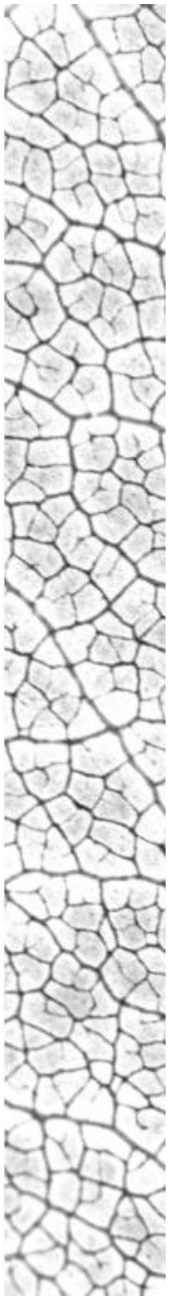

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Enhancement effect of sesame seeds oil on some physiological parameters in the serum of female mice treated with tamoxifen

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Abstract

The study aimed to investigate the protective effect of SSO against toxicity of TMX and enhances fertility criteria. The study included 25 female mice randomly divided to five groups, were rodent chow and water given ad libitum. First group served as control, 2nd group included ovariectomized mice, 3rd group received TMX 0.5 mg/kg, 4th group received SSO 5ml/kg, and 5th group received TMX + SSO.

Blood samples were collected and serum were kept in deep freeze (-20 c). Biochemical tests carried out for detection of ALT, AST, ALP, urea, uric acid and creatinin in addition to hormonal assay estrogen, progesterone, FSH and LH. Histological study carried out after laporatomy to estimate thickness of uterine wall, and diameter with number of uterine glands, while ovarian specimen used to calculate number of follicles and corpus luteum.

The results of the study recorded significant increase ($p<0.05$) in the hepatic enzymes and renal function test parameters in TMX group, also the same results were recorded in OVX group except AST, ALP and creatinin parameters. While SSO + TMX group not recorded significant differences ($p>0.05$) in above parameters and in the concentration of studied hormones. Number of ovarian follicles and corpus luteum recorded a significant decrease ($p<0.05$) in TMX treated group, but recorded non significant differences ($p>0.05$) when co-treated with SSO, but there was a significant increase ($p<0.05$) in the diameter and number of uterine glands. Concluded co-treatment SSO with TMX have protective and enhancement effect on some physiological and fertility parameters.

Keywords: sesame oil, fertility enhancement, concentration of hormones, physiological parameters, tamoxifen, female mice

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INTRODUCTION

Sesamum indicum L. one of the most important traditional healthy food, its seeds have highly valued ingredient particularly oil extract known sesame oil (SSO) (Abou-Gharbia et al., 2000; Sankar et al., 2006). Phytochemical components of sesame plant rich in phenolic compounds (phenol, lignans, and flavonoids), non-protein amino acids, glycoside, alkaloids, polyunsaturated fatty acids and lipids, mucilage, phospholipids, trace elements, minerals such as Ca, Fe, Mg, Cu, P and vitamins B1, B2 and E (Prasanthi and Rajini, 2005; Philip j.K 2010). Sesame oil has potential effects to prevent many disorders such as hypertension, hypercholesterolemia, cancer & aging (Boulbaroud et al., 2012; Reham, 2014). Additionally, useful in managing disease related to the oxidative stress such as neurodegenerative disease including Alzheimer, atherosclerosis, diabetes mellitus, chronic renal failure and rheumatoid arthritis (Azza et al., 2014; Anitha and Karuppasamy, 2011). Moreover, SSO has several physiological functions such as decreasing blood lipids, arachidonic acid levels, increasing antioxidant ability (RongChen et al., 2005) and y-tocopherol bioavailability, also provided anti-inflammatory function & potential estrogenic activity (Atef, 2018). SSO can promote synthesis of sex hormones, improves process of spermatogenesis and can recover male fertility, so advised to use this oil for cure some cases of male infertility (Alaauldeen, 2017). Moreover in male diabetic rats, SSO improved testicular microstructures & endocrine function concerned with testosterone concentration (Zahra et al., 2013).

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Higher exposure to estrogen increases incidence of breast cancer formation by two rout as genotoxic and mitogen (Amir et al., 2010). Since the existing surgical or medical procedure for ablation of the cancer don't permit elimination of estrogen completely (Boolbol and Cate, 2015), so an attention focused on estrogen blocker biosynthesis or action and on the estrogen mechanism action. One of the estrogen blockers drug known Tamoxifen widely used for the treatment of breast cancer (Deepika et al., 2018). Tamoxifen drug chemically 2-[4-(z)-1,2 diphenylbut -1-enylphenoxy]-N,N-dimethyl ethanamine, is synthetic non-steroidal anti-estrogen widely used for estrogen receptor-positive breast cancer treatment (EL-Beshbishy, 2005). Moreover, the drug decreases risk of recurrence and incidence of breast cancer but on the endometrium expressed partial estrogenic activity (Greet et al., 2011; Simsek, 2008). Also used as supplement for body building (Motrich, 2007).

Despite the beneficial effects of the drug, it has different side effects in patients with breast cancer (Liu et al., 2006), included jaundice, hepatitis, steatohepatitis, cholestasis & massive hepatic necrosis (Nishino et al., 2003). Moreover, the drug also associated with generation of ROS as an oxidative stress agent in cells leading tissue damage (Hanan et al., 2016). The treatment of cancer by chemotherapy agents not exclusively targeted to tumor cells, for their deleterious effects to the several vital tissues and organs such as reproductive system will remain an important aspect of cancer morbidity (Amir et al., 2010; Gebremeskel et al, 2016). As more people achieve long-term survival after cancer, so sexual consequences that will impact quality of life. The main aim of the study was performed to explore the modulator and protective effect of SSO against toxicity of anticancer drug TMX with special reference to improve hepato-renal parameters and enhances fertility criteria.

MATERIALS AND METHODS

Materials: Sesame seed oil prepared by (Maheen–Pakistan) and purchased from local market in Kirkuk city stored in cool place and given once in a day orally by gavages needle at volume of 5ml/kg B.W (Hussien, 2013). While Tamoxifen drug represented as tamoxifen citrate 12.5 mg (equivalent to 10 mg tamoxifen) under trade name Nolvadex (AstraZeneca UK.) and dosed as manufacturer information.

Experimental design: Twenty five female albino Swiss mice, approximately two month old, weighing 24±2 gm were housed (5 mice/cage) at controlled condition were temperature 24±2 °C and daily light / dark 12:12 hrs. Mice were fed rodent chow and tap water ad libitum . Animals of one group were ovariectomized bilaterally under anesthesia by ether inhalation while intact mice were used in all other groups.

