THE TREATMENT EFFECT OF MARJORAM LEAVES ON LETROZOLE INDUCED-POLYCYSTIC OVARY SYNDROME

Hoda S. Ibrahim, Alaa O. Aboraya, Aml F. Elgazar and Esraa S. Helala*

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Abstract

Polycystic Ovary Syndrome (PCOS) is a common condition of infertility in females and its major hallmark features are hyperandrogenemia and hyperinsulinemia.Marjoram (*Origanum majorana*) is traditionally used to restore hormonal balance and to regulate the menstrual cycle and was confirmed to have positive effect on the hormone profile of females with PCOS. The present study was conducted to evaluate the effect of marjoram on Letrozole induced-PCOS.Twenty-one female ratswere used and divided into three groups (n = 7/each): (group I): negative control group fed on basal diet. Group II and III were orally administrated LTZ 1mg/kg/day for 3 weeks. Group II kept as positive control group (PCOS-group) and fed on the basal diet only. While, the remaining group was fed on supplemented diet with 1.5% of marjoram. The obtained resultsrevealed thatfeeding PCOS-rats on 1.5% of marjoram hadsignificant increase ($p<0.05$) in Estradiol (E2) and decreased ($p<0.05$) in serum levels of insulin and glucose,luteinizing (LH), Total testosterone,Dehydroepiandrosterone sulfate (DHEA-S) hormones, andactivity of liver enzymes (AST, ALT, and ALP), while there is no significant change in Follicle-stimulating hormone (FSH) and Progesterone hormone level as compared to the positive group of 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 marjoram 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–Marjoram - Hitopathology– Rats.

Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt.
***Corresponding author:** Esraa Samy Helala **Email:** dresraasamy363@gmail.com

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a heterogeneous syndrome characterized by menstrual dysfunction, chronic anovulation, hyperandrogenism and polycystic ovaries. It commonly affects women of childbearing age; about 7 to 10 percent worldwide. The co-morbidities such as infertility, hirsutism and insulin resistant diabetes are often associated with PCOS. Multifactorial causes have been implicated for PCOS. Abnormalities in the hypothalamic-pituitary axis, hyperandrogenism, hyperinsulinaemia, chronic inflammation, environmental, genetic factors and obesity, play a significant role in the pathogenesis of this disorder. **Fatima et al., (2015)**

Plants have many properties that enable to modify the levels of female sex hormones by the action of the biologically active substances such as Origanum majorana L.Marjoram belongs to the Lamiaceae family.O. majorana L. contains biologically active substances such as (tannins, phenols, flavonoids, alkaloids, carbohydrates, terpenes, glycosides, essential oils, and phytoestrogens such as {biochanin a, daidzein, genistein, formononetin}. It has a role in reducing hyperandrogenism, insulin resistance, ovarian weight, and thus the return of natural hormones to their normal levels and ovulation**Abasian et al., (2018).**The present study was conducted to evaluate the effect of marjoram on Letrozole induced-PCOS.

MATERIALS AND METHODS

Marjoram: Dried marjoram leaves 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. Marjoram leaves were cleaned, sorted and washed from dust and all invalid parts were remove, Then,all leaves were dried in a hot air oven at 50^{o}C for 3 hrs. A grinder mill and sieves were used to obtain a powder particle size of less than 0.4mm of all leaves.

Rats and Diet: Twenty-oneadult 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)**.

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

Experimental Design and Assembly of Rats:

All female 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 three groups, Group I: negative control group (non-treated group) fed on the normal basal diet only. Group II and III were orally administrated LTZ at a dose of 1mg/kg/day for 3 weeks. Group II was kept as positive control group (untreated PCOS-group) and fed on the normal basal diet only. While, the other group were fed on the supplemented diet with 1.5% of marjoram.

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. 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.

BIOCHEMICAL ASSAY:

Estimation of serum Total testosterone, E2 and FSH Concentrations: Serum levels of total testosterone (TS), Estradiol (E2) and Follicle-stimulating hormones (FSH) were determined according to**Parker, (2006).**

Estimation of serum LH, Progesterone and DHEA-S Concentrations: Serum levels of luteinizing hormone (LH), Progesterone and Dehydroepiandrosterone sulfate (DHEA-S) were determined according to the instruction of **Jahan et al., (2018)**

Estimation of Blood glucose and Insulin hormone: Levels of blood glucose and insulin hormone were determined according to the methods of**Mohamad et al., (2018)**

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).**

Histopathological Examination: 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)**.

