

STUDY THE EFFECT OF FLAX LIGNAN EXTRACT OF *LINUM USITATISSIMUM* AND CONJUGATED ESTROGEN ON PHYSIOLOGICAL PARAMETERS IN FEMALE RATS

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ABSTRACT

This study was carried out in the animal house of the College of Veterinary Medicine /Basrah University to evaluate the effect of lignan extract of flax seed on haematological, biochemical parameters and histological examination in female rats, compared with conjugated estrogen drug.

For this purpose, eighteen female rats weighed (180-200g) and aged (4-5 months) was divided into 3 equal groups (6 rats / group) for 14 days.

First group: Drenched 1ml of normal saline (0.9% of NaCl)for 14 days.

Second group: Drenched 0.10mg/kg B.W of conjugated estrogen dissolved in normal saline (0.9% of NaCl) for 14 days.

Third group: Drenched 20mg/kg B.W of lignan extract of flaxseeds dissolved in normal saline (0.9% of NaCl)for 14 days.

Blood samples were collected from heart by cardiac puncture from all experimental animals. These samples are used for the measurement of haematological and biochemical parameter as well as hormonal assay. Thyroid glands, liver, kidneys, ovaries and uterus were removed for histopathological study.

The results revealed that, the female rats treated with lignan extract of flax seeds showed an increase in body weight.

The result also showed that treated female rats with phytoestrogen had no significant effect on blood parameters except a significant effect on lymphocytes. Also results revealed that lignan extract treatment caused significant decrease ($P \leq 0.05$) in serum lipid profile except HDL compared to control.



Moreover the results of biochemical parameters were indicated the affected in female rats treated with lignan extract that the revealed a significant ($P \leq 0.05$) decrease in serum lipid profile except HDL-Ch of female rats treated with lignan extract compared with the control group. Also the treatment with lignan extract caused significant ($P \leq 0.05$) increase in LH hormone, estradiol and progesterone compared with .While it has no significant effect on FSH.

The treatment showed some ameliorative effect on histological structure of studied organs.

INTRODUCTION

Phytoestrogens are any plant substance or metabolite that induce biological responses in vertebrates and can modulate the actions of endogenous oestrogens usually binding to estrogen receptors. The phytoestrogens have a similar structure to oestradiol and are able to bind the estrogen receptor (ER), preferably the ER β , although their binding affinity is lower than that of endogenous estradiol. Lignans, a type of phytoestrogens, are constituents of many plants and form the building blocks for the formation of lignan in the plant cell wall.

Lignan is a polymer of aromatic subunits usually derived from phenylalanine. Terrestrial vascular plants may, therefore, have appeared only after the evolution of lignan biosynthesis, because structural support and water transport functions are central to the biology of higher land plants [1].

Linum usitatissimum (Flax seeds), also known as Linseeds, are commonly used as a source of protein supplements in the rations of dairy animals. According to [2], feeding of Flax seeds to dairy cows increased the first service conception rates by 17%. Flax seeds also show many health benefits [3]. Flax seeds have been shown to have antioxidant property [4] and prevent diabetic complications [5], while lignan concentrate extracted from flax seeds showed cardioprotective effects in rats[6] . Chemically, these seeds contain 41% oil, 20% proteins and 28% dietary fiber. They are rich in essential omega-3 fatty acid and polyunsaturated linolenic acid[7].

The primary sources of estrogens during reproductive life are the theca and granulosa cells of the ovaries. The principal and most potent estrogen secreted is 17 β -



estradiol (estradiol). Estrogens are best known for the effect on the female reproductive organs. The principal function is to cause cellular proliferation and growth of the external female sex organs and the uterus. Moreover, changes of the endometrium and Fallopian tubes take place under the influence of estradiol. Further, estradiol has profound effects on osteoblast and -clast activity, as illustrated by the increased prevalence of osteoporosis in postmenopausal women and on cardiovascular function [8-9]. Besides actions in the reproductive tract, blood vessels and bone, estradiol have a major impact on the brain. Animal studies revealed that estradiol is able to modulate almost all neurotransmitter systems, as demonstrated for the serotonergic, dopaminergic, adrenergic, GABA-ergic, and cholinergic system [10]. Moreover, estradiol induces differential limbic activity and has a major influence on HPA-axis function [11]. Estrogen receptors (ERs) belong to the steroid hormone super family of nuclear receptors. It was assumed that there was only one ER. It was thought that this receptor was responsible for mediating all the effects of natural and synthetic estrogens and anti-estrogens. However, in 1996 a second ER was cloned from the c DNA library of the rat prostate [12].

Estrogen replacement therapy (ERT) is widely used for the treatment of peripheral menopausal symptoms, and the prevention of osteoporosis. Since there seems to be a strong relation between mood and hormonal status in women, estrogen replacement therapy has also been used for its antidepressant effect during the menopause [13].

Present study aimed to determine the effect of phytoestrogen extract on physiological performance and to compare the effect of phytoestrogen extract with synthetic estrogen.

MATERIALS AND METHODS

Plant Material.

Lignan had been extracted from flax seeds that were used in this study. The flax seeds was hand-picked from local market with full skin intact. It was washed with tap water ,the skin and fleshes were removed and the seeds are dried. The seeds of the flax were turned to powder with the help of an electric grinder and kept in dark container at 25c°.



Preparation Lignan Extract From Flax Seeds.

Fifty grams of dried flax seeds powder were defatted with (500 ml) of n-hexane for 4 hours by soxhlete. The combined n-hexane extract was concentrated below 50°C under reduced pressure in a rotary evaporator to get 10 ml of yellow oily mass. This mass was dried at room temperature and further (35gm) of flax seeds were refluxed in (500ml) methanol (80%) in water with 3% hydrochloric acid for one hour then filtered by Buchner funnel and filter paper (Wattman No.185). The filtrate was extracted with an equal volume of chloroform to remove pigments. The alcoholic layer was extracted with an equal volume of ethyl acetate treated with 2% of hydrochloric acid, the ethyl acetate layer was concentrated by rotary evaporator at 40°C and dried at room temperature [14]. After hydrolysis, the samples were cooled down to room temperature, and pH was adjusted to 5–6 by adding 10 mol/l sodium hydroxide was extracted for 60 min under constant stirring with 3 ml of methyl-*tert*-butyl ether (MTBE) and ethyl acetate (1:1, v/v), followed by centrifugation (2000 rpm, 10 min) to separate the organic layer. The organic layer was transferred to a separate flask and the remainder was washed twice with 2 ml. The supernatants were combined and the organic extract. The resultant extract (5gm) was brown color and dry material. The extract was kept in dark glass container at 6°C, then dissolved in distilled water each (20mg :1 ml) and given to the rats.



Fig(1): Stages of lignan extract of flaxseeds: flax seeds(1), flax seed powder (2), defatted of flaxseed (3), lignan (4).



Experimental animals:-

Eighteen healthy adult female rats with body weight ranged (180mg-200mg) were brought from the university of Qadisiya. Rats were kept for an adaptation period for 1 month in the animal house of Veterinary Medicine College/ Basrah University. The experimental animals were kept in individual cages, provided with ration composed fodder in addition to annelid protein and pure water and given, these animals were maintained in air-conditioned quarters (24°C) under standard husbandry conditions with alternate 12 hours light /dark.

-Drug and Dilution.

- Conjugated estrogen 0.10mg/kg suspended in 1ml of D.W.
- Lignan extract of flaxseeds 20 mg/kg suspended in 1ml of normal saline.

Experimental Design:-

The animals were divided into three equal groups ,each group(6 female rats /group).

First group: Drenched 1ml of normal saline (0.9% of NaCl)for 14 days.

Second group: Drenched 0.10mg/kg B.W of conjugated estrogens dissolved in normal saline(0.9% of NaCl)for 14 days.