Ovariectomy procedure: Under ether anesthesia, the bilateral ovariectomy was performed in female mice by making two dorsolateral incisions using sharp dissecting scissor, the skin and dorsal muscles were then cut and the peritoneal cavity was thus reached and fatty tissue around the ovaries were removed . The connection between the fallopian tube and the uterine horn was clamped by artery forceps and cut was made under the clamped area to remove the ovaries. Skin was closed bilaterally with one simple catgut suture. Tincture iodine solution and penicillin powder was applied locally on the skin at both sites of the operation (Lasota and Danowska-Klonowska, 2004).

Treatment: Animals were randomly assigned to the following groups:

1- Intact group treated with normal saline alone o.1 ml/mice daily served as control group.
2- Ovariectomized group treated orally with vehicle 0.1ml/mice daily.
3- Intact group treated with TMX (0.5 mg/kg B.W.) diluted and dosed daily 0.1 ml/mice.
4- Intact group treated with SSO (5ml/kg B.W.) = 0.12ml/mice orally once in a day.
5- Intact group treated with SSO (5ml/kg) + TMX (0.5mg/kg) /mice once in a day.

All animals were treated orally by gavages needle once daily for 30 days. At the end of experiment, blood were gathered by cardiac puncture in jell tubes, left for 15 minutes to coagulate and centrifuged by Centrifuge (centrion UK) for 5000 rpm for 10 minutes. Serum were kept in eppendrof tubes and kept at deep freeze (-20c) until the biochemical analysis carried out.

Biochemical assays: Levels of liver enzymes in the serum, aspartate amintransferase (AST), alanine amintransferase (ALT), and alkaline phosphatase (ALP). Renal function tests included serum urea, uric acid and creatinin. All diagnostic tests carried out with application commercially available AGAPPE kits (AGAPPE Diagnostic Switzerland GmbH) and assayed by Semi-Auto Chemistry Analyzer (Mindray BA-88A) at 540 nm as prescribed by the manufacturer information.

Serum total estrogen, progesterone, FSH & LH levels were assessed by commercial kits, and detected with Automic fluorescence Immuno-Assay (AFIAS 6) as depicted by manufacturer information.

Animals were killed by decapitation under basal conditions in a longitudinal fashion. Ovaries, uterus from each animal were weighted, immersed in 10% buffered formalin for routinely processed in a tissue processor and embedded in paraffin. Sections 5 µm were cut by microtome and stained with hematoxylin and eosin stain (Luna, 1968). Examination of prepared slides was performed by light microscope (Optica, Italy). Thickness of uterine wall and diameter of uterine glands were carried out by ocular micrometer, after calibration with micrometer stage (Galigher and Kozolof, 1964) and number of uterine glands also determined. While ovarian

Table 1. Effect of OVX, TMX, SSO and TMX+SSO treatment on some biochemical levels (mean ± SE) in the serum of female mice

Groups	Control	OVX	TMX	SSO	TMX+SSO
AST (U/L)	133.5 ± 25.5 a	169.7 ± 3.9 a	234.5 ±13.0 b	139.7 ± 2.01 a	140 ± 4.1 a
ALT (U/L)	17.2 ± 0.4 a	24.5 ± 0.6 b	20.5 ± 0.6 c	16.2 ± 0.4 a	17.7 ± 0.4 a
ALP (U/L)	336.0 ± 5.3 a	366.7 ± 5.7 a	626.0 ± 12.5 b	302.2 ± 6.9 a	342.0 ± 26.6 b
Urea (mg/dl)	35.5 ± 0.6 a	41.7 ± 0.8 b	48.5 ± 0.6 c	40.7 ± 1.3 b	39.5 ± 1.04 b
Uric acid (mmol/l)	175.5 ± 2.7 a	271.7 ± 4.3 b	384.2 ± 3.7 c	181.5 ± 7.3 a	167.7 ± 12.3 a
Creatinin (mg/dl)	0.55 ± 0.03 a	0.62 ± 0.02 a	0.67 ± 0.02 b	0.45 ± 0.03 c	0.55 ± 0.03 a

a, b, c: Small letters refer to the significant differences ($p<0.05$) between groups at the horizontal arrows.

