

Original article



Toxic Effects Of Orlistat And Green Tea Extract On Testes of Adult Albino Rats:(Comparative Study)

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ABSTRACT

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Background: Obesity is a serious medical health problem in the world. Orlistat drug and green tea herb have an important anti-obesity effect. **Aim of the work:** This study evaluated the chronic toxic effects of orlistat and green tea extract on testes of 36 adult male albino rats after 45 & 90 days with orlistat & green tea extract (GTE). **Material and methods:** The study was conducted for 90 days on 36 male adult albino rats, divided into three groups; (each one subdivided into 2 equal subgroups). Group I: control group, group II: treated orally with orlistat at a dose of 500 mg/kg/day. Group III: treated orally with GTE at a dose of 700 mg/kg/day. At the start of the experiment, after 45 & 90 days body parameters of all rats were calculated before scarification then, their blood and testicular tissues were subjected to biochemical and semen analysis, while the remaining testicular tissues were stained for histopathological and immune-histochemical studies. **The results:** administration of orlistat for 45 days showed mild histopathological changes. after 90 days, there were a significant decrease of testosterone with deterioration of sperm motility & shape abnormalities percentage & sperm count without effect on LH, moderately positive cytoplasmic and nuclear caspase 3 reaction & no changes in periodic acid schiff (PAS) stain. GTE administration showed time dependent decreased testosterone, increased serum LH, deteriorated semen parameters, marked histopathological changes & thickening in basement membrane of seminiferous tubules with PAS. Strong positive cytoplasmic and nuclear caspase 3 reaction. Oxidative stress on testes occurred after 90 days only in GTE group. **Conclusion:** chronic consumption of GTE induced more weight loss and testicular toxicity compared with orlistat. **Recommendation:** Abuse of orlistat and GTE should be avoided and restricted by medical prescription to avoid their toxicities.

Keywords: Obesity – Orlistat – Green tea – Testicular toxicity – Oxidative stress – Apoptosis

I. INTRODUCTION:

Obesity is a serious medical health problem in the world. Its prevalence has dramatically increased over several decades (Xu et al., 2015). It has many adverse effects on health such as type 2 Diabetes mellitus (T2DM), cardiovascular diseases, non-alcoholic fatty liver disease and fertility problems in both sexes (Du Plessis et al., 2010, Haslam, 2016 and Joseph et al., 2019). Drugs, diet, behavioral therapy, and physical activity are used for reducing body weight. Multiple anti-obesity drugs on the market pose significant health risks and few of them are food and drug administration (FDA) approved (Maksimov et al., 2016).

Orlistat, an anti-obesity drug approved by FDA, is the most commonly used worldwide. It reduces weight via inhibition of gastric and pancreatic lipase Mahmoud and Elnour (2013). Ahmad and Mahmud (2016) reported several side effects of orlistat such as fatty stool, nausea, insomnia and occasionally high blood pressure, irregular menstrual cycle, upper respiratory tract infections, pancreatitis, cholestatic hepatitis and tubular necrosis.

Green tea as a herbal supplement is considered the second beverage after water to be consumed. Recently, the anti-obesity effects of green tea in animals and humans have slowly become an important issue for molecular nutrition and food research (Grove et al., 2010). Several studies noted that extra intake of green tea complements induced many toxicities such as hepatotoxicity, nephrotoxicity and geno-toxicity (Lambert et al., 2010, Li et al., 2010 and Inoue et al., 2011). Kao et al. (2000) and Adhami et al. (2003) reported testicular affection after administration of green tea, which caused significant reduction in testosterone hormone level.

Although a toxic effect of orlistat on testes was reported by Sakr (2010) references to male reproductive toxicity induced by

orlistat in the literature are few. Consequently, the aim of this work was to evaluate the chronic toxic effects of orlistat on hormonal, biochemical parameters, semen analysis, histopathological and immunohistochemical changes in the testes of adult male albino rats and to compare it with the effects of green tea extract.

II.MATERIALS AND METHODS

II.1. Materials:

II.1.1. Chemicals:

Xenical capsules, each containing 120 mg orlistat and manufactured by the Switzerland-based company Hoffmann-La Roche Pharmaceuticals, can be purchased over-the-counter from retail pharmacies. Green Tea Fat Burner capsules, each containing 400 mg green tea extract (with 50 % epigallocatechin gallate (EGCG) and 160 mg caffeine, and manufactured by the Applied Nutrition Company of Los Angeles, are available over-the-counter from retail pharmacies.