Third group: Drenched 20mg/kg B.Wof lignan extract of flaxseeds dissolved in normal saline(0.9% of NaCl)for 14 days.

Collection of Blood Samples.

Blood samples (7ml) were collected from each animals at end of experiment by the heart (cardiac puncture). The (5ml) of blood was deposited into tube without anticoagulant and then the blood samples were centrifuged at (3000 rpm) for 10 minutes and serum samples stored in polyethylene eppendorff tubes at (-20°C), until used to study biochemical parameters. The remaining (2ml) of blood was deposited into tube with anticoagulant for heamatological analysis. The specimen from thyroid glands, liver, kidneys , ovaries and uterus were taken for histopathological study.



Study parameter:-

-Measurement of body weight.

-Heamatological analysis.

RBC,WBC, Hb, MCV, MCH, MCHC, PCV, differential WBC were measured by count 60 (Genex laboratories, Germany) apparatus.

-Measurement of Lipid Profile:

Total cholesterol measured by using method CHOD-POP, France.

Triglycerides measured by using Triglycerides–liquizyme\GPO-POP, Germany.

HDL-C measured by CHOLESTEROL liquid color test kit. Serum LDL-C

Concentration was calculated according to [15].

$LDL-C = Total\ cholesterol - [(HDL-C) + Triglyceride / 5]$.

The serum very low-density lipoprotein concentration was calculated by dividing

Serum triglyceride by five [16].

$VLDL = Triglyceride / 5$.

Hormonal Assay.

Serum samples assayed for FSH, LH, estradiol and progesterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.[17]

Histological study was done according to [18] and [19].

Statistical Analysis:

The results of the present study were analyzed by using two-way covariance (ANOVA) test in all study. All statistical calculations were carried out by the aid of the statistical package SPSS V. 17 (SPSS Inc.). The data were expressed as means \pm standard deviation ($X \pm SD$). Least significant different test (LSD) was calculated to test difference between means of groups and sub groups [20].



RESULT

1-Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on Body Weight of Virgin Female Rats.

The result in Table(1) revealed significant ($P \leq 0.05$) increase in final body weight of female rats treated with phytoestrogen (lignan) extract compared with the control group and non-significant ($P \leq 0.05$) increase in final body weight of female rats treated with phytoestrogen (lignan) extract compared with the group treated with conjugated estrogen.

Table(1): Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on Weight of Virgin Female Rats. Mean \pm SD , N=6

Parameters Treatment	Initial Body Weight of Female Rats (g)	Final Body Weight of Female Rats (g)
Control(Normal saline)	189 \pm 1.14 NS	200.17 \pm 4.91 B
Synthetic Estrogen (Conjugated Estrogen) (0.10mg/kgB.W.)	195 \pm 17.88 NS	223.50 \pm 8.21 AB
Phytoestrogen (Lignan) 20mg/kg B.W.	200 \pm 7.73 NS	245.17 \pm 20.10 A

N=number of animals., Capital letters denote differences between groups, $P \leq 0.05$ vs. control, NS=non-significant.

2-Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on RBC Counts and RBC Index in Virgin Female Rats.

The obtained results in Table (2) revealed non-significant ($P \leq 0.05$) increase in RBC, Hb, MCH and MCHC of female rats treated with lignin extract compared with the control group and group treated with conjugated estrogen.

The results of PCV% and MCV revealed a significant($P \leq 0.05$) decrease in female treated with conjugated estrogen compared with the control group and group treated with lignan extract while no significant difference were recorded in PCV% and MCV values between the control group and group treated with lignan.



Parameters Treatment	RBC × 10 ⁶ /μL	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %
Control(Normal saline)	6.50±0.31 A	12.56±0.14 A	38.57±1.80 A	59.70±2.09 A	20.80±2.04 A	34.25±1.60 A
Synthetic Estrogen (Conjugated Estrogen) (0.10mg/kgB.W.)	6.2±0.18 A	11.8±0.41 A	33.37±5.46 B	46.98± 7.69 B	18.12±1.74 A	34.30±2.84 A
Phytoestrogen (Lignan) 20mg/kg B.W.	6.96±0.82 A	12.90±0.69 A	40.3±3.51 A	55.7± 6.74 A	19.68± 2.48 A	34.41±3.20 A

Table (2): Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on RBC Counts and RBC Index in Virgin Female Rats. Mean ± SD N=6

N=number of animals., Capital letters denote differences between groups, $P \leq 0.05$ vs. control, NS=non-significant.

3-Effect of Synthetic Estrogen and Phytoestrogen (Lignan) on WBC Counts and Percentage of Differential Count of WBC in Virgin Female Rats.

The obtained results in Table (3) revealed significant ($P \leq 0.05$) increase in WBC in females rats treated with conjugated estrogen compared with phytoestrogen (lignan) treated group and control while non significant difference were observed between lignan and control group.

The results of neutrophils%, basophile % and esonophils%, monocyte showed no significant ($P \leq 0.05$) changes in females rats treated with phytoestrogen (lignan) extract and treated with conjugated estrogen compared with the control group.

The results of lymphocytes% showed significant ($P \leq 0.05$) increase in females rats treated with lignan and treated with conjugated estrogen compared with the control group.



Table (3): Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on WBC Counts and Percentage of Differential Count of WBC in Virgin Female Rats. Mean \pm SD , N=6

Parameters Treatment	WBC \times 10 ³ / μ L	Neutro %	Eosino %	Baso %	Lymph %	Monocyte %
Control(Normal saline)	8.30 \pm 0.58 B	48.7 \pm 5.7 A	0.33 \pm 0.81 A	0.2 \pm 0.63 A	46.41 \pm 2.59 B	5.62 \pm 1.06 A
Synthetic Estrogen (Conjugated Estrogen) (0.10mg/kgB.W.)	11.83 \pm 1.10 A	46.3 \pm 5.2 A	0.4 \pm 0.89 A	0.46 \pm 0.05 A	51.36 \pm 2.81 A	4.31 \pm 1.08 A
Phytoestrogen (Lignan) 20mg/kg B.W.	9. 8 \pm 3.03 B	50.6 \pm 3. 3 A	0. 20 \pm 0.83 A	0.26 \pm 0.05 A	53.05 \pm 10.6 A	5.83 \pm 0.60 A

N=number of animals., Capital letters denote differences between groups,P \leq 0.05 vs. control, NS=non-significant.

4-Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on Lipid Profile in Virgin Female Rats.

The obtained results in Table (4) revealed significant (P \leq 0.05) decrease of total cholesterol, triglyceride, LDL and VLDL in serum of females rats treated with lignan extract compared with the control group and females rats treated with conjugated estrogen while HDL showed non-significant difference between both treated groups compared with the control group.

Table(4): Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on Lipid Profile in Virgin Female Rats Mean \pm SD , N=6



Parameters Treatment	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control(Normal saline)	130.68±39.12 A	75.34±19.62 A	51.06±6.23 A	30.23±8.01 A	20.15 ±4.87 A
Synthetic Estrogen (Conjugated Estrogen) (0.10mg/kgB.W.)	118.9±26.11 A	74.63±27.85 A	55.72±4.45 A	27.31±01.2 A	19.03±2.92 A
Phytoestrogen (Lignan) 20mg/kg B.W.	94.50±28.32 B	64.60±12.01 B	58.32±4.43 A	24.13±4.05 B	15.57±4.27 B

N=number of animals., Capital letters denote differences between groups, P≤0.05 vs. control, NS=non-significant.

5-Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on FSH, LH, Estrogen and Progesterone in Virgin Female Rats.

The results in Table (5) revealed significant (P≤0.05) increase of LH, estradiol and progesterone in serum of females rats treated with lignan extract compared with the control group and females rats treated with conjugated estrogen while FSH showed non-significant (P≤0.05) increase in serum of females rats treated with lignan extract compared with the control group and females rats treated with conjugated estrogen.

