Treatment Effect of Licorice and Flaxseed on Letrozole Induced-Polycystic Ovary Syndrome

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Abstract

Polycystic ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age. Recently use of herbs for the management of polycystic ovary syndrome is interesting. Flaxseed is a rich plant-derived α-linolenic acid of omega-3 polyunsaturated fatty acids, has been proven to benefit for PCOS. Also Licorice is one of the natural antioxidants used for the treatment of infertility.The aim of the present study was to evaluate the effectiveness of licorice and flaxseed in the treatment of Letrozole-Induced PCOS.Twenty-eight female ratswere divided into four groups (n = 7/each): (group I): negative control group fed on basal diet.Group II,III and IV were administrated onLTZ 1mg/kg/day orally by gastric gavage for 3 Weeks.Group II kept as positive group (PCOS group) and fed on the basal diet only. While, the remaining other two groups were fed onsupplemented diet with 1.5% of flaxseed and licorice, respectively.The obtained resultsrevealed thatthe groups of rats were fed on flaxseed and licorice had significant increased ($p<0.05$) in Follicle-stimulating hormone (FSH), Progesterone and Estradiol (E2) and had significant decreased ($p<0.05$) in Insulin, Glucose, luteinizing hormone (LH), Total testosterone,Dehydroepiandrosterone sulfate (DHEA-S), AST, ALT, and Alkaline Phosphate (ALP), compared to the positive group of rats (PCOS rats). Microscopically, results showed improvement in Graffian follicle and corpus luteum in the groups of PCOS rats and were fed on diet supplemented with 1.5% of flaxseed and licorice, respectively as compared to the positive control group. Finally, the existing study illustrated that licorice and flaxseed could improve PCOS syndrome via affecting the serum levels of different hormones and morphology.

Keywords: Polycystic ovary syndrome – Letrozol - Licorice – Flax seed –Hitopathology– Rats.

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1.INTRODUCTION

Polycystic ovary syndrome (PCOS) isa hormonal imbalance and metabolicdisorder affecting about 8-13% of allwomen during their reproductive age.Anovulation, hyperandrogenism, polycysticovary, hyperinsulinism, hirsutism, and elevatedconcentrations of luteinizing hormone (LH) are some of themajor implications of PCOS **Shamsi et al., (2020).**

Licorice (*Glycyrrhiza glabra*) has been used to treat different diseases for many years. Multiple biological activities such as the powerful anti-oxidative, anti-fatigue, anti-proliferative, and estrogenic activity of licorice have been demonstrated in several experimental evidence. Therefore, some studies have suggested licorice extract as a possible candidate for the treatment of infertility as well as PCOS**.**Licorice has an anti-testosterone activity that may be beneficial in treating women with PCOS. Additionally, licorice exhibits estrogenic properties that could result in aromatase stimulation activity **Shamsi et al., (2020).**

On the other hand,Flaxseed is also recommended for the treatment of endocrine disorders and the regulation of female sex hormones. The most important ingredients in flaxseed are fibers, minerals, vitamins, and phytoestrogen.Flaxseed is an exceptionally rich source of dietary ligninmay facilitate the binding of testosterone in the enterohepatic circulation and increase its excretion. Also increases levels of sex hormone-binding globulin (SHBG) thereby decreasing free testosterone levels **Mina et al., (2020)**. The aim of the present study was to evaluate the effectiveness of licorice and flaxseed in the treatment of Letrozole-Induced PCOS.

2. MATERIALS AND METHODS

2.1. Flaxseed and Licorice: Dried flaxseed and licorice were purchased from the local herbalist shops in Cairo, Egypt and classified in the Herbarium, Botany Department, Faculty of Sciences, Cairo University, and Giza, Egypt. Both flaxseed and licorice cleaned, sorted and washed from dust and remove all invalid parts and dried in a hot air oven at

105^{o}C for 3 hrs. A grinder mill and sieves were used to obtain a powder particle size of less than 0.2mm of all leaves.

2.2. Rats and Diet: Twenty-eight Adult of female rats (Sprague Dawley Strain), weighing about 200± 10 g were obtained from Agriculture Research Center, Giza, Egypt. Basal diet constituents were purchased from El-Gomhorya Company for Pharmaceutical and Chemical, Cairo, Egypt. The normal basal diet (AIN-93M) consisting of protein (14%), corn oil (5%), minerals mixture (3.5%), vitamins mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and corn starch was being thoroughly mixed and formulated according to **Reeves et al., (1993)**.

2.3. Induction of Polycystic ovary syndrome (PCOS):
Polycystic ovarian was performed in rats according to method described by **(Fatma, et al., 2019)**which LTZ tablets dissolved in normal saline, letrazol tablets (LTZ) was purchased from the Gamma Trade Company for Pharmaceutical and Chemical, Dokki, Egypt.