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 ($P< 0.05$)

Results

The Effect of Supplemented Diet with Marjoram on FSH, LH and TS hormones in rats with PCOS.

The end outcome in Table 1 shows that rats treated with LTZ alone had a significant ($p <0.05$) decrease in serum levels of FSH hormone and increase in the levels of LH and TS hormones as compared to that of the negative control rats. on the other hand, feeding PCOS-rats on the supplemented diet with 1.5% of marjoram caused almost a significant ($p<0.05$) decrease in the serum LH and TS hormones levels and no significant change in the FSH hormone level, compared to the untreated PCOS-group and fed on the normal basal diet.

Table1: The effect of marjoram on FSH, LH and TShormones in PCOS-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 + 1.5% of marjoram	0.22 ± 0.019^{c}	0.17 ± 0.013^{b}	0.55 ± 0.30^{ab}

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.

The Effect of Supplemented Diet with Marjoram on BG and insulin hormone in rats with PCOS.

Table 2 represents the effect of Marjoram on the BG and insulin hormone in PCOS-rats. Our results revealed that oral administration of LTZ induced a significant ($p<0.05$) rise in BG and insulin levels, in comparison to the negative control group. Combining the Marjoram in the diet with the oral administration of LTZ, not significantly reduced serum levels of BG and insulin hormone, compared to positive control rats fed on the basal diet alone.

Table 2: The effect of marjoram on the levels of BG, insulin hormone in PCOS-rats

Parameters / Groups	Glucose 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 + 1.5% of marjoram	69.33 ± 17.92 [ab]	0.23 ± 0.14 [ab]

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

The Effect of Supplemented Diet with Marjoram on Progesterone, DHEA-S and E2 hormones in rats with PCOS:

The effect of oral administration of LTZ alone and with feeding supplemented diet with 1.5% of marjoram on the serum levels of Progesterone, DHEA-S and E2 hormone is recorded in Table 3. Rats treated with LTZ alone (positive control group) had a significant ($p<0.05$) increase in the serum level of DHEA-S hormone and decrese the levels of Progesterone and E2 hormones, comparable to the negative control group. Co-combined oral administration of LTZ with the supplemented diets with 1.5% of marjoram results in a significant decrease in the serum level of DHEA-S hormone, non-signifcant increase in serum level of Progesterone and significant increase in the level of E2 hormone, compared to the rat-group who received LTZ orally and fed on the basal diet alone.

Table 3: The effect of marjoram on serum levels of 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 + 1.5% of marjoram	5.28 ± 1.48 [b]	1.13 ± 0.60 [b]	35.86 ±.613 [d]

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

The Effect of Supplement Diet with marjoram on the liver enzymes AST,ALT andALP in rats with PCOS:

The attained results in Table 4 exhibit the effect of provision LTZ orally without or with feeding on the complemented diet with marjoram on the liver function in rat groups. Delimited results showed that the LTZ-treated 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 a normal control group. However, combining a supplemented diet with 1.5% of marjoram and the administration of LTZ orally, results in a significant ($p<0.05$) lowering in the liver enzymes, compared with the positive control group.

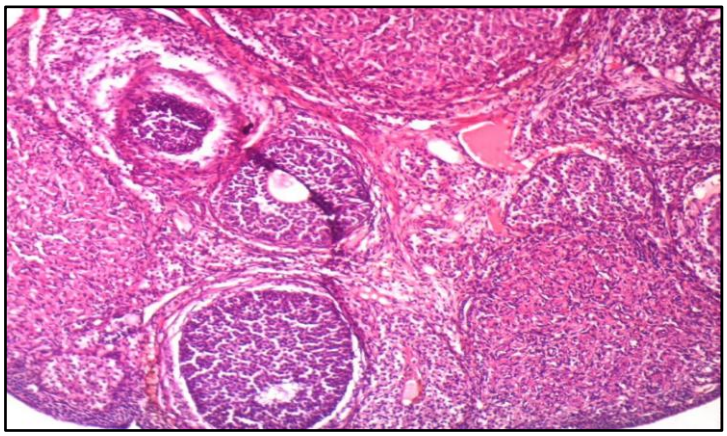
Table 4: The effect of marjoram on serum Activities of liver enzymes (AST, ALT and ALP) in PCOS-rats

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 + 1.5% of marjoram	17.87±0.344[b]	15.57±0.401[b]	102.01±3.158[c]