Table (5):Effect of Conjugated Estrogen and Phytoestrogen (Lignan) on FSH, LH, Estradiol and Progesterone in Virgin Female Rats. Mean±SD , N=6

Parameters Treatment	FSH (mlU/ml)	LH (mlU/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)
Control(Normal saline)	6.03±1.56 A	8.02±1.83 B	44.02±8.12 B	7.43±2.34 B
Synthetic Estrogen (Conjugated Estrogen) (0.10mg/kgB.W.)	5.79±0.21 A	7.45±1.58 B	54.25±9.57 A	6.03±2.72 B
Phytoestrogen (Lignan) 20mg/kg B.W.	6.11±0.65 A	10.84±0.32 A	50.69±10.2 A	9.65±1.41 A

N=number of animals., Capital letters denote differences between groups, P≤0.05 vs. control, NS=non-significant.

Histological Examination:-

1-Thyroid gland

The thyroid gland of control group rats appeared normal thyroid tissue composed of thyroid follicles of varies size and filled with colloid and lined by cuboidal thyriocytes, parafollicular cells can be distinguished as shown in Fig.(2). While



thyroid gland of female rats treated with Conjugated Estrogen appeared histopathological changes as shown in Fig. (3). The changes included of different size thyroid follicles, dilated thyroid follicles lined by flatted thyriocyte and vacuolation of some follicles depletion of parafollicular cells. The thyroid gland of female rats treated with Lignan revealed normal of follicular cells and Full with colloid of follicle, present of parafollicular cells as shown in Fig. (4).

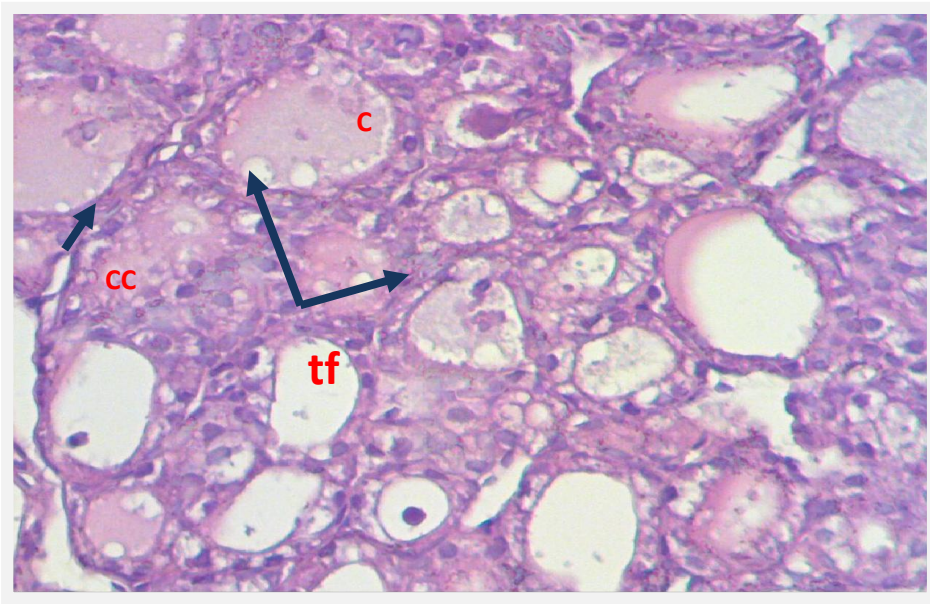


Fig.(2):-Section of thyroid gland of control female rats. Showing normal thyroid follicle(tf), full with colloid and present C-cells(CC), Stained with H&E 400X.

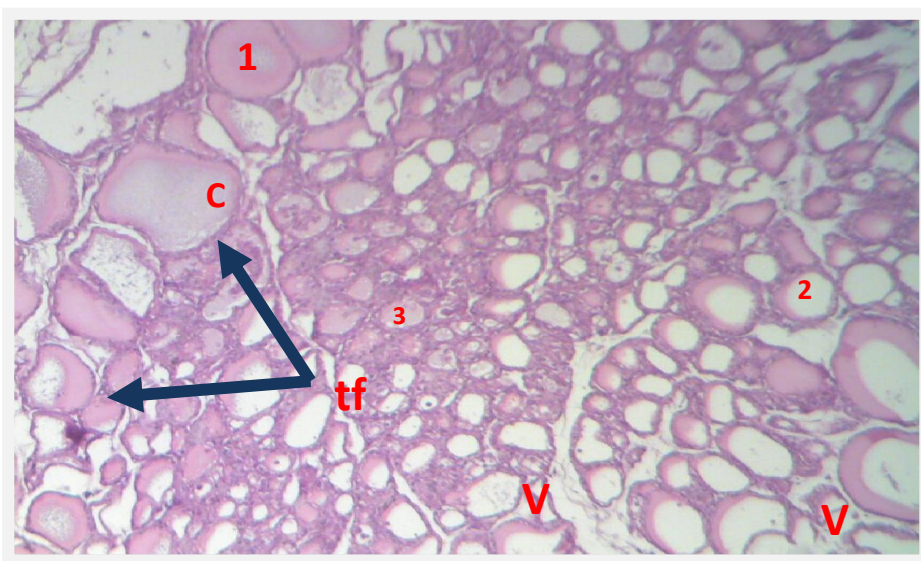
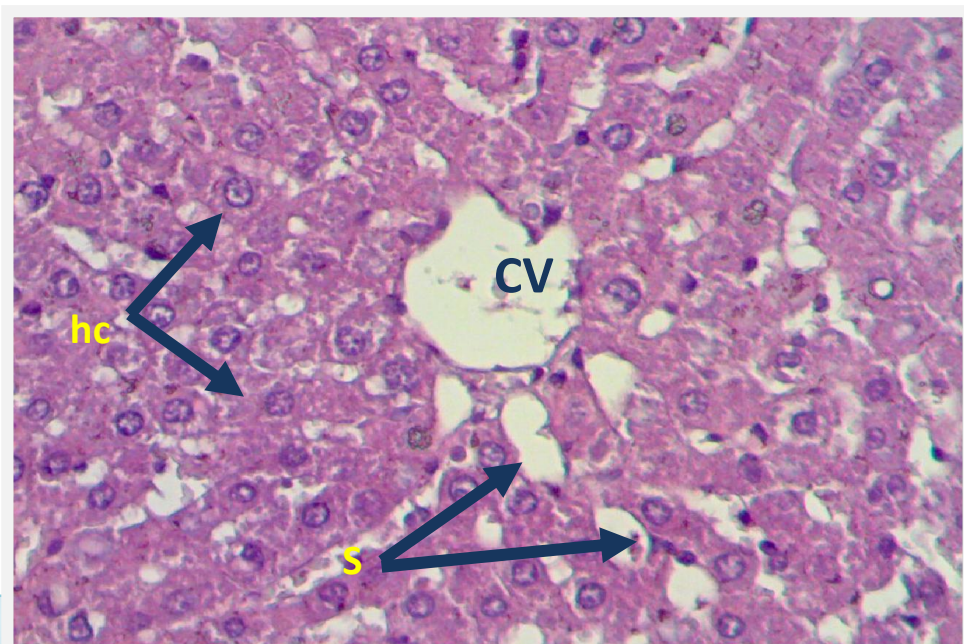


Fig.(3):-Section of thyroid gland of female rats treated with conjugated Estrogen. Showing different size thyroid follicle(tf), dilated thyroid follicle lined by flatted thyriocyte and vacuolation of some follicles depletion of parafollicular cells , Stained with H&E 100X.