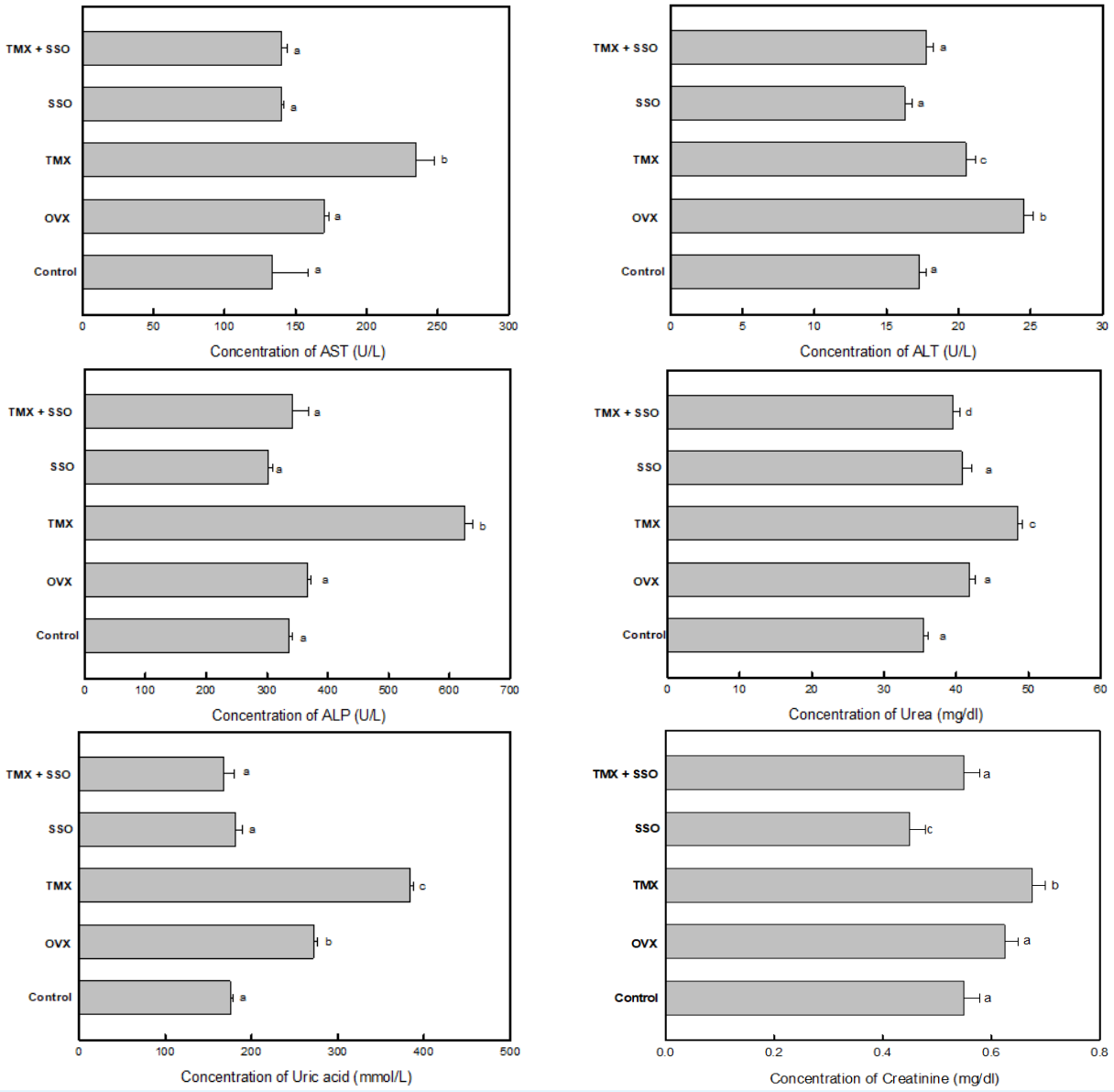

Fig. 1. Effect of OVX, TMX, SSO, and TMX+SSO treatment on the concentration of some biochemical parameters in the serum of female mice

tissues were examined for number of primary, secondary, tertiary, and graafian follicles in addition to the number of corpus luteum (Guo, 2004).

Statistical Analysis: Conventional statistical methods were used to calculate means and standard errors. Analysis of variance (ANOVA) applied to test for any significant differences ($P<0.05$). All statistics were carried out used SigmaPlot (version 12).

RESULTS

Results of Physiological and biochemical parameters in the study revealed in **Table 1** and **Figure 1** concerned

Table 2. Effect of OVX, TMX, SSO, TMX+SSO treatment on the concentration of hormones FSH, LH, estrogen, and progesterone (mean ± SE) in the serum of female mice

Groups	Control	OVX	TMX	SSO	TMX+SSO
FSH (mIu/ml)	0.85 ± 0.02 a	1.2 ± 0.2 b	0.5 ± 0.04 c	0.7 ± 0.04 a	0.7 ± 0.04 a
LH (mIu/ml)	0.46 ± 0.02 a	0.55 ± 0.06 a	0.55 ± 0.06 a	0.75 ± 0.02 a	0.77 ± 0.08 a
Estrogen (mIu/ml)	0.87 ± 0.04 a	0.42 ± 0.06 b	0.92 ± 0.09 a	1.27 ± 0.14 a	1.17 ± 0.12 a
Progesterone (ng/ml)	1.6 ± 0.1 a	0.75 ± 0.06 b	2.42 ± 0.13 c	2.22 ± 0.12 c	1.6 ± 0.14 a

a, b, c: Small letters refer to the significant differences (p<0.05) between groups at the horizontal arrows.

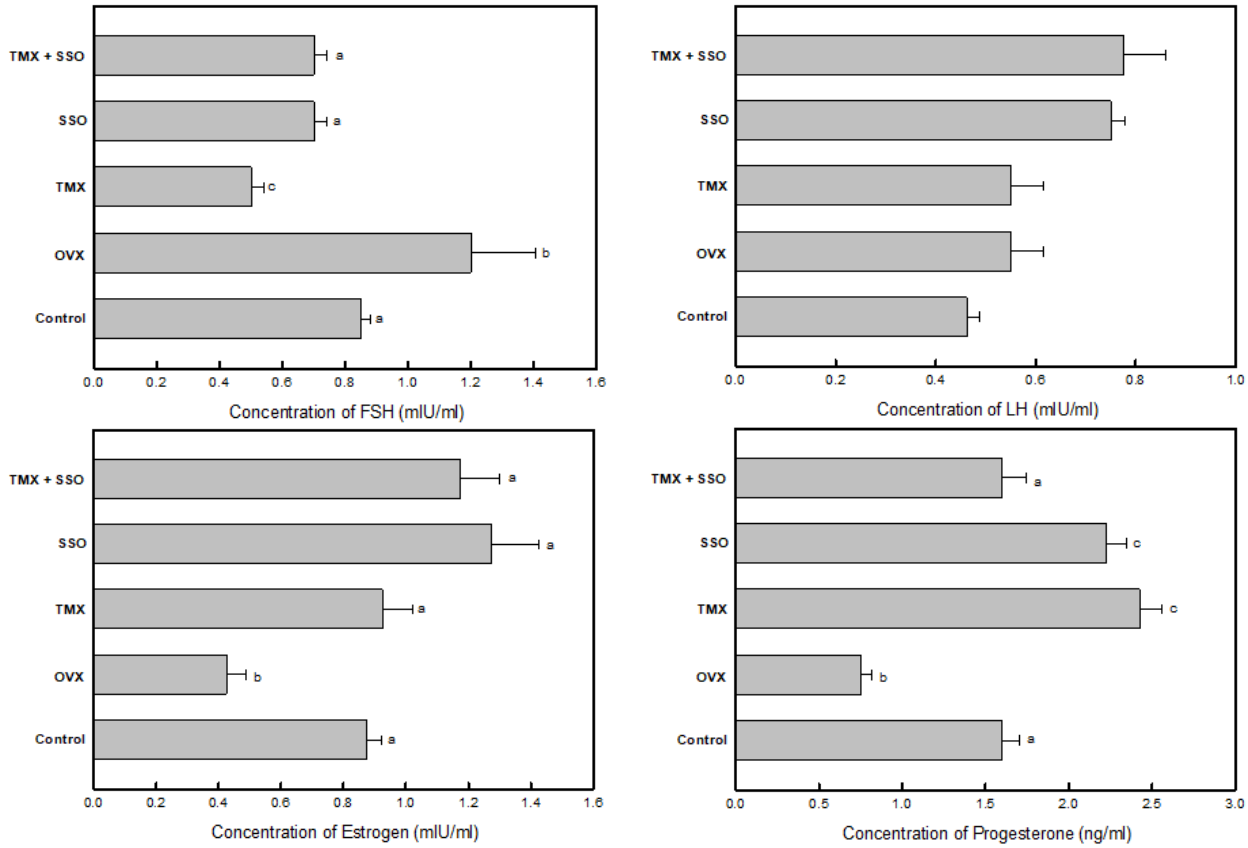

Fig. 2. Effect of OVX, TMX, SSO, and TMX+SSO treatment on the concentration of hormones FSH, LH, estrogen and Progesterone in the serum of female mice

with the concentration of some hepatic enzymes, were ALT recorded a significant increase (p< 0.05) in the OVX (24.5±0.6) and TMX (20.5±0.6) treated groups when compared with control and other groups. While the level of AST (234.5±13.05) and ALP (626.0±0.4) were recorded a significant increase (p< 0.05) in TMX treated group as compared with control and other groups.