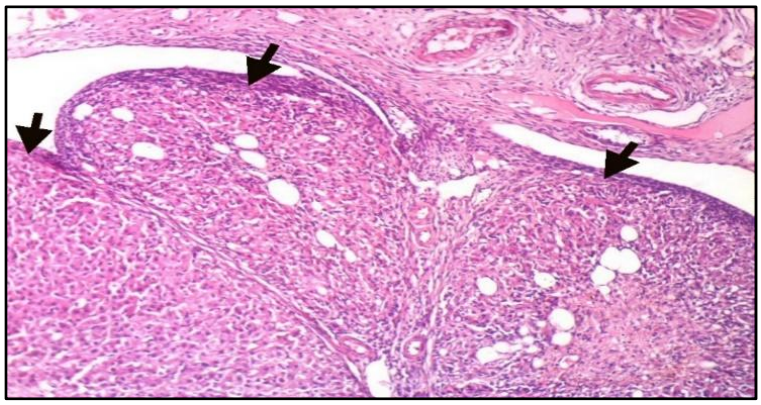
Values expressed as means ± SD; Means with different letters in each column are significantly differs at p< 0.05. **PCOS =** Polycystic Ovary Syndrome; **AST** = Aspartate Aminotransferase serum; **ALT** = Alanine aminotransferase; **ALP**= Alkaline phosphatase.

Histopathological examination of ovary:

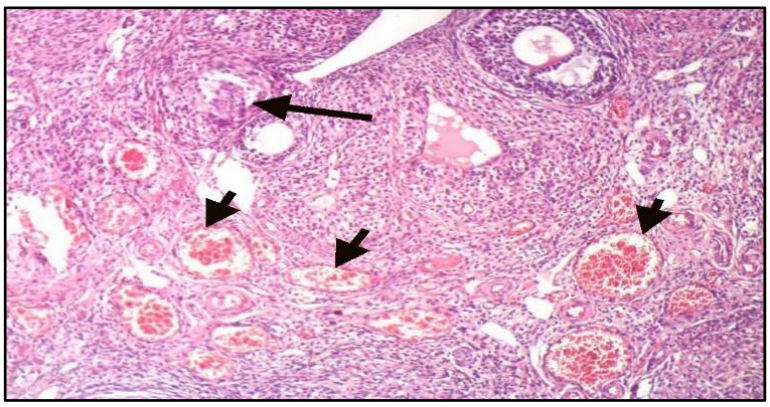
Microscopic investigation of ovary sections of the negative control group (normal rats) discovered a normal histological arrangement without any pathological amendment (numerous follicles of different types, Graafianfollicles and Corpus luteum) as shown in Picture 1. On contrary, ovary sections from LTZ-treated rats (positive control group) and fed a normal diet pronounced multiple degenerated corpus luteum (Picture 2), marked congestion interstitial blood vessels, atretic follicles ((Picture 3) and ovarian cyst (Picture 4). On the other hand, ovary sections from LTZ-treated rats and fed on supplemented diet with marjoram exhibited mild congestion interstitial blood vessels and interstitial edema (Picture 5). whereas, other sections revealed normal Graffian follicle and corpus luteum (Picture 6).



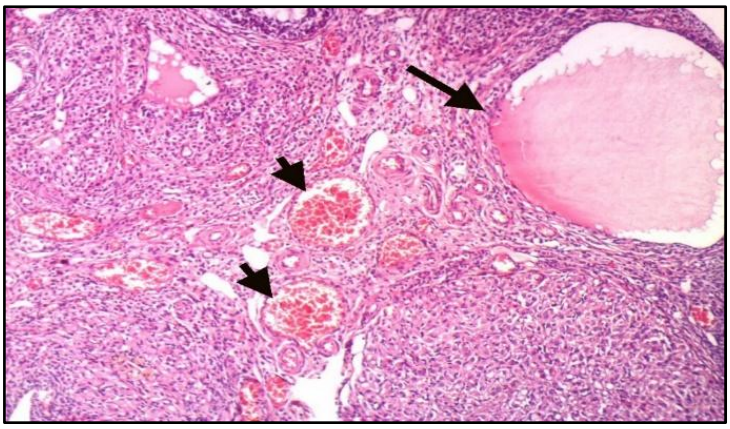
Picture 1: Ovary of rat from group 1 showing the normal histological structure (H & E X 100).