2-Liver:-

The liver of Control female rats appeared, normal architecture of liver, to be divided into the classical hepatic lobules, each was formed of cords of hepatocytes radiating from the central vein to the periphery of the lobule. The plates hepatic cells were separated by narrow blood sinusoids and normal hepatocyte (parenchymal cells) normal central vein with no congestion, normal hepatic artery in Fig. (5). While female rats treated with Conjugated Estrogen revealed histological changes including the moderate enlargement of hepatic cells, some of them have dark pyknotic nuclei as well as some sinusoidal dilatation as shown in Fig. (6). Moreover the liver of female rats treated with Lignan extract showed the moderate enlargement of hepatic cells, some of them have dark pyknotic nuclei as shown in Fig. (7).



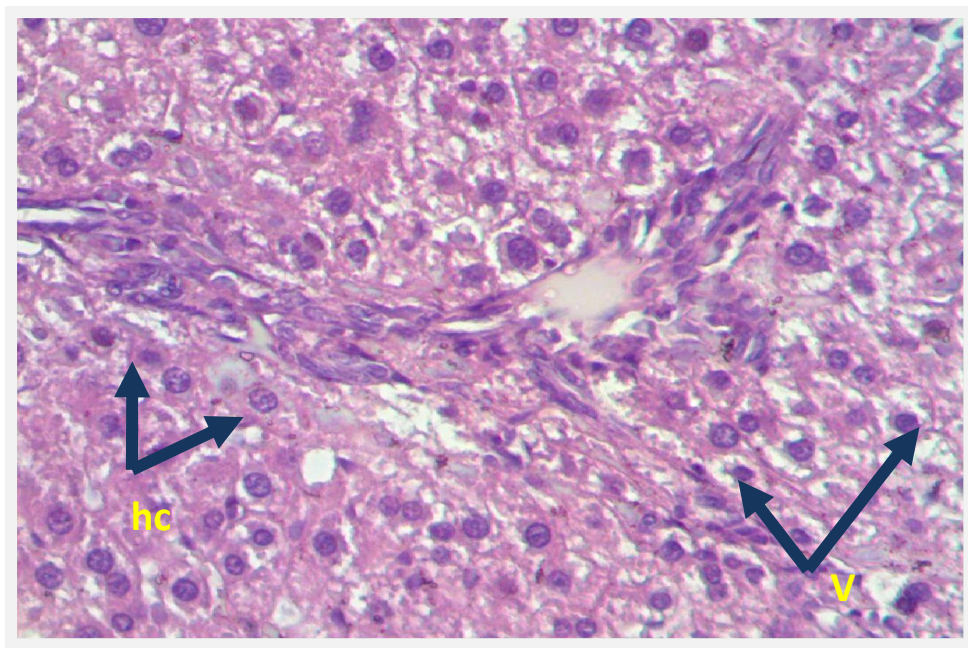


Fig.(6):Section of liver of female rats treated with conjugated estrogen. Showing hepatocyte (hc), pyknotic nuclei and vacuolation (V), Staiend with H&E 400X.

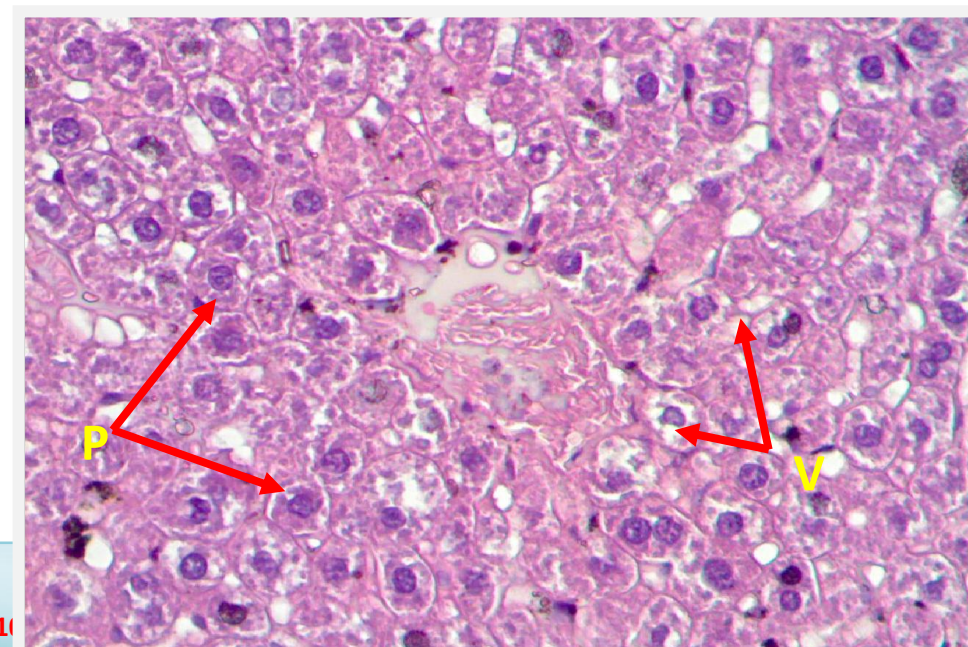


Fig.(7):Section of liver of female rats treated with Lignan. Showing hepatocyte, pyknotic nuclei(P), and vacuolation (V), Staiend with H&E 400X.



3-Kidneys:-

As shown in Fig.(8) kidneys of Control rats showed normal glomeruli, normal renal cortical tubules and normal epithelial cells lining of the renal tubules. While the rabbits treated with Conjugated Estrogen revealed histological changes in kidneys as shown in Fig. (9). 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 rats treated with Lignan extract showed normal architecture Fig. (10).

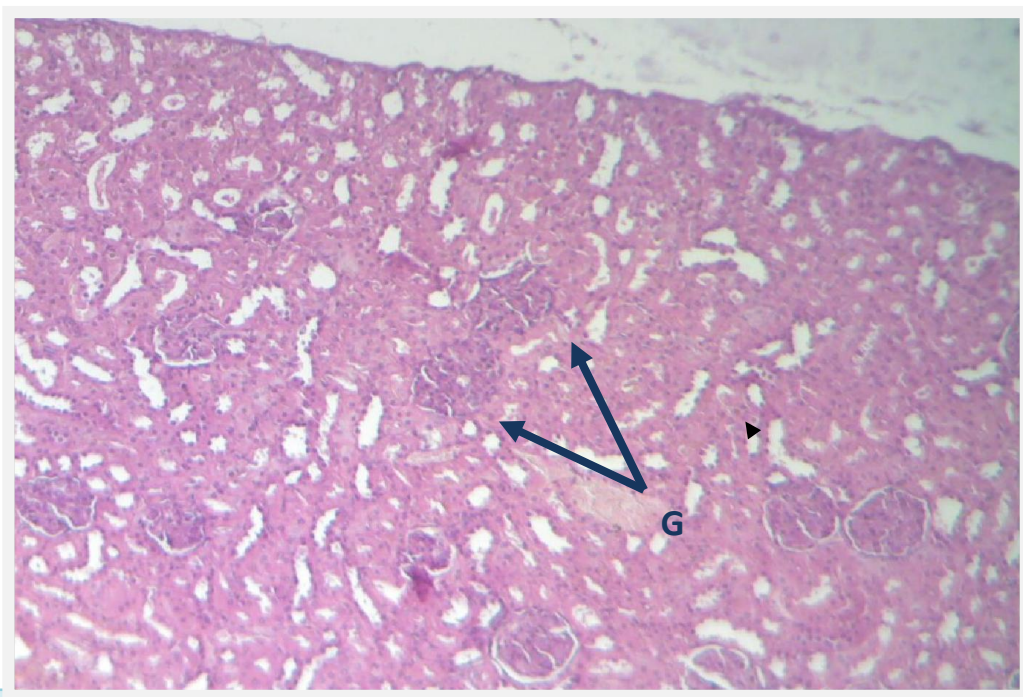


Fig.(8):Section of kidneys of control female rats. Showing normal glomeruli(G), normal cortical tubules. Stained with H&E 100X.