On other hand results of some renal function tests included urea, uric acid and creatinin concentration; were urea level recorded a significant increase (p< 0.05) in the TMX treated group (48.5 ± 0.6) when compared with the control & other groups. While in OVX (41.7±0.8), SSO (40.7±1.3) and TM and SSO (39.5±1.04) groups recorded a significant increase (p< 0.05) when compared with the control. Moreover, uric acid level recorded a significant increase (p< 0.05) in the TMX group (384.2±3.7) when compared with control and other groups. While in OVX group recorded (271.7 ± 4.3) a significant decrease (p< 0.05) when compared with the TMX group (384.2 ± 3.7) & an increase when compared with the control & other groups. Also creatinin level recorded a significant increase in TMX group (0.67 ± 0.02) when compared with the control and other groups. While when treated with the SSO treated group recorded (0.45±0.03) significant decrease (p< 0.05) when compared with the control & other groups.

Table 2 and **Figure 2** showed hormonal results of the study were FSH level recorded a significant (p< 0.05) increase in the OVX group (1.2±0.2) when compared with the control & other groups. While TMX group recorded a significant (p< 0.05) decrease (0.5 ± 0.04) when compared with the control and other groups. On other hand estrogen concentration recorded a significant decrease (p< 0.05) in the OVX group (0.42± 0.06) when compared with the control & other groups. Serum LH level recorded non-significant (p> 0.05) differences between control and other treated groups. However progesterone concentration increased significantly

Table 3. Effect of OVX, SSO, TMX and TMX+SSO treatment on thickness of uterine wall (µm), diameter of uterine gland (µm), and number of uterine glands (mean ± SE) in the female mice.

Groups	Control	OVX	TMX	SSO	TMX+SSO
Thicknesses of uterine wall (µ m)	506.09 ± 5.4 a	450.8 ± 1.94 b	498.0 ±3.67 a	685.3 ± 3.1 c	523.8 ± 2.82 a
Diameters of uterine gland (µm)	73.6 ± 6.7 a	30.1 ± 1.4 b	98.6 ± 1.2 c	131.2 ± 14.1 d	130.3 ± 4.1 d
Numbers of uterine gland	20.5 ± 1.7 a	9.5 ± 1.1 b	11.5 ± 1.3 b	31.0 ± 1.2 c	27.5 ± 1.3 c

a, b, c: Small letters refer to the significant differences (p<0.05) between groups at the horizontal arrows.

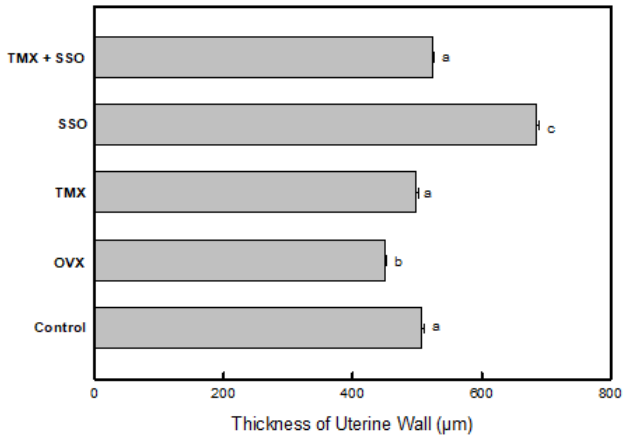

Fig. 3. Effect of OVX, TMX, SSO and TMX+SSO treatment on the thickness of uterine wall (µm) of female mice.
a, b, c: Small letters refer to the significant differences (p<0.05) between groups

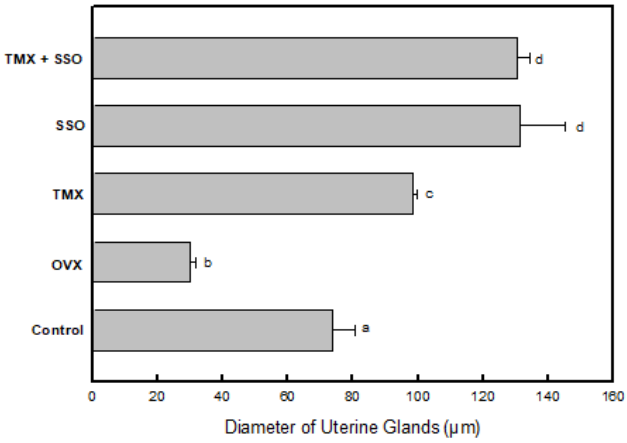

Fig. 4. Effect of OVX, TMX, SSO and TMX+SSO treatment on the diameter of uterine glands (µm) of female mice.
a, b, c: Small letters refer to the significant differences (p<0.05) between groups.

(p<0.05) in SSO (2.22 ± 0.12) & TMX (2.42± 0.13) treated groups respectively when compared with the control & other groups, while recorded a significant decrease (p< 0.05) in the OVX group (0.75± 0.06) when compared with the control and other groups.

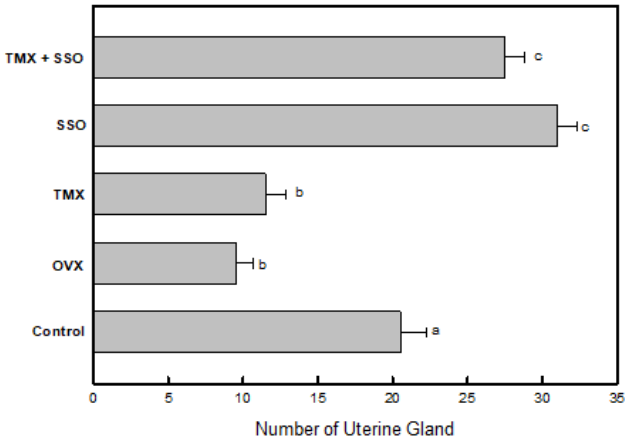

Fig. 5. Effect of OVX, TMX, SSO and TMX+SSO treatment on the number of uterine glands of female mice.
a, b, c: Small letters refer to the significant differences (p<0.05) between groups.

Table 4. Effect of TMX, SSO and TMX+SSO on the number of ovarian follicles and corpus luteum (mean ± SE) in the ovaries of female mice.