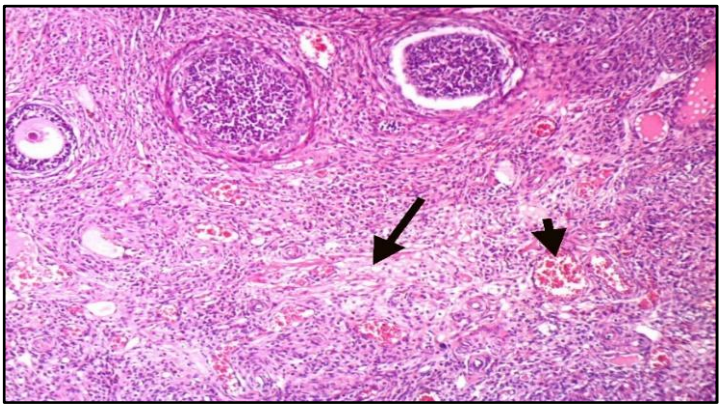
Picture 2: Ovary of rat from positive control group 2 showing multiple degenerated corpus luteum (H & E X 100)



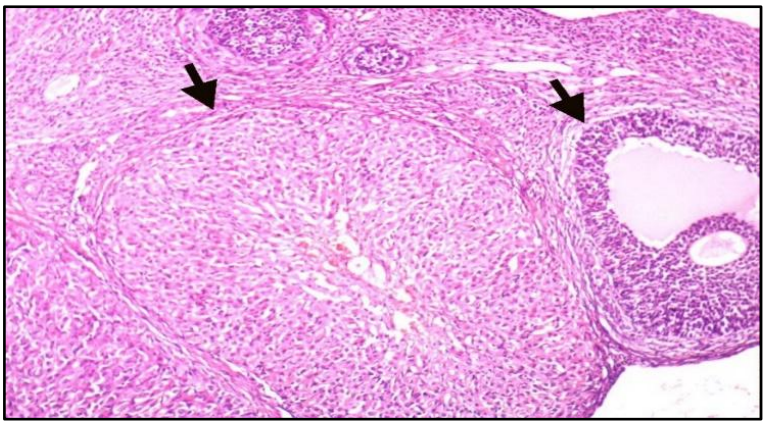
Picture 3: Ovary of rat from positive control group 2 showing congestion interstitial blood vessels (Small arrow) and atretic follicles (large arrow) (H & E X 100).



Picture 4: Ovary of rat from positive control group 2 showing congestion interstitial blood vessels (Small arrow) and cyst formation (large arrow) (H & E X 100).



Picture 5: Ovary of rat from LTZ-treated rats and fed on supplemented diet with marjoram showing mild congestion interstitial blood vessels (Small arrow) and interstitial edema (large arrow) (H & E X 100).



Picture 6: Ovary of rat from LTZ-treated rats and fed on supplemented diet with marjoram showing normal Graffian follicle and Corpus Luteum (H & E X 100)

DISCUSSION

An existing study was conducted to find out the treatment effect of Marjoram on Polycystic Ovary Syndrome Induced in Female Rats.

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**Fatmaet 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 letrazoleadmenesteration 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 enhance 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. **M.A.Morsy et 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 hepatocytesPolycystic ovary syndrome (PCOS) is a complex metabolic–endocrine disorder that affects 4–18% of women at the age of reproduction. The genetic predisposition to PCOS is uncertain, and no genetic screening test has been validated. PCOS is characterized by anovulation, menstrual irregularity, amenorrhea, hirsutism and infertility. Furthermore, various metabolic and clinical complications have been reported such as insulin resistance and diabetes, obesity, extensive coronary artery disease, hypertension, endometrial hyperplasia, ovarian and breast cancers. The secretion and metabolism of estrogens and androgens are disturbed in PCOS. The leading cause of PCOS is the excessive androgen level that induced by overstimulation of ovarian theca cells by gonadotropin - releasing hormone (GnRH).**Asmaa et al., (2021)**