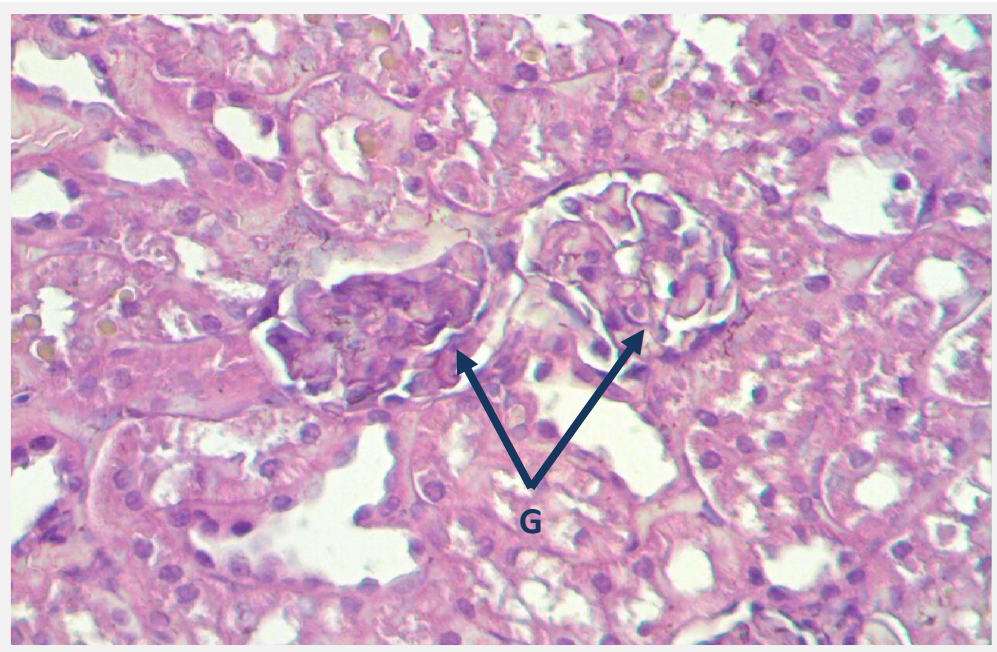


Fig.(9):Section of kidney of female rats treated with conjugated estrogen. Showing some glomeruli atrophy(G). Stained with H&E 400X.

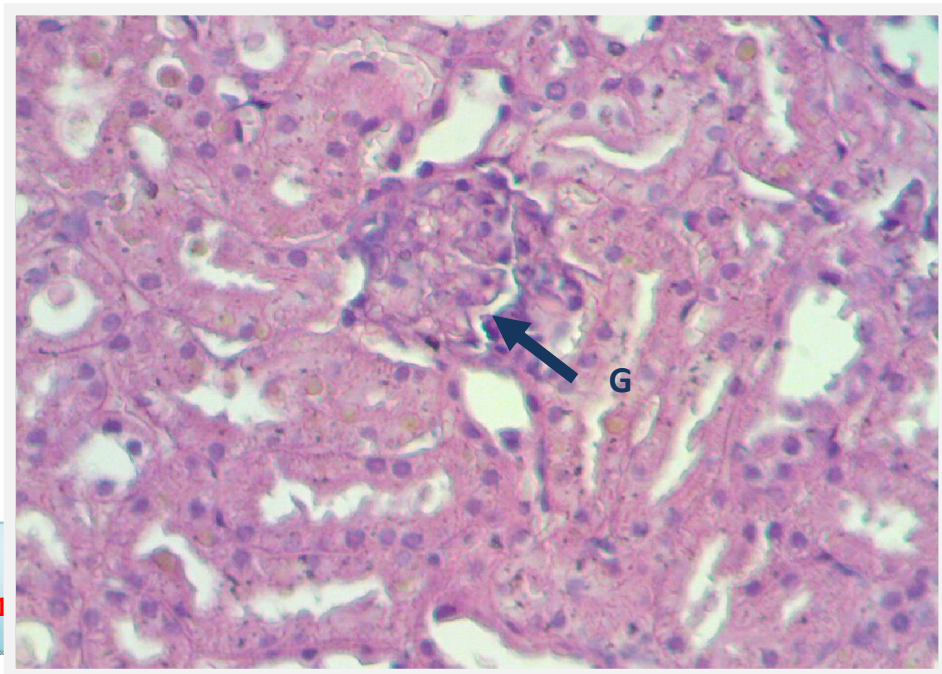


Fig.(10):Section of kidney of female rats treated with Lignan. Showing normal glomeruli, normal cortical tubules. Stained with H&E 400X.

4-Ovaries:-

The ovary of control rats appeared normal ovarian cellular tissue with normal Graafine follicles. In addition, there are normal primary follicles and secondary follicles in Fig. (11). While the ovary of rats treated with Conjugated estrogen revealed histological changes included very clear disturbance in ovarian tissue or parnchymia, high proliferation of denase fibrous tissue with no primary follicle formation. There is only two Graafine follicle containing dense matrix like tissue structure as shown Fig. (12). But the ovary of rat treated with Lignan extract appeared normal active ovarian tissue including well nourished blood capillaries, primary, secondary and Graffain follicles. The large follicles are at the beginning of atretic follicles Fig.(13).

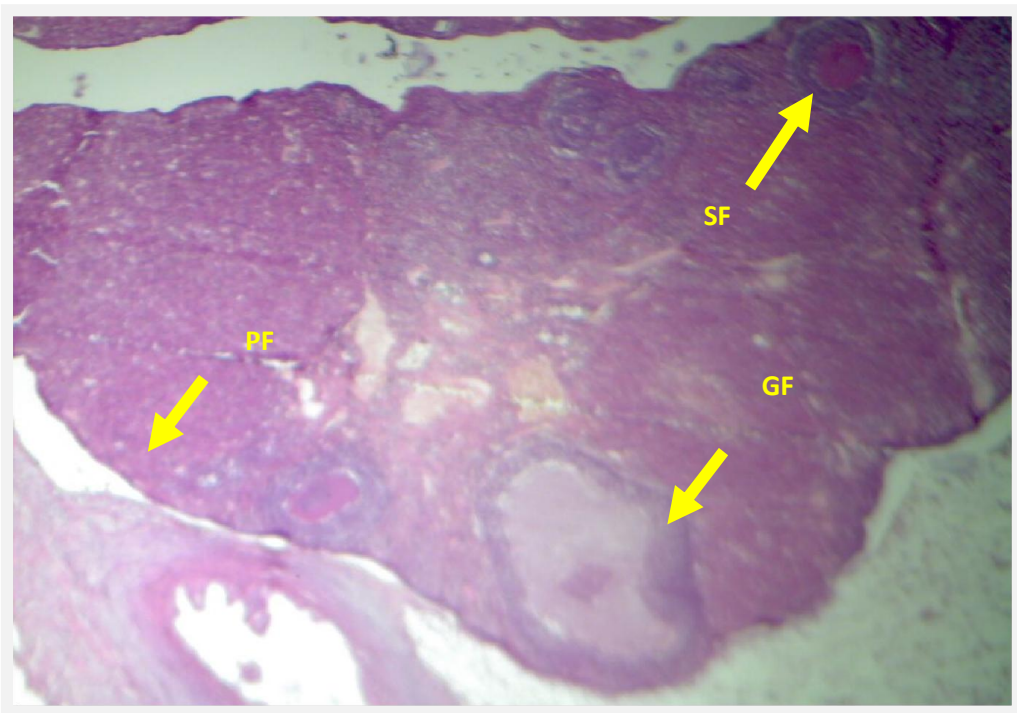


Fig.(11):Section of ovary of control female rats. Showing normal ovary present primary follicles (PF), secondary follicles (SF) and Graffain follicles (GF), stained with H&E.400X.



