal of Biochemistry Research*, 5 (7): 188 - 196.
30) Solati, S. M., Attaei, L and Azizi, F. 2007. Lipid oxidation, antioxidants and paraoxonase enzyme activity in patients with subclinical thyrotoxicosis. *Ghudad –I Darunriz Va Mitabulim Iran*, 8 (4): 317 - 323.
31) Soukup, T., Zacharova, G., Smerdu, V and Jirmanova, I. 2001. Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status. *Physiological Research*, 50: 619 - 626.
32) SPSS Statistical Packages for the Social Sciences. 2007. Statistical software for windows version 13.0 Microsoft. SPSS®, Chicago, IL, USA.
33) Subudhi, U., Das, K., Paital, B., Bhanja, S and Chainy, G. 2008. Alleviation of enhanced oxidative stress and oxygen consumption of L-thyroxine induced hyperthyroid rat liver mitochondria by vitamin E and curcumin. *Chemical Biology Interaction,* 173 (2): 105 - 114.
34) Suchetha, K., Sanda, N and Mamodara, G. 2011. Oxidative Stress in Hypo and Hyperthyroidism. *Al Ameen Journal of Medical Science*, 4 (1): 49 - 55.
35) Tzang, B. S., Yang, S. F., Fu, S. G., Yang, H. C., Sun, H. L and Chen, Y. C. 2009. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. *Food Chemistry*, 114: 1450 - 1455.

36) Wagner, M. S., Wajner, S. M and Maia, A. L. 2008. The role of thyroid hormone in testicular development and function. *Journal of Endocrinology*, 199: 351- 365.
37) Zhao, G., Etherton, T. D., Martin, K. R., West, S., Gillies, P., Kris Etherton, P. 2004. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholestrolemic men and women. *Journal of Nutrition*, 134: 2991 - 2997.

Access this Article in Online

Quick Response Code

Website www.jpsscientificpublications.com
DOI Number DOI: 10.22192/lsa.2017.3.2.10

How to Cite this Article:

Muna H. AL - Saeed and Abdullah Anas. 2017. Evaluation of the protective role of seeds oil of *Linum usitatissimum* on oxidative stress of physiological parameters and some aspect of heart tissue in hyperthyroidism female rabbits. *Life Science Archives*, 3 (2): 1035 – 1049.

DOI: 10.22192/lsa.2017.3.2.10