The gained results showed that, the group of rats were fed on supplemented diet with 1.5% of marjoram powder/day had significantly increased in 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 marjoram powder/day and this result was conformance with **Haj et al., (2015)** who approved that Marjoram tea significantly reduced the levels DHEA-S ,LH and Total Testosterone. Therefore, it is suggested that marjoram herb may have mechanisms (that affect the adrenal androgen production**. Duha et al., (2022)** proved that significant increase in the follicle-stimulating hormone (FSH), estrogen and progesterone when treated with an aqueous extract of Origanum majorana leaves. The gonads of mature rats, where the researcher explained that the methanolic extract of Origanum majorana leaves at the maximum dose gave a stimulating effect of sex hormones and could affect the activity of different levels of the pituitary-gonadal axis and increase the secretion of reproductive hormones. **Rababa'h et al., (2020)** conspicuous that increase in progesterone and estradiol levels as a result of the increase in the activity of cytochrome p450, 17 α-hydroxylase and regulatory protein steroidogenic acute in females, enhances the synthesis of hormonal steroids when treated with alcoholic extract of marjoram**. El-Wakf et al., (2015)** Illustrated that increase the conversion of progesterone due to the high availability of aromatase with an excess of adipose tissue and thus, recording a relationship between increased aromatase activity and increased estrogen with a decrease in both the percentage of testosterone, which is particularly responsible for the development of infertility in obese, and leads to increased estrogen production who Obese people have a negative feedback effect on LH secretion through the presence of E2 receptors**. Neda et al., (2021)** demonstrated that Chlorogenic acid (CGA), the major phenolic content of marjoram,increased the concentration of FSH and progesterone level hormone production and reduced the concentration of LH and Testosterone level hormone in CGA-supplemented group in comparison with non-supplemented groups.**Chen et al., (2014)** mentioned that the low levels of LH in serum of PCOS-CGA group could be attributed to the inhibitory effects of CGA on the activity of nitric oxide synthase.31 Reducing the level of LH is necessary in order to balance LH/FSH ratio and exert a stimulatory effect of FSH on follicle growth, therefore, it seems that the low level of LH in mice with CGA-treated PCOS has helped to initiate the development of follicular groups and ovulation. **Mohd et al., (2022)** explained that gallic acid (GA), another major phenolic content of marjoram, reverses the metabolic as well as endocrine abnormalities which are linked to LETZ-induced PCOS by regulation of androgen and adiponectin circulation. Also approved that testosterone levels were lowered by GA treatment**. M. Daimon et al., (2017)** also showed a significant rise in the LH/FSH ratio, which suggests a coping mechanism against the inhibition of estrogen synthesis and estrogen circulation which in turn has an impact on metabolic metrics, particularly IR. **Q. Wang et al., (2012)** studies showed lower expression of aromatase enzyme and estrogen release in granulosa cells of DHT -treated rats were in agreement with the decrease of CYP19A1 (Aromatase (Cyp19a1) converts testosterone into estrogen in the granulosa cells of ovarian follicles) in ovaries of LETZ treated mice and found that mice given GA coupled with letrozole had higher levels of steroid synthesis-related gene mRNA expression, including Cyp19a1, required in converting androstenedione into estrogen. **Bibi et al., (2017)** GA-treated PCOS model increases significantly the concentrations of FSH and progesterone level hormones. Also, GA in dose manner decreases the serum level of LH, Estradiol and testosterone level hormones.

The achieved results demonstrated that, the group of rats were fed on supplemented diet with 1.5% of marjoram 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 marjoram powder/day and this result was conformance with **Haj et al., (2015)** who found that reduced the levels of fasting insulin and suggested to have an insulin-sensitising effect, as is evident by the significant reduction of HOMA-IR, despite the majority of participants demonstrating normal indices

of insulin sensitivity.**Rau et al., (2006)** explain that Marjoram herb has the capacity to activate PPAR-a and PPAR-c, with a stronger agonistic activity and a significant dose dependency for the gamma subtype for the ethanolic extract of marjoram. Hence, the activation of PPARs comprises the suggested mechanism justifying the observed insulin-sensitizing effect of the herb. Also **Ding et al., (2010)** illustrated that the phenolic content of marjoram aqueous extract mainly includes caffeic acid derivatives, such as rosmarinic acid, as well as glycosides of luteolin and hydroquinone. Rosmarinic acid and luteolin are among the phenolic compounds reported to exhibit an insulin-sensitizing effect via the activation of PPARs and other suggested mechanisms. Mohd **et al., (2020)** pertinence that reduction in the FBG and insulin levels as compared to the LETZ-induced PCOS group.

Our results demonstrated that, the group of rats were fed on supplemented diet with 1.5% of licorice 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 licorice powder/day and this result was settlement with **Ghaidafeh et al., (2018)** who verified that decreased ALT, AST and ALP enzymes in GA treated group. Abdolmomen et al., (2020) Confirmed that the levels of ALT and AST notably decreased after the treatment with 150 mg/kg/day and 450 mg/kg/day dosages of Origanum majorana.

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