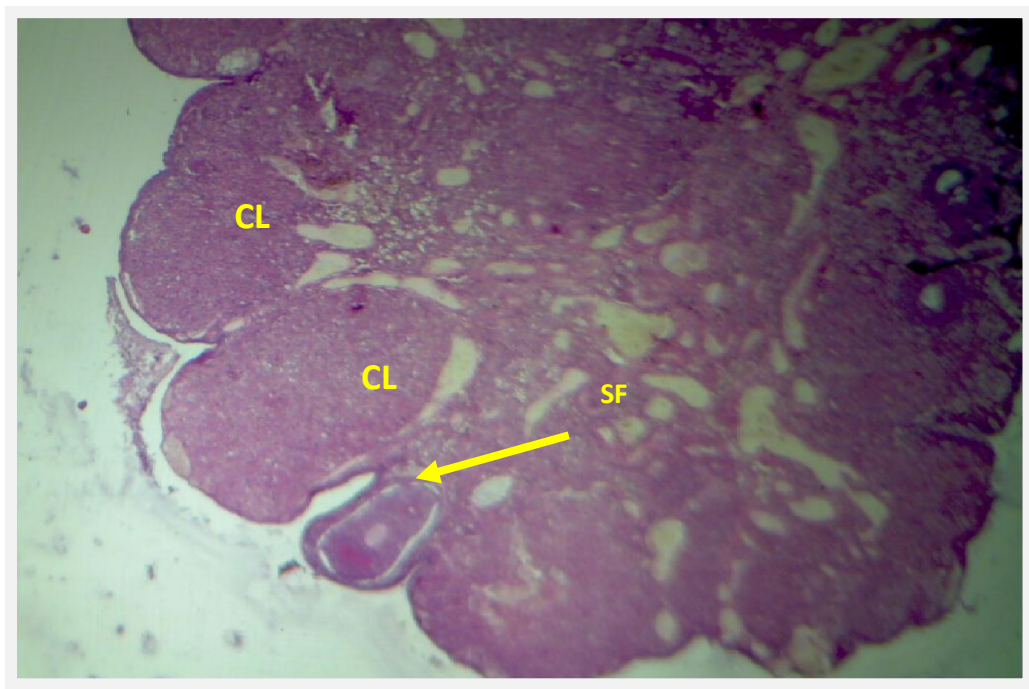


Fig.(12):Section of ovary of female rats treated with conjugated estrogen. Showing defect ovary absent primary follicles(PF) and Graffain follicles(GF) but present secondary follicles (SF), atretic and Corpora leuta (CL),stained with H&E.400X.

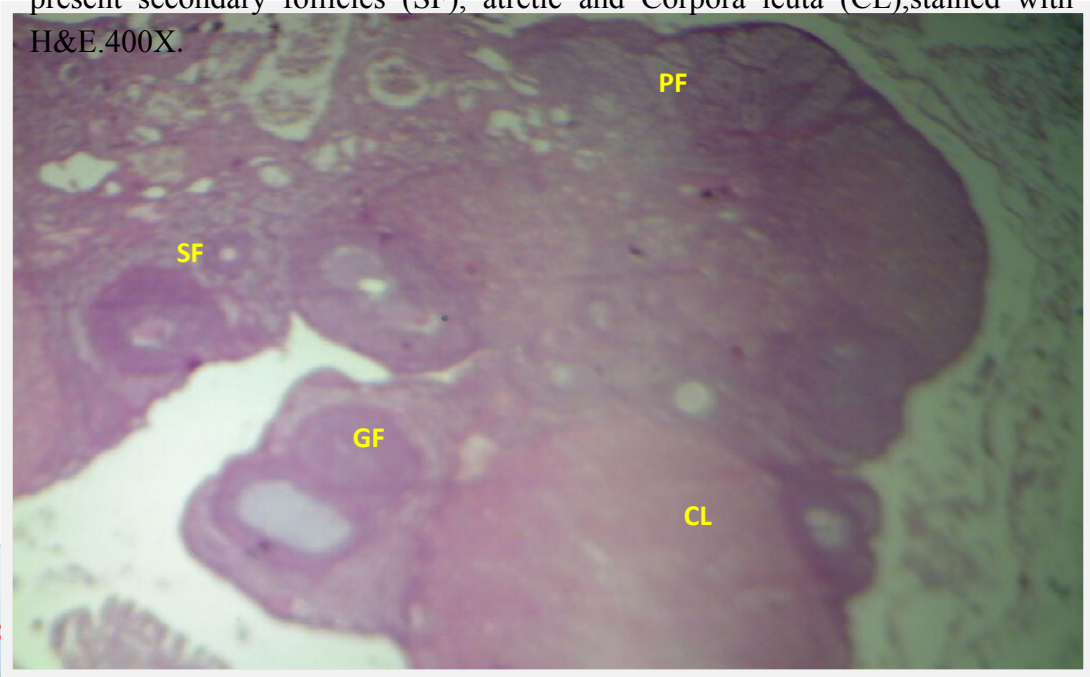


Fig (13):Section of ovary of female rats treated with Lignan Showing normal ovary

5-Uterus:-

As shown in Fig.(14), uterus of control rats showed normal architecture endomterium, (proliferative phase), uterine gland, uterine lumen. While the uterus of rat treated with Conjugated Estrogen revealed histological changes including thin superficial layer of endomterium, miss shape of uterine gland and uterine lumen is enlarged, Fig. (15). But the uterus of rat treated with Lignan extract appeared normal uterine architecture Fig. (16).

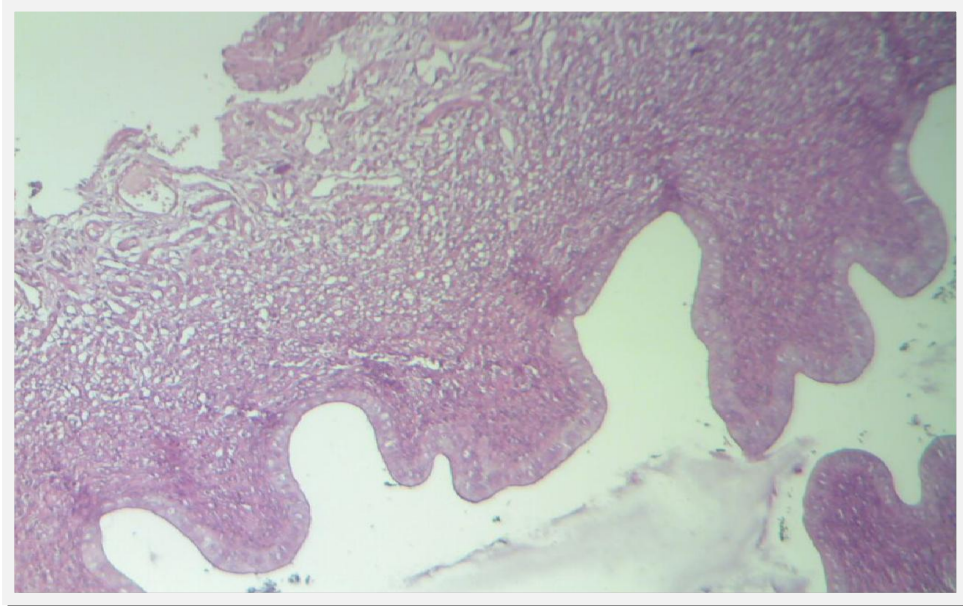


Fig.(14):- section of uterus of control female rats. Showing normal tissues, stained with H&E.400X.