2.4. Experimental Design and Assembly of Rats:
All rats were housed at a room temperature of 25 ± 2 °C, relative humidity of 50–55% and light/dark cycles (12/12) in the animal house of the Agriculture Research Center, Giza, Egypt for one week for acclimatization. After acclimatization period, animals were divided into four groups, Group I:negative control group (non-treated group) fed on the normal basal diet only. Group II, III and IV were administrated on LTZ 1mg/kg/day orally by gastric gavage for 3 Weeks. Group II kept as positive group (PCOS group) and fed on the basal diet only. While, the remaining other two groups were fed on supplemented diet with 1.5% of flaxseed and licorice, respectively.
At the end of the experiment period (4 weeks), animals were fasted for 12-hr., except of water and then rats were sacrificed. Blood samples were collected from the posterior vena cava into dry clean centrifuge tubes. Blood samples were left at room temperature to clot, and then centrifuged for 15 minutes at 3000 rpm for serum separation. **Sahibzada et al., (2021)**Serum samples were carefully aspired using a needle and transferred into dry clean test tubes and frozen at -20°C for biochemical analysis. Ovary was removed from all animals was removed immediately, washed with saline solution, dried, and immersed in buffered formalin 10% for histopathology examination.

2.5. Biochemical Assay:
All chemicals and Kits for biochemical analysis were purchased from the Gamma TradeCompany for Pharmaceutical and Chemicals, Dokki, Egypt.

2.5.1.Estimation of serum Total testosterone,E2 and FSH Concentrations: Serum levels ofTotal testosterone, Estradiol (E2) and Follicle-stimulating hormone (FSH) was determined according to**Parker, (2006)**

2.5.2.Estimation of serum LH, Progesterone and DHEA-S Concentrations: Serumlevels of luteinizing hormone (LH), Progesterone and Dehydroepiandrosterone sulfate (DHEA-S)was determined according to **Jahan et al., (2018)**

2.5.3.Estimation of Blood glucose and Insulin hormone: Serumlevels ofBlood glucose and Insulin hormones determined according to **Mohamad et al., (2018)**

2.5.4.Estimation of Liver Functions: Serum activities of AST and ALT enzymes were estimated colorimetric using kits instruction (Diamond Co, Hannover, Germany) as described by **Young, (2001)**. Serum activity of ALP enzyme was determined according to the methods of **Roy, (1970).**

2.6. HistopathologicalExamination: Specimens from the ovary were dissected out, washed with normal saline solution to remove blood and placed in 10% neutral buffered formalin for histopathological examination according to **Bancroft et al., (1996)**. Histopathological examination was done in Animal Health Research Institute, Dokki, Egypt.

2.7. Statistical analysis:
All data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, Version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to **Armitage et al., (1987).** All differences were considered significant if P-values were ($P< 0.05$)

3.RESULTS

3.1.The Effect of Supplemented Diets with Flaxseeds and Licorice on FSH, LH and TSHormones in rats with PCOS:
Theeffects of supplementeddiet with flaxseed and licorice on Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Total testosterone (TS) hormones in rats with Polycystic Ovary Syndrome (PCOS)are recorded in **Table (1).** The results revealed that the positive control group of rats (PCOS group) had significant decrease ($P<0.05$) in the serum levels of FSH and increase in LH and TS hormones, compared to the negative control group of rats (normal group).In contrast, feeding groups on the supplemented diet with 1.5% of flaxseeds and licoricehad significant increase ($P<0.05$) in the serum levels of FSH and decrease in the levels of LH and

TS hormonescompared to the positive group fed on the normal basal diet.

Table1: The Effect of Flaxseeds and Licorice on FSH,LH and TSHormones inPCOS-rats.

Parameters / Groups		FSH mIu/ml	LH mIu/ml	TS ng/ml
Negative group		0.47 ± 0.033^{a}	0.12± 0.008^{e}	0.17 ± 0.01^{c}
Positive group (PCOS)		0.22 ± 0.015^{c}	0.19 ± 0.009^{a}	0.62 ± 0.36^{a}
PCOS + Supplemented diet with 1.5% of	**Flaxseed**	0.27 ± 0.014^{b}	0.15 ± 0.013^{c}	0.32 ± 0.12^{bc}
	Licorice	0.27 ± 0.029^{b}	0.14 ± 0.014^{d}	0.31 ± 0.11^{bc}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS =** Polycystic Ovary Syndrome; **FSH**= Follicle Stimulating Hormone; **LH**= luteinizing Hormone; **TS**= Total Testosterone Hormone.

3.2.The Effect of Supplemented Diets with Flaxseeds and Licorice on BG and Insulin Hormone in rats with PCOS

The effects of supplement diet with flaxseeds and licorice on blood glucose (BG) and insulin hormone in rats with PCOS arepresented in **Table (2)**. The obtained results revealed that the positive control group of rats had significant increase(P<0.05) in the blood glucose and insulin hormone level, compared to the negative control group.However, groups with PCOS and fed on the supplemented diet with1.5% of flaxseedsand licorice had significant decrease (P<0.05) in thelevels of blood glucose and serum insulin hormone, compared to the positive control group fed on the normal basal diet. As exhibited, there is better improvement in BG and serum insulin levels in PCOS-rats fed on the supplemented diet with flaxseeds, compared to that fed on diet with licorice.

Table 2: The Effect of Flaxseeds and Licorice on BG and Serum insulin in PCOS-rats.

Parameters / Groups		BG mg/dl	Insulin u/ml
Negative group		35.33 ± 8.96 ^{c}	0.10 ± 0.00 ^{c}
Positive group (PCOS)		78.33 ± 15.13 ^{a}	0.27 ± 0.19 ^{a}
PCOS+Supplemented diet with 1.5% of	**Flax seed**	58.67 ± 16.74 ^{b}	0.10 ± 0.00 c
	Licorice	62.67 ± 13.87 ^{ab}	0.13 ± 0.05^{bc}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. PCOS = Polycystic Ovary Syndrome. BG= Blood glucose.