Groups	Control	OVX	TMX	SSO	TMX+SSO
Primary follicles	12.8 ± 0.3 a		5.2 ± 0.3 b	13.4 ± 0.2 a	13.0 ±0.4 a
Secondary follicles	10.8 ± 0.3 a		3.8 ± 0.3 b	10.2 ± 0.3 a	11.0 ± 0.7 a
Tertiary follicles	6.2 ± 0.3 a		2.4 ± 0.2 b	6.6 ± 0.5 a	5.8 ± 0.3 a
Graafian follicles	6.4 ± 0.5 a		2.4 ± 0.2 b	7.2 ±0.3 a	6.2 ± 0.3 a
Corpus luteum	1.8 ± 0.3 a		2.6 ± 0.2 b	2.0 ± 0.4 a	2.2 ± 0.3 a

a, b, c: Small letters refer to the significant differences (p<0.05) between groups at the horizontal arrows.

Histological study included some uterine parameters, were thicknesses of the uterine wall recorded a significant (p< 0.05) increase in SSO group (685.3 ±3.1) and decrease in the OVX group (450.8 ± 1.94) respectively when compared with the control & other groups (**Table 3**, **Figures 3** and **6**). Whereas diameter of uterine glands recorded a significant increase (p< 0.05) in the SSO ((131.2±14.1) and in the TM + SSO group (130.3±4.1) respectively when compared with the control and other groups, while in the OVX group recorded (30.1±1.4) significant decrease (p< 0.05) when compared with the control and other groups (**Table 3**, **Figures 4** and **7**). On other hand number of uterine glands recorded a significant increase (p< 0.05) in the SSO (31.0±1.2) and in the TMX+SSO (27.5±1.3) groups when compared with the control and other groups. While in OVX group (9.3±1.1) and in the TMX group (11.5±1.3) recorded a significant decrease (p< 0.05) when compared with control and other groups (**Table 3** and **Figure 5**).

However (**Table 4** and **Figure 8**) shows the results of the ovarian parameters included numbers of different stages of the ovarian follicles and corpus luteum, were TMX treated group recorded a significant decrease

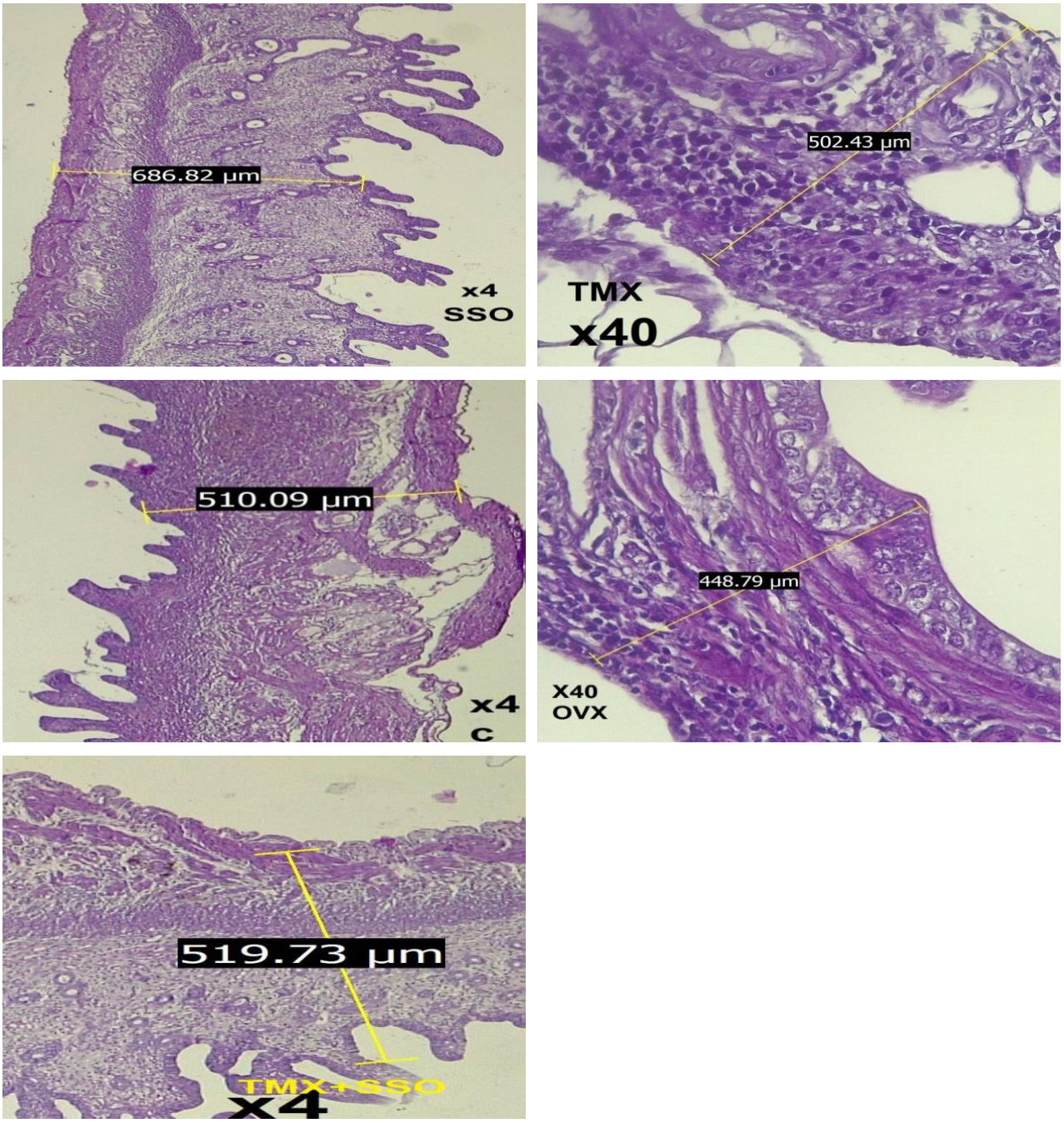

Fig. 6. Effect of OVX, TMX, SSO and TMX+SSO treatment following hematoxylin and eosin staining for measurement of uterine wall thickness (µm) from following groups of female mice:
C : control group.
OVX : ovariectomized group.
TMX : Tamoxifen treated group.
SSO : Sesame oil treated group.
TMX+SSO : Tamoxifen co-treated with Sesame oil group.

($p<0.05$) in the numbers of primary, secondary, tertiary and graafian follicles and significant increase ($p< 0.05$) in the number of corpus luteum when compared with the control and other treated groups. While there was no significant increase ($p< 0.05$) in the number of different ovarian follicles and corpus luteum in the SSO and TMX+SSO treated groups when compared with control group (**Figure 7**).