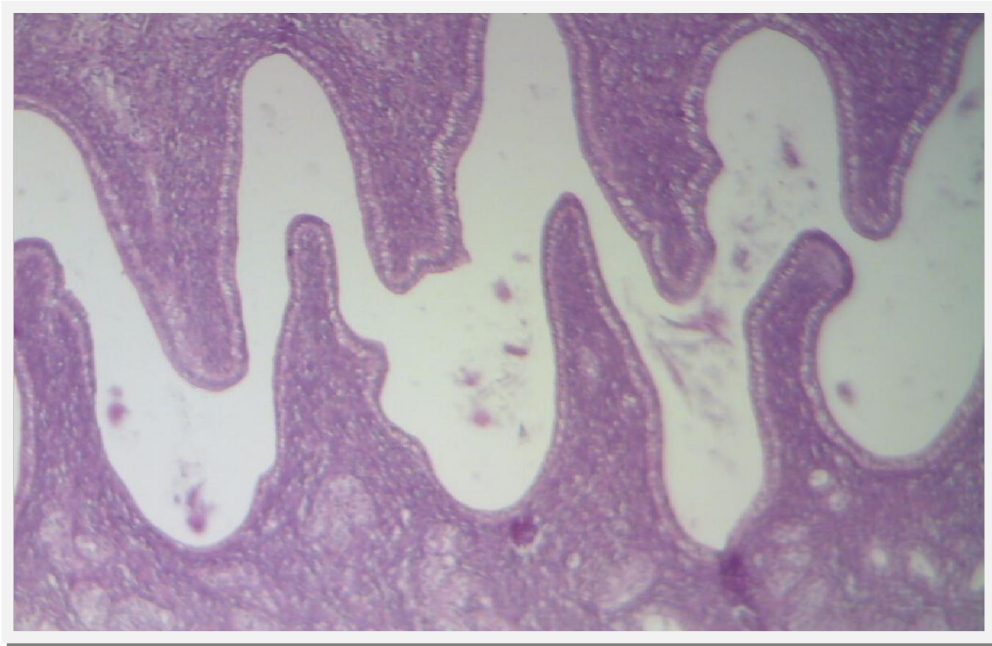
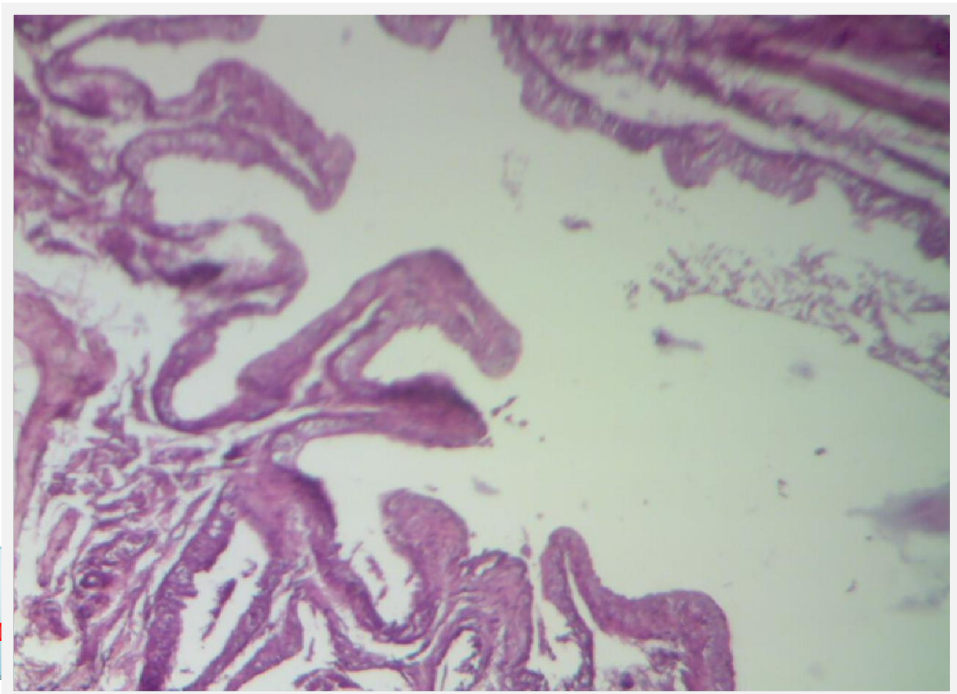


Fig.(15):- section of uterus of female rats treated with conjugated estrogen. Showing normal tissues, stained with H&E.400X.



DISCUSSION

The present study revealed that an increase in body weight of rats treated with phytoestrogen (lignan) extract of flax seeds orally daily for 14 days compared to rats of control. This result agreed with [21] observed significant increase in the body weight of mice given aqueous methanol extract of flax seeds at 200 or 300 mg/kg body weight for 25 days; the increase was dose dependent. The increase in body weights was attributed to the phyto-estrogenic activity of the flax seeds. According to [22] Flaxseeds contain secoisolariciresinol diglycoside, which is changed to phytoestrogens by the ruminal microflora in compound stomach animals and by the microflora of the hind gut in rats and mice. [23] have indicated that treatment of immature female mice with estradiol benzoate significantly increased body weight compared to the control.

Lignans is considered a functional food, producing metabolic and physiological health benefits, in addition to its nutritional properties. Many studies have shown its positive effects when used as a supplemental feeding. Despite the increasing popularity of flaxseed as a dietary supplement, no large randomized clinical trials of its effects [24]. Flaxseed is rich in fat (36%), protein (24%) and dietary fiber (32%), as we evaluated in our laboratory in lots of flaxseed used in previous studies of our laboratory [25-27].

This result disagreement with [28] who said the maternal consumption of flaxseed during pregnancy and lactation is associated with reduced body mass at birth and hormonal changes in offspring.

The analysis of blood parameters is one of the most valuable modern methods because it has been shown that these physiological values are species-specific and age-dependent [29]. An evaluation of the hematological profile usually furnishes vital



information on the body's response to injury and is also a good indicator of the physiological status of animals[30]. RBC and WBC counts as well as RBC indices such as MCV, MCH and MCHC are valuable in monitoring feed toxicity, especially with feed constituents that affect the blood and health status[31]. The result obtained of the effects of lignan extract (20 mg/kg body wt orally daily for 14dys) on the red blood cells (RBC) count, mean corpuscular volume (MCV), hematocrit (Hct),

hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and counts of white blood cell (WBC), granulocytes, lymphocytes, monocytes in virgin female rats that showed no significant differences between control and lignan extract due to duration period lead to no changes in haematological parameters.

This result agreement with[32]results who said that hemoglobin, and RBC counts unaltered after flaxseed diet (flaxseed does not have deleterious effects on the hemopoietic system). This result disagreement with[33] who reported that flaxseed significantly increased the total RBC count and hemtocrit in rats but Hb was not affected by flaxseed diet. This result agreement with[34] that the reported showed that lignan complex had no adverse effects of counts of RBC, WBC, granulocytes, lymphocytes and monocytes in both the normo- and hyper-cholesterolemic rabbits. The values for MCV, RDW, Hct, Hb, MCH and MCHC were similar in lignan complex-treated or untreated normo- and hypercholesterolemic rabbits. While [35] that reported the positive impact of diet with the addition of flaxseed was observed in the blood of rabbits, after 42 days of experimental feeding flaxseed, RBC and HCT indicators presented higher values compared with the control group. Also[36] found differences in hematological indices after inclusion of flaxseed in the diet of laying hens. They reported an increase in HCT and decrease in MCHC values at ambient pressure after the inclusion of flaxseed in the diet, while there was no difference in hemoglobin concentrations. In the present study, hemoglobin levels changed as a result of diet, time and the time \times diet interaction. Partially similar results were observed by[37] when ground flaxseed was included in the diet of rats. They showed higher total RBC counts and HCT than in controls; however, hemoglobin was not affected.