3.3. The Effects of Supplemented Diets with Flaxseeds and Licorice on Progesterone, DHEA-S and E2 Hormones in rats with PCOS.

The effects of supplement diets with flaxseeds and licorice on progesterone, Dehydroepiandrosterone sulfate (DHEA-S) and Estradiol (E2) hormones in rats with PCOS arerecorded in **Table (3).**The results revealed that PCOS-rats(positive control group) fed on the basal diet had significant increase (P<0.05) in the serum levels of DHEA-S and decrease in the levels of Progesterone and E2hormones as compared to the negative control group.On the other hand, PCOS-rats fed on the supplemented diet with 1.5% of flaxseeds and licorice had significant decrease (P<0.05) in the serum levels of DHEA-S and increase in the levels of Progesterone and E2hormones, compared to PCOS-rats fed on the basal diet.

Table 3: The Effect of Flaxseeds and Licorice on Progesterone, DHEA-S and E2 Hormones in PCOS-rats.

Parameters / Groups		Progesterone ng/ml	DHEA-S ng/ml	E2 pg/ml
Negative group		14.19 ± 6.98 ^{a}	0.41 ± 0.23 ^{b}	55.39 ± 1.479 ^{a}
Positive group (PCOS)		4.22 ± 0.57 ^{b}	2.93 ± 0.64 ^{a}	28.86 ± 2.615 ^{e}
PCOS + Supplemented diet with 1.5% of	**Flaxseeds**	12.98 ± 5.63 ^{a}	0.95 ± 1.02 ^{b}	47.26 ± 1.711 ^{b}
	Licorice	10.47 ± 8.73 ^{ab}	0.95 ± 0.44 ^{b}	48.61 ± 1.909 ^{b}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS =** Polycystic Ovary Syndrome; **DHEA-S** = Dehydroepiandrosterone sulfate; **LH**= luteinizing Hormone; **E2**= Estradiol

3.4.The Effects of Supplemented Diets with Flaxseeds and Licorice on serum levels of, AST, ALT and ALP in rats with PCOS.

The attained results in Table **4** exhibit the effect of supplement diets with flaxseed and licorice on the activities of Aspartate Aminotransferase serum (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP)enzymes in rats with PCOS.

Delimited results showed that the PCOS-group feeding on the basal diet alone had a significant ($P<0.05$) increment in activities of liver enzymes (AST, ALT, and ALP), compared to the normal rats. However, PCOS-group feeding on the supplemented diet withflaxseeds and licorice had significant ($P<0.05$) decrease in the serum activities of liver enzymes (AST, ALT, and ALP), compared to PCOS-group feeding on the normal basal diet. In addition to PCOS-group feeding on the supplemented diet with licorice had significant decrease in serum AST and ALT, and increase on serum ALP, compared to PCOS-group feeding on the supplemented diet with Flaxseeds.

Table 4: The Effect of Flaxseeds and Licorice on The Activities of AST, ALT and ALP enzymes in PCOS-rats.

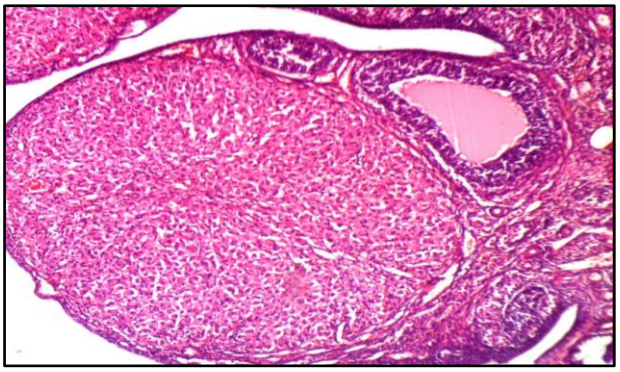
Parameters / Groups		AST) (U/L)	ALT (U/L)	ALP (U/L)
Negative group		13.57±0.371^{d}	11.6±0.401^{d}	99.5±1.260^{d}
Positive group (PCOS)		19.87±0.262^{a}	17.6±0.193^{a}	120.51±3.445^{a}
PCOS + Supplemented diet with 1.5% of	**Flaxseeds**	13.15±0.391^{d}	11.3±0.355^{d}	103.01±3.109^{C}
	Licorice	15.6±0.293^{c}	13.29±0.287^{c}	107.49±2.158^{b}

Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS =** Polycystic Ovary Syndrome; **AST** = Aspartate Aminotransferase serum; **ALT** = Alanine aminotransferase; **ALP**= Alkaline phosphatase.

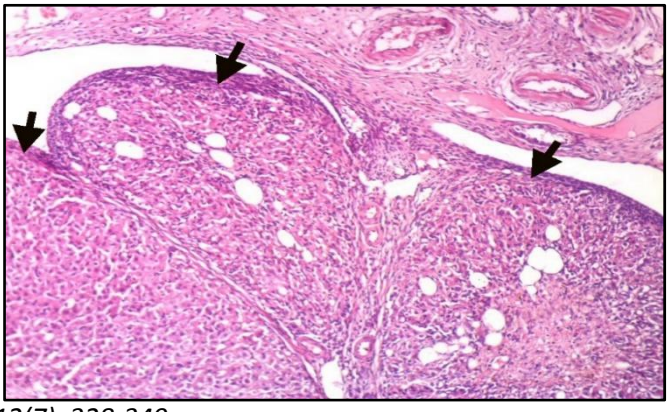
3.5. HistopathologyExamination of the Ovary:

Microscopical examination of ovary of rats from normal rats revealed the normal histological structure (numerous follicles of different types; Graafian follicles and Corpus luteum) **(Pictuers 1).** Meanwhile, ovary of rats from positive control rats showedmultiple degenerated corpus luteum **(Pictuers 2)**, marked congestion interstitial blood vessels, atretic follicles and ovarian cyst **(Pictuer 3).**

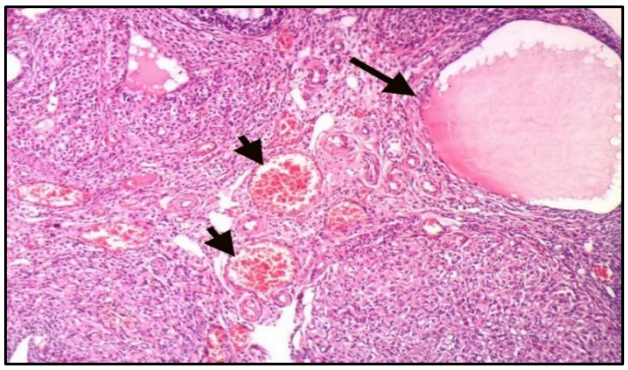
Moreover, ovary sections from PCOS-rats fed on supplemented diet with flaxseeds showed normal Graffian follicle and corpus luteum **(Pictuer4)**and interstitial edema and hyperplasia of interstitial cells **(Pictuers 5).**Ovary of rats from PCOS-rats fed on supplemented diet with licoricerevealed multiple normal graffian follicles **(Pictuers6).**



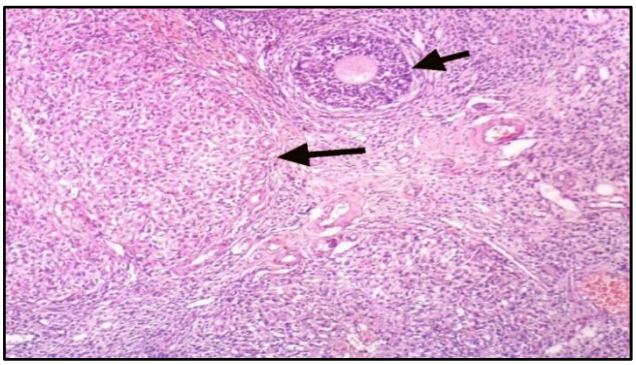
Picture 1: Ovary of rat from normal rats showing the normal histological structure.Note numerous follicles of different types (Graafian follicles and Corpus Luteum) (H & E X 100).



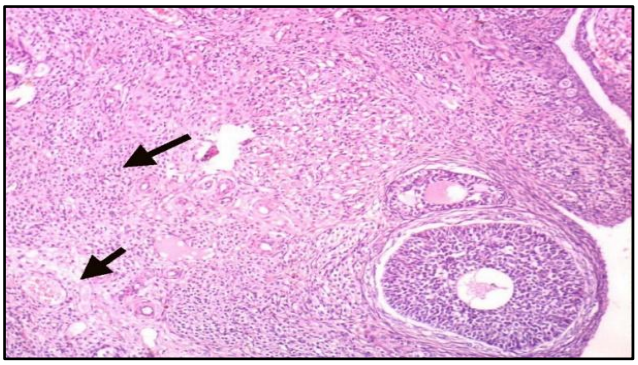
Picture 2: Ovary of rat from positive control rats showing multiple degenerated corpus luteum (H & E X 100)



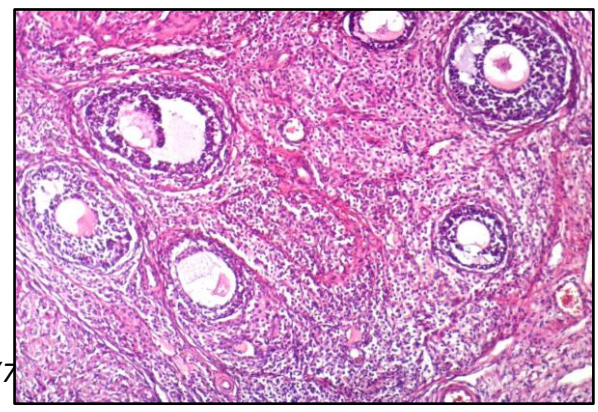
Picture 3: Ovary of rat from positive control rats showing congestion interstitial blood vessels (Small arrow) and atretic follicles (thin arrow) and cyst formation (large arrow) (H & E X 100).



Picture 4: Ovary of rat from PCOS-rats + 1.5 flaxseeds showing normal Graffian follicle and corpus luteum (H & E X 100).



Picture 5: Ovary of rat from PCOS-rats + 1.5 flaxseeds showing interstitial edema (short arrow) and hyperplasia of interstitial cells (H & E X 100).



Picture 6: Ovary of rat from PCOS-rats + 1.5 licorice showing multiple normal graffian follicles (H & E X 100).