II.1.2. Animals:

Thirty-six adult male rats, weighing between 220-300 grams were obtained from the Animal House of the Faculty of Medicine, Zagazig University. These weights are suited to the purposes of the study of anti-obesity agents, according to Mack et al. (2006) and Tavana et al. (2015). All animals were treated humanely in compliance with the Animal Care Guidelines and Ethical Regulations in 'The Guide for the Care and Use of Laboratory Animals' (Institute of Laboratory Animal Resources, 2011). The Institutional Review Board on animal research at Faculty of Medicine, Zagazig University, Egypt granted approval for the use of animals (No. 3400/12-2-2017). They were exposed passive preliminaries over a period of 14 days in order to acclimatize themselves to their new environment.

II.1.3. The Experimental design

The rats were randomly and similarly divided into three equal main groups; each

group was equally subdivided into two equal subgroups. Subgroup A: 6 rats were sacrificed after 45 days. Subgroup B: 6 rats were sacrificed after 90 days. Group I (control group): Each rat received only regular diet and tap water to measure the basic parameters. Group II (Orlistat treated group): Each rat received 500 mg/kg B.W (represents 1/10 of LD50 of orlistat in rat) once daily given by gavage. LD50 of orlistat in rat is (> 5000 mg/kg) so, 1/10 LD50 equals 500 mg/kg B.W. according to EMEA, (2005). Group III (Green tea extract treated group): Each rat received 700mg/kg (represents 1/10 of LD50 of green tea extract in rat) once daily given by gavage. LD 50 of green tea extract in mice (1000 mg/kg) according to Besnard et al. (2008) then calculated by using Paget table to obtain used dose in rats, 1000 mg/kg in mice equal to 7000 mg/kg in rat. One tenth (1/10) of LD₅₀ of green tea extract in rat is equal to 700 mg/kg (Paget and Barns, 1964).

Both orlistat and green tea were dissolved in distilled water.

II.2. Methods

II.2.1. Body measurements

At the start of the experiment, after 45 and 90 days, rats from all groups were weighed. Their weights were recorded and body lengths were measured. Body mass index (BMI) was calculated as follows:

II.2.2. Luteinizing hormone (LH) and Testosterone estimation :

Venous blood samples were gathered from animals in micro-capillary glass tubes from the retro-orbital plexus and stored deep frozen at -20°C (Johnson, 2007) for estimation of LH using Rat ELISA kit with Cat. No. (KT-21064) from Kamaya Biomedical Company. and testosterone hormone levels using Mouse / Rat Testosterone ELISA kit with Cat. No. (55- TESMS-E01) from Alpco Diagnostics according to manufacturers' protocols.

II.2.3. Oxidative stress parameters in testicular tissue

Immediately after the rats' scarification, one testis from each creature was taken, then homogenizing about 1 gram of one testis in cold phosphate buffer (pH 7.4) using tissue homogenizer, prepared according to Kheradmand et al. (2009) to obtain the supernatants, which were separated and used immediately for assessment of malodialdehyde (MDA) (Catalogue No. K739) and reduced glutathione (GSH) (Catalogue No. K261). They purchased from Biovision Company, Dar El Salam, Cairo, Egypt.

II.2.4. seminal collection for semen analysis

Cauda epididymis of one testis was macerated in a sterilized petri dish holding 2 ml warm normal saline to obtain the epididymal contents (Hafez, 1970). the following examinations were done:

II.2.5. Sperm motility assay:

This assay was carried out according to Slot et al. (1991). A drop of the suspension was put on a clean glass slide pre-warmed at 37° C, covered by a pre-warmed glass cover slide at 37° C, and was examined under high power X40 of light microscope for evaluating the individual motility of spermatozoa. Several microscopical fields were examined to evaluate the percentage of motile spermatozoa.

II.2.6. Sperm cell count per ml of semen:

The concentration of sperm cells was performed according to the method of Robb et al. (1978); where 200 µl of the semen were taken by automatic pipette, put in plastic tube containing 800 µl of normal saline previously added to it few drops of formaline 40% to kill spermatozoa and mixed well, then used for counting the spermatozoa in an improved hemocytometer counting chamber.

II.2.7. Sperm shape abnormalities:

Sperm abnormalities were observed in eosin-negrosin stained smears, a drop of 5% aqueous bluish eosin solution about its double size, then a drop of negrosin 10% twice the size of eosin was placed on the slide, followed by brief, careful mixing of semen with eosin then negrosin was included to the mixture. The mixture was smeared out on slides and examined under oil immersion lens after drying. One hundred sperm were examined randomly in different fields, the percentage of abnormal spermatozoa was determined according Filler (1993).