In the previous study, no significant differences were observed for either MCV or WBC values. MCV represents the average volume of RBCs. The cell size may be described as normocytic with normal MCV, microcytic with less than normal MCV and macrocytic with greater than normal MCV [33]. We can conclude that the inclusion of lignan extract of flaxseed in the diet had no adverse effects on RBC deformability. Flaxseed lignans also are shown to have cholesterol-lowering effect

and could regress the atherosclerotic process. Previous animal studies suggest that flaxseed reduces both total and LDL cholesterol. Owing to the promising results in preclinical models, many clinical trials have been performed to determine the outcomes of flaxseed intervention (whole flaxseed, flaxseed oil, or lignans) on various cardio metabolic risk factors, particularly blood lipids. The administration of flaxseed or its derivatives could improve blood lipids (total, LDL, and HDL cholesterol and triglycerides). Flaxseed is the richest food source of lignans, one of the major groups of phytoestrogens [38], and is increasingly being incorporated into human diets because of its reported health benefits. Lignans have been implicated as having estrogenic and/or anti-estrogenic [39], and antioxidant properties [40-42]. [43] reported that rabbits receiving secoisolariciresinol diglucoside, the major lignan found in flaxseed, had reduced hypercholesterolemic atherosclerosis that could be partly attributed to lower total and low-density lipoprotein (LDL)-cholesterol concentrations. A recent population study also found an inverse association between serum lignan concentrations and the risk of acute coronary heart disease [44]. However, the hypocholesterolemic effects of whole flaxseed can also be attributed to its -linolenic acid and fiber components[45-47].

The oral administration of estrogens was compared with respect to the effects on lipid metabolism. Comparable significant decreases in total and low density lipoprotein (LDL) cholesterol (mean range -6.5/-18.0%) were observed in women on estrogen replacement therapy. High density lipoprotein (HDL) cholesterol significantly diminished in estradiol group, but it rose slightly in the oral estrogen group. Thus the fraction of HDL cholesterol over LDL cholesterol did not change in the transdermal group whereas it significantly rose in subjects treated with oral



estrogens. It remains to be established to what extent these differences on lipid metabolism are relevant for the prevention of cardiovascular diseases.

In this experiment, it was observed that supplementation with integral flaxseed flour brought a significant improvement in lipid profile of the evaluated animals. Lower levels of total cholesterol and triglycerides were observed. Therefore, a beneficial effect of the seed used in this study on the serum lipids levels of animals was noted.

Estrogen-like activity of Flax seeds was also evident in the histological sections of ovaries. Ovaries of rats treated with lignan extract of *Linum usitatissimum* revealed the presence of mature Graffian follicles, together with corpora lutea (Fig.13). Similarly, rats given conjugated also exhibited mature, as well as growing, follicles. However, no mature follicles were seen in rats of control group; these rats had only growing Graffian follicles, with a corpus luteum (Fig 12). Increased levels of serum progesterone in can be attributed to the corpora lutea seen on the ovaries in histological sections. Phytoestrogens are strikingly similar in chemical structure to the mammalian estrogen, estradiol, and bind to estrogen receptors α and β with a preference for the more recently described estrogen receptor β [48-50]. These receptors after binding with ligand are able to move from cytoplasm to the nucleus, bind and affect the transcription-control regions of DNA or small RNAs and therefore the expression of specific genes. Furthermore, steroids are able to bind to receptors of cell surface, promote formation of cytoplasmic cyclic nucleotides and related protein kinases, which in turn via transcription factors control the expression of target genes [51-52]. Therefore, phytoestrogens can potentially affect all the processes regulated by estrogens including induction sex hormone binding globulin and inhibition aromatase[53]. Estrogen receptors are present in different tissues –central nervous system (including hypothalamo–hypophysial axis), gonads, reproductive tract, placenta, mammary gland, bones, gastrointestinal tract, lung. This suggests that phytoestrogens may exert tissue specific hormonal effects [54]. The main function of estrogen is to cause cell proliferation and growth of tissues of the sexual organs and other tissues related to reproduction. Once ingested phytoestrogens are metabolized in the body, some of them are bound to serum proteins and another portion is available



to occupy the estrogen receptor (ER). This free portion exerts its effect via two types of receptors ER α and ER- β have different tissue distribution, so that β is more ubiquitous than α . Activation of ER β -receptor is expressed in non-reproductive tissues such as bone, brain, pituitary, tract urinary, vascular system and prostate; and reproductive tissue such as ovary and uterus [55]. At this point, we cannot even say with certainty where the compounds from flax seeds involved. However it is believed that could have effect on luteinizing hormone and follicle stimulating hormone, as

Shown in previous studies in other plants species [55], or even on aromatize and converting precursor's estrogen into active estrogen process.

دراسة تأثير مستخلص اللكينان لبذور الكتان والاستروجينات الصناعية على المعايير الفسلجية في إناث الجرذان

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أجريت هذه الدراسة في البيت الحيواني- التابع لكلية الطب البيطري- جامعة البصرة لدراسة تأثير الاستروجينات النباتية المتمثلة بمستخلص اللكينان من بذور نبات الكتان على المعايير الدمية والكيمو حيوية التغيرات في التراكيب النسيجية للأعضاء المدروسة في إناث الجرذان. كما صممت هذه الدراسة لمقارنة بين الاستروجينات النباتية و الاستروجينات الصناعية وتضمنت الدراسة استخدام 18 من إناث الجرذان البالغة، تتراوح أوزانها ما بين (180-200غم) وبمعدل عمر ما بين (4- 5 شهر) قسمت عشوائيا بالتساوي إلى ثلاث مجاميع رئيسية (6 أنثى جرد / مجموعة). المجموعة الأولى:

(مجموعة سيطرة) وتجرع المحلول الفسيولوجي (0.9% من كلوريد الصوديوم) لمدة 14 يوم.
المجموعة الثانية :

تجرع الاستروجين الصناعي 0.10 ملغم/كغم المذاب في المحلول الفيسيولوجي 1(مل) لمدة 14 يوم.
المجموعة الثالثة :

تجرع الاستروجينات النباتية (20 ملغم / كغم) المتمثلة بمستخلص اللكينان من بذور نبات الكتان لمدة 14 يوم. بعد انتهاء فترة المعاملة تم سحب عينات الدم (7مل) من قلب الحيوانات بواسطة ثقب القلب باستعمال محاقن طبية معقمة اذ قسمت عينة الدم إلى جزئين. الجزء الأول (2 مل) يوضع في أنابيب تحتوي على مانع التخثر (EDTA) لغرض إجراء الفحوصات الدمية إما الجزء الثاني (5 مل) يوضع في أنابيب غير حاوية على مانع للتخثر ووضعت في جهاز الطرد المركزي (5000 دورة) لمدة 10 دقائق لغرض الحصول على مصل الدم



لقياس المعايير الكيموحيوية كقياس نمط الدهون وقياس هرمونات الغدة النخامية وهرمونات القند. وتم التضحية بالحيوانات لدراسة التغييرات النسيجية (للغدة الرقية والأعضاء الأخرى مثل الكبد و الكلى والمبايض والرحم). وتوصلت الدراسة إلى النتائج الآتية:
حصول زيادة معنوية في اوزان إناث الجرذان المعاملة بمستخلص الليكنان مقارنة مع مجموعة السيطرة.
عدم تأثر المعايير الدمية بالمعاملة بالاستروجينات النباتي والصناعية مقارنة مع مجموعة السيطرة بينما لوحظ انخفاض معنوي في جميع نمط الدهون عدا الدهون الجيدة (عالية الكثافة).

وكذلك لوحظ ارتفاع معنوي ($p \leq 0.05$) في تركيز الهرمون اللوتيني (LH) لمصل دم إناث الجرذان المعاملة بالاستروجينات النباتية وكذلك زيادة في هرمونات القند (الاستروجين والبروجستيرون) بينما لوحظ عدم حصول تغير في تركيز هرمون (FSH) مقارنة مع مجموعة السيطرة وكما لوحظ حصول تحسن في التغييرات النسيجية للأعضاء المدروسة.

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