4.DISCUSSION

An existing study was to evaluate the effectiveness of licorice and flaxseed in the treatment of Letrozole-Induced PCOS. On the other hand, The obtained results showed that letrozole (LTZ) administration significantly decreased in FSH, Progesterone and E2 level hormones and increased in total testosterone , LH and DHEA-s level hormonesof rats in positive control group compared to negative group, this result related to the induction of letrozole (LTZ) and this result was agreement with **Morales et al.,(2017)** who showed that LTZ administration could induce a model of PCOS in female rats as evidenced by significantly increased testosterone level, LH and LH/FSH ratio. LTZ is a competitive inhibitor to aromatase enzyme by binding to heme subunit of cytochrome P450 which subsequently decreases estrogen and increases the ovarian androgens which leads to hyperandrogenism and abnormal follicular development. The ovarian androgen increases the number of pycnotic granulosa cells and degenerate's oocytes. Also,**JSOG. (2007)** showed that testosterone secretion by theca cells is stimulated in this condition might be attributed to the increase of LH level. Pituitary production of LH is increased because of the decreased estrogen production with LTZ administration and subsequently weakens the negative feedback exerted by estrogen on LH production in pituitary gland. Increased LH and LH/FSH ratio are the main factors contributing to the anovulatory state in PCOS as LH increases ovarian androgen which suppresses follicle growth and maturation. Also **Fatma.et al., (2019)** reported that LTZ administration decreased plasma adiponectin level which testosterone hormone has a down regulatory role on adiponectin secretion. IN addition, LTZ administration significantly enhanced the occurrence of apoptosis in ovarian tissues and affected the related protein expression as it increased Bax expression and Bax/Bcl2 ratio but it decreased Bcl2 expression in ovarian tissue compared with normal control group. Cell apoptosis has an essential role in follicular development, oocyte degeneration, follicle selection and follicle atresia. PCOS patients show dysregulation of cell apoptosis which could be due to dysregulation of FSH-granular cell axis with elevation of androgen level leading to impairment of follicle selection and aggregation of small follicles to form cysts inside the ovary. Also,**Yarak et al., (2005)** reported that the frequency of pulsatile gonadotropin releasing hormone (GnRH) release may be the cause of reduced FSH level in the present study. In L group, the increased frequency of GnRH pulse favors the transcription of LH over FSH. The mechanism of GnRH secretion deregulation may be related to the weak peripheral aromatization of the androgen which affects blood level of sex hormone which may increase sensibility of GnRH receptors as well as pituitary sensibility to GnRH. Another study, **Selim et al., (2019)** showed that decreased aromatase activity in the ovary may be the cause of PCOS development. Letrozole reduces conversion of androgens to estrogens in the ovary, resulting in increased testosterone and decreased estrogen production. In addition, the low estrogen level weakens the negative feedback on LH production in the pituitary, resulting in increased LH levels, which further stimulates ovarian theca cells to secrete testosterone. This is compatible with the increased serum testosterone in Letrozole group. **Yasmine et al., (2020)** showed marked elevation in LH and testosterone levels in comparison to control animals reflecting the hyperandrogenism state in PCOS condition which the hormonal alternations of letrozole induced rats exhibit a hyper-androgenized state responsible for disrupted ovarian physiology. The disturbance in the usual hypothalamic-pituitary gonadal axis increases both LH and testosterone progressing into a disease status. As evidenced, LH triggers testosterone secretion in a thecal layer of ovarian follicles initiating such abnormalities.

Our results also showed that letrazoleadministration significantly increased in glucose and insulin level hormones of rats in positive controle group compared to negative group this result related to the induction of letrazole this result was agreement with **Yasmine et al., (2020)** who reported that remarkable increase in fasting blood glucose levels and insulin resistance declared by a significant increase in HOMA/IR.**Lauterbach et al., (2017)** explain the mechanism of IR and hyperglycemia in LTZ group one of them may be related to the ability of testosterone to change directly the muscle structure in female rats with PCOS. It may decrease the amount of highly oxidative insulin sensitive type I muscle fibers, increase the amount of glycolytic type II less insulin sensitive muscle fibers, and inhibit glycogen synthase enzyme. In addition, excess visceral fat accumulation is responsible for an increase in circulating adipocytokines, which have implications for IR, hyperglycemia and dyslipidemia. IR may be also related to TNF-α induced serine phosphorylation of insulin receptor, leading to inhibition of signaling. Another research, **Asmaa et al., (2021)** who showed High testosterone concentrations in PCOS lead to pancreatic β cell dysfunction, insulin resistance and thus hyperglycemia. **Fatma.et al., (2019)** reported that

LTZ administration decreased plasma adiponectin level, Adiponectin is a protein secreted from adipose tissue and has insulin sensitizing effects as it enhances glucose transport via glucose transporter 4 (GLUT4), and fatty acid oxidation. Low plasma level of adiponectin has been associated with Insulin Resistance (IR). Also, insulin could enhance the biosynthesis of testosterone in theca cells by affecting the expression of low density lipoprotein cholesterol receptors in granulosa cells. **Mohd et al.,(2022)** Revealed that letrozole administration resulted in the significant decrease ($p < 0.001$) in the adiponectin levels in comparison to the normal control. **Morsyet al., (2022)**showed that Insulin resistance is one of the most important contributing factors in the pathogenesis of PCOS. Insulin resistance in PCOS is characterized by obesity, hyperandrogenism, and increased insulin cretion in response to metabolic abnormalities. Hyperinsulinemia, in turn, stimulates fat storage and disturbs cholesterol and lipoprotein metabolism. Furthermore, insulin directly stimulates the steroidogenic enzyme cytochrome P450c17 and promotes the conversion of cholesterol to progesterone and subsequently into androgen. In addition, insulin directly promotes the pituitary secretion of luteinizing hormone, which activates its receptors on theca cells to increase androgen production. On the other hand, abdominal obesity associated with elevated androgen leads to metabolic disorders, promoting more insulin production.