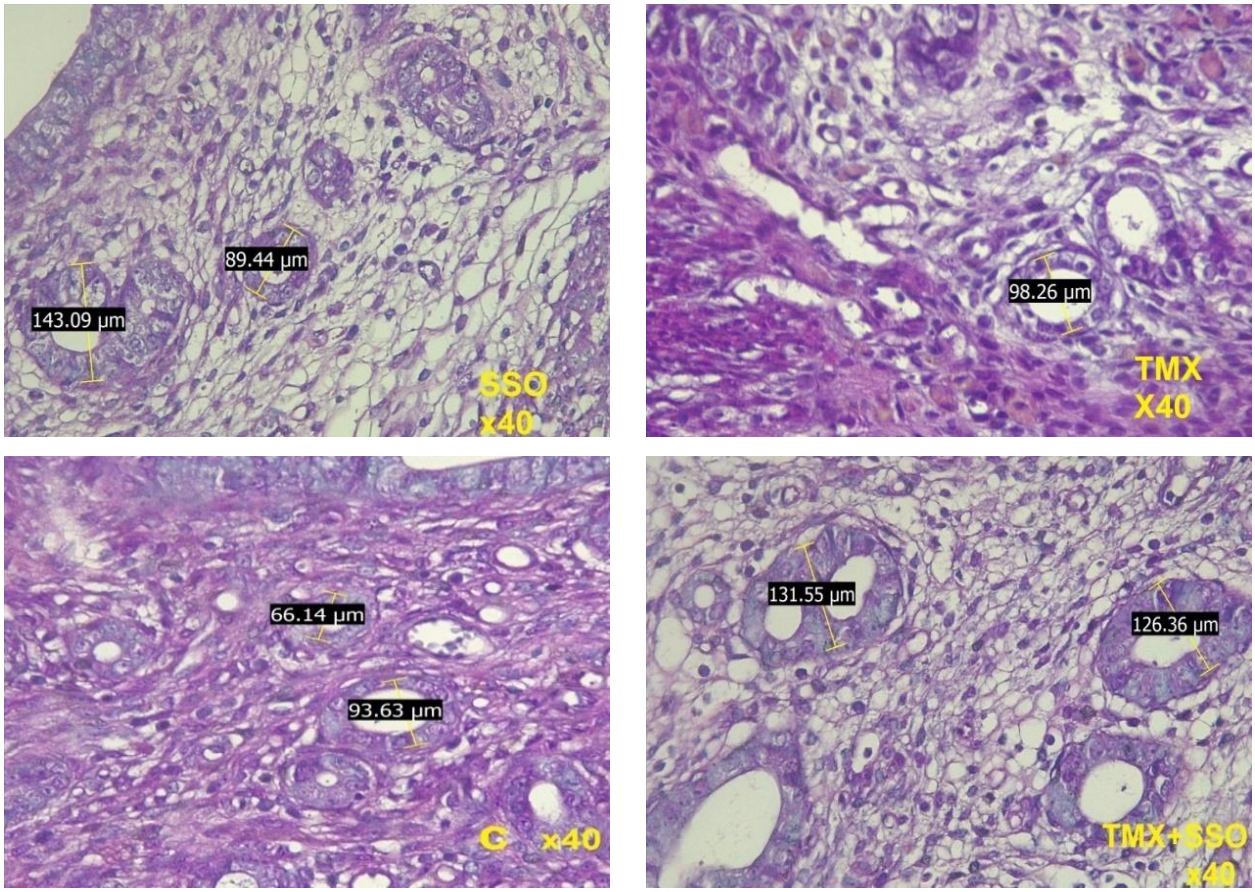

Fig. 7. Effect of TMX, SSO and TMX+SSO treatment following hematoxylin and eosin staining for measurement diameter of the uterine glands (µm) from following groups of female mice:
C : control group.
TMX : Tamoxifen treated group.
SSO : Sesame oil treated group.

DISCUSSION

The study aimed to investigate enhancement and supportive effect of sesame oil (SSO) when treated female mice with anti-estrogenic breast cancer drug Tamoxifen.

The results of the study revealed in (**Table 1** and **Figure 1**) when treated group of mice with tamoxifen recorded a significant increase ($p<0.05$) in the level of ALT, AST and ALP. Similar results were reported in many experimental investigations on female rats when treated with TMX (Faried, 2007; Ghada, 2014).

Hepatic enzymes ALT, AST and ALP are included in amino acids metabolism. Transaminases have a great role in conversion of ketoacids to amino acids (Abolfazl et al., 2014), thus disturbance in their activity can appeared damage effects. Two amino acids, glutamic and aspartic acids have great role in transferring ammonia to urea cycle, thus disturbance of enzymes included in the amino acids metabolism would be deleterious and increase of ALT level indicated to hepatic damage.

However, treatment of mice with 0.1mg TMX daily for 28 days recorded a significant elevation in hepatic enzymes ALT, AST and LDH in the serum (Zaina et al., 2011).

Also rats treated with TMX by I/P rout for 7 days recorded remarkable elevation in hepatic transaminases enzymes and TBARs with decrease in antioxidant enzymes GSH, SOD and catalase (Hesham et al., 2005).

In the present study the OVX group recorded a significant increase ($p<0.05$) in the concentration of ALP, urea and uric acid as compared to the control group (**Table 1** and **Figure 1**).

We suggest that the ovariectomy operation damages some tissues around injury sites or may be oxidative stress takes place and generate ROS so increases liberation of transaminases enzymes and elevated their concentrations in the serum of ovariectomized group.

Previous investigations indicate that p- Nonylphonel causes elevation of ALP activity in the cell culture (Wober J.et al., 2002; Kanno S. et al., 2004) which is in agreement with present study.