Also, our results showed that letrazole administration significantly increased in liver functions (ALT, AST and ALP level hormones) of rats in positive control group compared to negative group this result related to the induction of letrazole this result was agreement with **Asmaa et al., (2021)**who showed a minor hepatic changes demonstrated by elevated ALT and AST levels with a moderate vacuolation of hepatocytes

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects almost 15% of women of reproductive age. As this disease is a syndrome, it affects different aspects of women health; it is much more prevalent in obese patients and affects different body systems leading to reproductive and metabolic complications. It is also the most common cause of chronic anovulation and hyperandrogenism in young women. It is also the most common cause of infertility in women of reproductive age.PCOS is typically first identified during the early reproductive years, its clinical manifestations usually include: oligo oranovulation, hyperandrogenism (either clinical or biochemical), presence of polycystic ovaries.Women with PCOS have an increased proportion of primordial follicles and a corresponding increase in activated growing (primary) follicles. Small follicles do not develop into ovulatory follicles because growth of these follicles is arrested before they mature. In PCOS, there is an abnormal follicular development and apparent failure to select a dominant follicle results in anovulation. PCOS is associated with long term-health complications, including diabetes, obesity, heart disease and endometrial hyperplasia or cancer**. Selim et al., (2019)**

The obtained results showed that, the group of rats were fed on supplemented diet with 1.5% of licorice powder/day had significantly increased in FSH, Progesterone and E2 level hormones and decreased in total testosterone, LH and DHEA-s level hormones compared to positive control group (PCOS), this result related to feed on supplemented diet with 1.5% of licorice powder/day and this result was agreement with **Yang et al. (2018)** investigated the effects of Glycyrrhiza glabra administration (0.3 g/kg) for 2 wk. on 6-wk old Sprague Dawley female rats with letrozole-induced polycystic ovary syndrome (PCOS) symptoms. The administration of Glycyrrhiza glabra resulted in increased serum FSH levels, a decreased LH: FSH ratio.Our results also agreement with **Nader et al., (2022)** who revealed that The amount of estradiol and progesterone level were significantly higher in the 30% of licorice root hydro alcoholic extract group compared to the control group, also showed that Estrogens as one of the most important sexual hormones are mainly produced in the ovarian internal theca cells through the conversion of cholesterol to androstenedione or testosterone, being subsequently aromatized to estrone and estradiol in granulosa cells, Phytoestrogens might act as natural selective estrogen receptor modulators, Since ancient times, the root of Glycyrrhiza glabra named licorice, as a high source of phytoestrogens, has been known to have a wide range of clinical applications.Licorice encompasses valuable constituents including Glycyrrhizic acid and Glabridin as well as lots of phenolic and flavonoid constituents main compound has a high structural similarity to steroidal hormones including estrone (E1), estradiol (E2), and estriol (E3). **Kaur et al., (2013)** proved that icorice inhibits two enzymes [3β-hydroxysteroid dehydrogenase (3HSD) and 17-hydroxysteroid dehydrogenase (17HSD)], stimulates the activity of aromatase, and also affects the activity of 5α- and 5β-reductase enzymes, all of which are involved in the synthesis and metabolism of androgens and estrogens. Due to the presence of phytoestrogens with aromatase-inducing and 17HSD-inhibiting activities, licorice can reduce testosterone synthesis and therefore can be used to treat women with PCOS. **Armanini et al., (2007)** showed that licorice extract will lead to a reduction in intraovarian androgen concentration when taken orally. Subsequently, this results in decreasing the levels of androgen synthetized from estrogen, causing positive feedback on the LH secretion and when 17-hydroxysteroid and 17-20-lyase are blocked and the activity of aromatase increases, there is a significant reduction in serum testosterone levels that indicated the licorice could be considered as adjuvant therapy of PCOS.

Also,**Bachler et al., (2014)** reported that the LH/FSH ratio significantly decreased following treatment with GRR extract. **Al-Muala et al., (2010)** reported that the effects of estrogen-like substances present in the licorice, the effects of estrogen-like substances present in the licorice. confirms the estrogen bioactivity of licorice., this may cause positive feedback action on gonadotropin, probably from both a direct effect of the estrogen on the pituitary gonadotropes to secrete more FSH & LH in response to GnRH and indirectly by stimulating the hypothalamic neurons that secrete GnRH with modulation of the frequency and magnitude of the pulses of GnRH. **Alani et al., (2004)** noticed that serum E2 level tends to rise significantly by ingestion of licorice extract, because it has an action similar to estriol and E2, and it binds to estrogen receptors in genital organs. **Shimoyama et al., (2003)** showed that licorice inhibits two enzymes [3β-hydroxysteroid dehydrogenase (3HSD) and 17-hydroxysteroid dehydrogenase (17HSD)], stimulates the activity of aromatase, and also affects the activity of 5α- and 5β-reductase enzymes, all of which are involved in the synthesis and metabolism of androgens and estrogens. **Vaya et al., (1997)** explained that Phytoestrogens are natural compounds derived from plants, which exhibit estrogen-like activities. They can be divided into the subclasses lignans, isoflavonoids, and coumestans. They are widely distributed in oil seeds, vegetables, and soybeans and hence are part of the normal human diet. Several isoflavans from the licorice root that presented antioxidant activity have been isolated in our laboratory. Of these, glabridin is the major constituent (11%) of the alcohol extract. **Tamir et al. (2001)** demonstrated that some licorice components and derivatives, such as glabridin, glabrene, and isoliquiritigenin, act like phytoestrogens that bind to the human ER in multiple estrogen responsive tissues, and noted a response to increasing concentrations of glabrene and isoliquiritigenin on the growth of estrogen-dependent cell lines, and determined that cellular response was biphasic, with flavonoids displaying ER-dependent growth-promoting effects at low concentrations and ER-independent antiproliferative activity at concentrations >15 mM. The binding affinities of isoliquiritigenin, glabrene, and glabridin for ER were found to be lower than estradiol. **Hillerns et al. (2005)** explain that the binding affinities of licorice-derived phytoestrogens to human SHBG and uterine ERs in Sprague Dawley rats. In line with previous findings, flavonoid and chalcones components, such as liquiritigenin and isoliquiritigenin, showed varying estrogenic affinities to ERs. Interestingly, GA was not able to sufficiently displace estradiol (E2) from the uterine ERs, further highlighting that estrogen-like activity exerted by licorice is mainly carried out by its flavonoid components. Notably, the tested compounds displaced E2 from SHBG much stronger than dihydrotestosterone, which is consistent with previous evidence reporting a much higher affinity between SHBG and dihydrotestosterone compared with E2.

The results we found demonstrated that, the group of rats were fed on supplemented diet with 1.5% of licorice powder/day had significantly decreased in Glucose and Insulin level hormones compared to positive control group (PCOS), this result related to feed on supplemented diet with 1.5% of licorice powder/day and this result was agreement with **Luan et al. (2015)** demonstrated a significant decrease in insulin resistance and androgen levels along with improvement of ovulatory cycles in women with polycystic ovary syndrome after glabridin treatment for 12 months. Also**Feihua et al., (2013)** reported that glabridin produced a significant decrease in FBG levels in diabetic mice. In addition, glucose tolerance also improved significantly after glabridin treatment. These results indicated that glabridin possesses hypoglycemic.

In our study we found that, the group of rats fed on the supplemented diet with 1.5% of licorice had significantly decreased in AST, ALT and ALP level hormones compared to positive control group (PCOS) this result was agreement with **Jalal et al., (2013).** Stronger Neo-Minophagen C (SNMC), a glycyrrhizin preparation, has been extensively used with considerable success. In two clinical trials, SNMC has been shown to significantly lower aspartate transaminase (AST) and alanine transaminase (ALT) concentrations, whilesimultaneously ameliorating histologic evidence of necrosis and inflammatory lesions in theliver.Also**Akamatsu et al., (1991)** reported that glycyrrhizin shows hepatoprotective effect by preventing changes in cell membrane permeability, inhibiting phospholipase A2 (PLA2) and increasing survival rate of hepatocytes. **Van et al., (1998)** approved that Glycyrrhizin induced significant reduction in serum aminotransferases and improved the liver histology when compared with the placebo. **Hajiagha et al., (2012)** demonstrated that consumption of 2 g/day aqueous licorice root extract for 2 months in patients significantly decreased the serum ALT and AST levels.

The discovered results showed that, the group of rats were fed on supplemented diet with 1.5% of licorice powder/day had significantly increased in FSH, Progesterone and E2 level hormones and decreased in total testosterone, LH and DHEA-s level hormones compared to positive control group (PCOS), this result related to feed on supplemented diet with 1.5% of licorice powder/day and this result was accordance to **Thompson et al., (1995)** who explained that flaxseed, a food renowned for its omega-3 fatty acid content, is one of the richest sources of dietary lignan also, having levels that are 800-fold more as compared to other foods These lignans have the potential to reduce the excess

testosterone which play a vital role in the development of PCOS. **Low et al., (2005)** indicated that high lignin foods increased testosterone exertion by binding it to enterohepatic circulation. Lignans could also reduce the bioavailability of free testosterone through increasing SHBG levels. **Haidari et al., (2020)** in contrast, reported a significant reduction in serum total testosterone level. **Ting et al., (2020)** in this study, abnormal elevated plasma T and LH/FSH ratio, as well as reduced levels of plasma FSH, estrogen, E2 and PROG in PCOS, was notably rectified by FO intervention, demonstrating that dietary FO supplementation was capable of improving the homeostasis of sex steroid hormones in PCOS. **Mamta et al., (2018)** showed that there was a statistically significant reduction in the mean LH levels and an improvement in the mean FSH levels of the subjects after intervention with flaxseed. **Fatima et al., (2015)** who have concluded that flaxseed supplementation is effective in reducing the LH levels. LH plays an important role in androgen production by theca cells and in PCOS high levels of androgen hormones (e.g., testosterone) from the Ovary and Adrenal gland, results in infertility. **Forouhi et al., (2015)** suggested that consumption of omega-3 fatty acids results in a decrease in testosterone concentration after 2 months' trial. This decrease can be due to the effect of omega-3 on LH levels. **Neda et al., (2015)** found that omega-3 supplementation might significantly reduce testosterone concentration when compared with placebo. **Adlercreutz et al., (1987)** also reported reduction in total and free testosterone levels following flax seed administration.21 The decrease in concentration of free circulating testosterone may be due to the lignan content of flax seed which has been found to increase levels of sex hormone binding globulin (SHBG) as described by Adlercreutz.29 There will be increased binding of testosterone to SHBG and hence the decline in its free level. Reduction in free testosterone may facilitate regularization of menstrual cycle, reduction of hirsutism and improve ovulation. **Shaimaa et al., (2021)** detected that the greatest increase in FSH and LH level hormones were obtained by supplementation with flaxseed powder. **Jelodar et al., (2018)** obtained that progesterone levels in the treatment group increased significantly in comparison with the PCOS group. **Ahmad et al., (2012)** achieved that hydroalcoholic flaxseed extract to immature rats has been reported to increase progesterone levels significantly. **Nazir et al., (2011)** acquired that serum estradiol and progesterone concentrations were higher in rats given extract of Flax seeds. **Sokola-Wysoczanska et al.,(2018)** proved that the composition of flaxseed oil, demonstrating that PUFAs such as linoleic acid and α-linolenic acid as the main component of flaxseed oil which regulate prostaglandin synthesis, PUFAs increase the sensitivity of the receptors toward estrogen and can modulate the binding of hormones. PUFAs are an elemental factor in the actions of estrogen due to altering the expression of receptors on the cells. **Tanideh. et al., (2021)** revealed that the effects of flaxessd oil (2.50 mg/kg body wt) on the OVX rats were shown; oestrogen and progesterone levels were improved in the OVX rat.