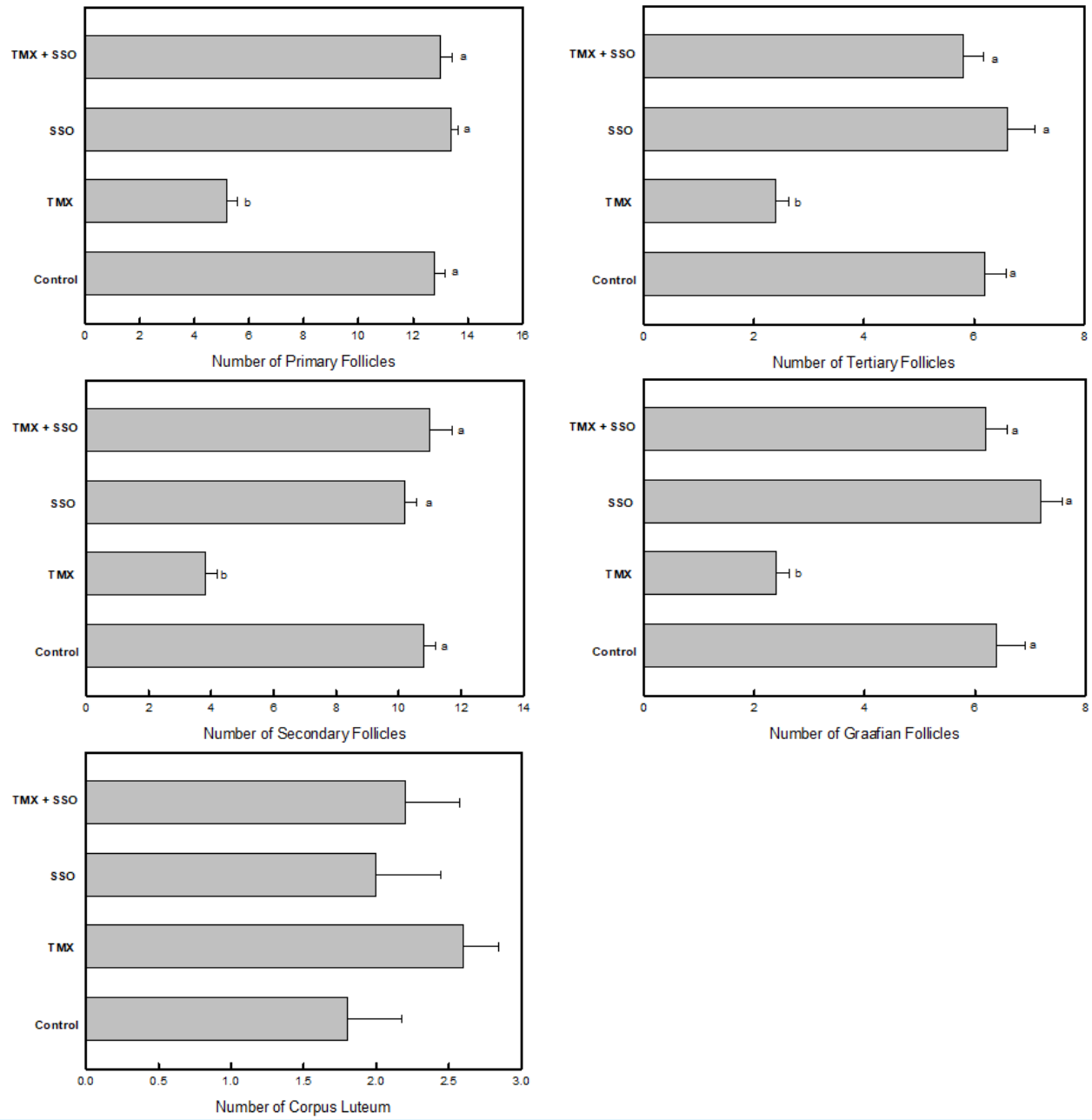

Fig. 8. Effect of TMX, SSO and TMX+SSO treatment on the number of ovarian follicles at different stages and corpus luteum (mean ± SE) in ovaries of female mice.
a, b, c: Small letters refer to the significant differences ($p<0.05$) between groups.

The study also showed Co-treatment of TMX group with 2ml SSO daily for 30 days ameliorate and compensate deleterious effects of TMX at the level of hepatic enzymes as shown in (**Table 1** and **Figure 1**), were their concentrations recorded non significant differences ($p>0.05$) as compared to the control group.

Co-administration of SSO to rats exposure to cyperomethrin recovered GOT and GPT concentration in the serum through ameliorate of some antioxidant enzymes such as GSH, SOD and catalase (Hussien et al., 2013; Sahar et al., 2019). However in other study, the treatment of female rats with TMX significantly inhibited the elevation of hepatic enzymes in serum when pretreated these rats daily with thymoquinone 50mg/kg. B.W (Ghada, 2014).

Also, administration of aqueous extract of chicory root to TMX treated rats markedly decreased ALT, AST and ALP concentration (Alaaudeen, 2017), the same results obtained in the study of Hamid et al. (2015) when treated rats with Silybum marianum plant extract for 24 days.

However, TMX- induced hepatic injury in rats recorded an improvement of hepatic and antioxidant

enzymes when treated these rats with green tea extract orally for 18 days (Hesham, 2005).

The study also revealed that, the treatment of mice with 2ml SSO /day alone did not effects the activity of liver enzymes and other biochemical constituents such as ALT, AST, ALP, urea, uric acid and creatinin (**Table 1** and **Figure 1**),these results were in agreement with other studies (Mohamed et al., 2015; Farhan et al., 2017).

Table 1 and **Figure 1** revealed results of some renal function test parameters when treated group of mice with TMX for 30 days were recorded a significant increase ($p<0.05$) in the level of urea, uric acid and creatinin as compared to the control & other treated groups. These results indicated mostly for renal injury caused by the TMX and/or with their metabolites and impaired renal function, these data were in agreement with (Hanan et al., 2016), who mentioned that the renal tissue damages of mice related with the impairment to the vasculature or/and microstructure of the kidney associated to the oral administration of TMX

The nephrotoxicity may be rises from TMX drug which generates ROS and cellular thiol system depletion that mediates kidney damage, moreover ROS interact with the renal mitochondria and generates oxygen radicals and causes damage to the renal architecture (Tebassum et, al; 2007).

While treatment group of mice with 2ml SSO recorded a significant decrease ($p< 0.05$) in the level of urea, uric acid and creatinin, suggested the preventive effect of SSO against degradation of protein, nucleic acid and creatine, by activation of antioxidant defense mechanism with the SSO supplementation (Palanisamy et al., 2012). Also in another study recorded in both TMX and OVX groups high levels of creatinin in their serum which considered marker of muscle wastage (Luo and Luo, 2009).

However, administration of green tea extract to long – term diabetic nephropathy recorded suppressing hyperglycemia & prevent accumulation of glycogen in the proximal convoluted tubules, so enhanced GFR and improved urea, uric acid and creatinin level (Renno et al., 2008; Hasan and Abass, 2020) .

Results of the study concerned with the hormonal alterations as shown in (**Table 2** and **Figure 2**) recorded a significant decrease ($p < 0.05$) in the concentration of estrogen & progesterone in the serum of OVX group, this decrement belongs to the removal of ovaries from mice. In fact ovaries were a major source for synthesis & secretion of steroidal hormones, so significant decrement of ovarian hormones would stimulate pituitary gland by feedback mechanism for synthesis and secretion of gonadotropic hormones FSH and LH, therefore the study recorded a significant increase ($p< 0.05$) in the level of FSH and non significantly in the level of LH (**Table 2** and **Figure 2**).

The results of (Clarke and Cummins, 1988) showed that, there was a direct effect of estrogen on the pituitary gland of Ewes for action of GnRH, while progesterone alone does not have such direct effect for release of LH or FSH , these results also proved in female Balb/C mice (Fazeleh et al., 2016).