The gained results demonstrated that, the group of rats were fed on supplemented diet with 1.5% of flaxseed powder/day had significantly decreased in Glucose and Insulin level hormones compared to positive control group (PCOS), this result relative to feed on supplemented diet with 1.5% of flaxseed powder/day and this result was conformance with **Haidari et al.,(2020)** Flaxseeds (linseeds) is food and fiber that are high in omega 6, 3 fatty acids (alpha linolenic acid, ALA) which have shown benefits in insulin concentration. **Pilar et al.,(2017)** show that omega 3 fatty acids supplemented from flaxseed oil when given for 12 weeks improve hyperinsulinemia by reducing the activation of the nuclear factor-kappaB (NF-kB) transcription factor. **Ruderman et al., (2013)** illustrated the beneficial effect might be due to the higher amounts of lignan and fiber that can improve insulin sensitivity by reducing glucose uptake speed and insulin release Furthermore, some researchers have suggested that omega-3 fatty acids in flaxseed could increase adiponectin level, which has antiatherosclerotic, antidiabetic, and anti-inflammatory properties by improving insulin sensitivity. It has also been shown that adiponectin increased AMP-Activated Protein Kinase (AMPK) enzyme activity. AMPK activity was diminished in adipose tissue of very obese insulin-resistant people and was associated with IR and oxidative stress. Also**WuJH et al., (2013)** expound that supplementation of fish oil rich in omega-3 fatty acids can elevate the adiponectin levels to stimulate AMPK in the muscle to downstream oxidative pathways and to finally improve insulin sensitivity. **Hutchins et al., (2015)** discuss the relationship between insulin resistance and hyperandrogenism is that insulin resistance can stimulate the production and secretion of androgens and ovarian failure. Therefore, improving insulin resistance is considered to be of quit importance for PCOS. Omega-3 fatty acids are the very substance that increases the sensitivity to insulin by producing and secreting anti-inflammatory adipokine (such as adiponectin) and reducing inflammation and proinflammatory cytokines.**Albert et al., (2014)**possible explanation for the necessity of long-term intervention (≥12 weeks) is that improving glucose control and insulin sensitivity require an increase in EPA and Docosahexaenoic acid (DHA) concentrations and ALA conversion to these two fatty acids is time-consuming. Also, the function of the gut microflora is enhanced gradually by flaxseed fiber consumption, which in turn led to improved blood glucose control and insulin function. **Elahe et al., (2012)** reported

that omega−3 fatty acids caused a considerable decrease in insulin resistance. **Navas et al., (2009)** explained that reduction in insulin levels and HOMA-IR in young women30 and decreased fasting insulin levels in non-diabetic, moderately hypertriglyceridaemic patients and hemodialysis patients after supplementation with omega−3 fatty acids. Lower plasma glucose and insulin concentrations were reported in studies on mice fed diets rich in EPA and DHA compared to other experimental diets.**Kondo et al., (2010)** Improvement in insulin sensitivity by omega−3 fatty acids in our patients might be resulted from elevated adiponectin level which has anti-diabetic, anti-atherosclerotic and anti-inflammatory effects. Adiponectin increases glucose utilization by activation of AMPK (AMP-activated protein kinase). Moreover, AMPK suppresses gluconeogenesis in the liver and stimulates glucose transport in muscle. It has also been suggested that inhibition of the activity and expression of glucose-6- phosphatase by n−3 fatty acids, decreases hepatic glucose output.

The revealed results demonstrated that, the group of rats were fed on supplemented diet with 1.5% of flaxseed powder/day had significantly decreased in AST, ALT and ALP level hormones compared to positive control group (PCOS), this result related to feed on supplemented diet with 1.5% of flaxseed powder/day and this result was settlement with **Kaithwas et al., (2010)** approves that flaxseed oil significantly reduced the AST and ALT enzyme levels and ablated the non-alcoholic fatty liver in hamsters along with acute and chronic arthritis in albino rat models. **Bhatia et al., (2007)** reported that flaxseed oil has been shown to lower the AST and ALT in radiation-induced hepatotoxicity in mice.

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