So the decrement effect of ovarian hormonal level in OVX group showed at the uterine microstructure, were histological study recorded a significant decrease ($p< 0.05$) in the thicknesses of uterine wall, also number and diameter of uterine glands (**Table 3** and **Figures 3-7**). In fact ovarian hormones have anabolic activities on uterine muscles & glands suggested that uterus being a responsive tissue to ovarian hormones especially estrogen (Ghadire and Zahra, 2017). 28 days after ovarioctomy, there was a marked decrease in the thickness of the endometrium as compared to intact mice, this decrement in endometrial thickness belongs to decrease in both the number & the size of the endometrial glands accompanied by a condensed endometrial stroma (Zougrou et al., 2018).

Hormonal study also showed the treatment group of mice with TMX drug recorded a significant increase ($p < 0.05$) in the level of progesterone & non significantly increase ($p> 0.05$) in the level of estrogen (**Table 2** and **Figure 2**).These alterations of hormonal levels suggested that it may be attributed to the adverse effects of TMX on the ovarian tissues certainly follicular theca and granulose cells causes atresia of follicles at different stages, so decreased in the number of ovarian follicles and increase in the corpus luteum numbers as shown in (**Table 4** and **Figure 8**) were considered as a main source of estrogen and progesterone.

Treatment of female rats with TMX drug recorded non-significant ovarian histological alterations with decrement of follicular numbers (Ibrahim et al., 2019). Also exposure of fischer rats to 100mg/kg B.W./day of methoxychlor (MXC) recorded a marked increment in the percentage of antral and preantral ovarian follicles with decline percentage of corpus luteum, were suggested that MXC had altered ovarian gene expression and folliculogenesis (Annmarie et al., 2008).

Fluctuation of ovarian hormones in the serum of TMX treated group were responded by the anterior pituitary gland, so level of FSH recorded a significant decrease ($p< 0.05$) with non-significant differences of LH level through feedback mechanism regulation (**Table 2** and **Figure 2**)

A significant decrease in plasma LH level were recorded in female rats treated with 0.1mg/kg TMX, while there was no significant changes in pituitary and plasma LH level when companied with an elevation in plasma estradiol (Natalia et al., 2015).

On the other hand, para-nonylphenol treatment does not effect to the FSH concentration which was not compatible with our results of the study. While different results recorded by other studies were FSH level

declined in (Masutomi et al, 2003), or increased in (Han et al, 2004) as we found, it is obvious that p-NP effects on hormones concentration which has a complex mechanisms (Soleimani, 2007). So we suggest all together alterations in anterior pituitary hormones could be effects to the ovarian histological components, therefore ovarian hormonal release and subsequently influences to the uterine tissues.

Histological study of the uterus in the TMX treated group recorded a significant increase ($p< 0.05$) in the myometrium thicknesses as compared to the OVX group. However, there was a marked increase in the diameter of uterine glands as compared to the control and OVX groups. While number of uterine glands were recorded a significant increase & decrease as compared to OVX and control groups respectively (**Table 3** and **Figure 5**).Tamoxifen showed antiestrogenic activities at breast tissue and on endometrium expressed estrogenic activity so processes hyperplasia and carcinoma in endometrium were induced (Simsek and Sever, 2008).

On the other hand, treatments of female mice with SSO recorded a significant increase ($p< 0.05$) in the level of progesterone and non-significantly ($p > 0.05$) in the level of estrogen (**Table 2** and **Figure 2**).

These results may be attributed to the active components of SSO which promotes synthesis and release of hormones directly by the gonads or indirectly enhances of some enzymes that have role in the synthesis of steroid hormones of the ovaries, or may be SSO have estrogenic activity for phytoesterol components. Phytoesterogens one of active constituents of SSO (Lewis et al., 2003; Nakari, 2005) can alter metabolism of the cholesterol (Ostlund et al., 2002), and has estrogenic activity or activate enzymes for synthesis of steroid hormones (Laurenzana et al., 2002). Moreover, flavonoids considered one of the most beneficial components of medicinal plants extract stimulate and activate an enzyme known aromatase which stimulate conversion of androgens to estrogen and progesterone, then enhances folliculogenesis (Van et al., 2007).

So alterations of ovarian hormones in the SSO treated group in this study, may be belonged to the ovarian histological changes, and number of corpus luteum were non significantly ($p> 0.05$) increased but significantly ($p < 0.05$) increased in the number of ovarian follicles as compared to TMX treated group and subsequently synthesis and release of FSH from anterior pituitary gland were significantly increased ($p< 0.05$) as a result of alterations in the concentration of ovarian hormones.

Follicular stimulating hormone in female stimulates growth and maturation of ovarian follicles for synthesis of estrogen by granulose cells, while LH stimulated theca cells for secretion of androgens and ovulation of mature follicles (Bernard, 2010). Treatment of female mice with date palm pollens (DPP) significantly increase estrogen and progesterone level but this increment were no significantly recorded in the pituitary hormones FSH and LH (Annmarie et al., 2008).

Moreover, significant increase of estrogen level in the SSO treated group may correlate to the follicular proliferation and development in this study. Influences of ovarian hormones on the uterine tissues in the SSO treated group were clearly revealed from histological study, were recorded a marked increase ($p <0.05$) in the uterine wall thickness and diameter with number of uterine glands as compared to the control and other treated groups (**Table 3** and **Figures 3-7**). In fact ovarian hormones have anabolic activities on uterine tissue through induction of protein transcription mechanism (**Figure 6**).

Co-treatment of TMX with SSO recorded non-significant increase ($p > 0.05$) in the level of estrogen, progesterone, FSH and LH as compared to the control group. These results referred to the ameliorating and normalizing effect of SSO against adverse effects of TMX concerned with the hormonal and histological level. So the results of the study showed hormonal and histological data in the (**Table 4** and **Figure 8**), were number of ovarian follicles and corpora lutea also recorded non-significant differences as hormonal parameters when compared to the control group, while at the uterine study, co- treatment of SSO with TMX recorded a significant increase ($p<0.05$) in the diameter and number of uterine glands but not significantly ($p>0.05$) in the thickness of uterine wall as shown in (**Table 3** and **Figures 3-7**).

We suggest the SSO have enhancement effect on the functional glandular components of the uterus but have protective role for the uterine muscle through regulation of protein transcription process in the myometrium fibers, additionally to the partial estrogenic effect of TMX on the estrogen responsive tissues as uterus (Greet et al., 2011; Al-Mafrajy and Abass, 2019).

Damage of germ cells in male rats exposure to acrylamide for 54 days with significant decrement in somniferous tubules diameter documented by (Shler et al., 2015), while such rat when treated with 2ml SSO/day improved release of sex hormones and increase spermatogenesis (Alaaudeen et al., 2017) so recommended to use SSO for treatment of male infertility .

Finally, we concluded from the results of the study that exogenously administrated SSO have modulator effects on the toxicity of the anticancer drug TMX with special reference to the protection role for hepato-renal parameters with enhancement of some fertility criteria.

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