II.2.8. Histopathological studies:

The other testis was fixed in 10% formalin, and then the samples were prepared using the Paraffin Technique. The samples were blocked in hard paraffin and cut into sections of 5 micron thickness and prepared for Haematoxylin (H) & Eosin (E) and periodic acid schiff (PAS) stains according to (Bancroft and Gamble, 2008). The slides were examined under light microscope. Johnsen's testicular score system was used to calculate the histopathological alterations. The score between 1 (very poor) and 10 (excellent) was estimated in each tubule.

II.2.9. Immuno-histochemical analysis:

After deparaffinization, tissue sections were treated with H₂O₂, heated in citrate buffer, blocked in normal serum and then sections were incubated with rabbit polyclonal IgG anti-caspase-3 antibody (Catalogue No. PA5-16335, Thermo Fisher Scientific, Rockford), which was diluted at 1:200 in PBS. Sections then were hatched with a goat anti-rabbit IgG biotin-conjugated secondary antibody (1:2,000). After further incubation with horseradish peroxidase-labeled streptavidin, binding of antibody was visualized using diaminobenzidine, and the sections were counterstained with hematoxylin (Ramos-Vara et al., 2008).

II.2.10. Statistical analysis

Data were analyzed by Statistical Package of Social Science (SPSS), software version 19 (Chicago, USA). Independent t-test was used for comparison between means of two groups. Comparison between means of two groups to test the difference between the two periods of the study was done by paired t-test. Comparison of several means were done by One Way Analysis of Variance (ANOVA), followed by Least Significance Difference (LSD) test for multiple comparisons between groups.

III. RESULTS

III.1. Body parameters and testicular weight:

Orlistat and green tea groups showed significant decrease in the mean values of total body weights, BMI and testicular weight after 45 days and 90 days when each of them compared with initial values and even when compared with the control group. GTE caused high significant reduction in total body weights and BMI and testicular weights when compared with orlistat-treated group after 45 and 90 days ($p < 0.001$) (figure 1,2).

III.2. Biochemical results (LH and testosterone levels):

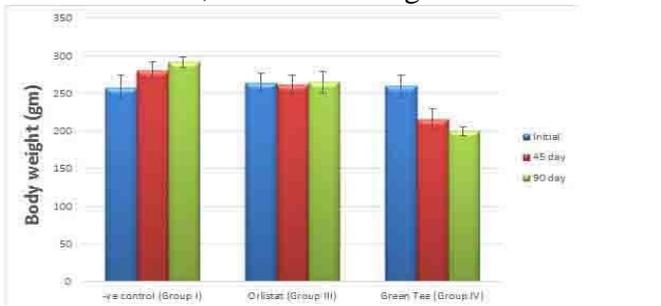
Orlistat treated group showed non-significant changes in LH and testosterone levels after 45 days. However, there were significant reductions in serum testosterone hormone level after 90 days when compared with control group. Green tea extract (GTE) caused a significantly high reduction in serum testosterone hormone levels together with significant increase in serum LH levels after 45 and 90 days when compared with the control group. There were significantly high decreases in serum testosterone hormone and significant increase in serum LH in the GTE-treated group after 45 days and 90 days, compared with the orlistat-treated group (table 1).

III.3. Oxidative stress parameters:

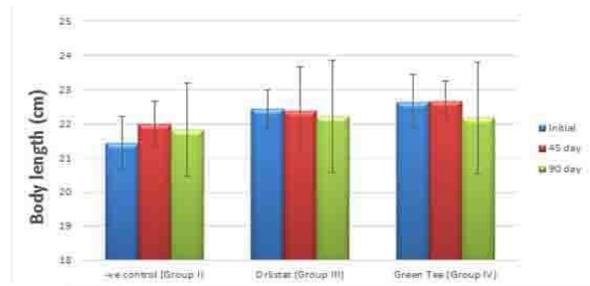
Orlistat caused significant reduction ($p < 0.05$) of testicular MDA level and high significant

increase in testicular GSH level ($p < 0.001$) after 45 & 90 days when compared with control group (table 2). This means that orlistat was a strong antioxidant. Green tea extract (GTE) caused non-significant difference ($p > 0.05$) of testicular MDA and GSH levels when compared with control group after 45 days. This indicates absence of oxidative stress after 45 days. However, it showed high significant ($p < 0.001$) increase in testicular MDA with high significant ($p < 0.001$) decrease in testicular GSH when compared with control group after 90 days indicating oxidative stress process. In addition, there was a significant increase in

the mean values of testicular MDA in GTE treated group when compared with orlistat-treated group after 45 days ($p < 0.05$) and after 90 days ($p < 0.001$) (table 2). As for testicular GSH, there was a significantly high increase in the orlistat-treated group when compared with the GTE-treated group after 45 and 90 days ($p < 0.001$) (table 2). In the present study orlistat acts as antioxidant all over the experimental period, whereas GTE exerted oxidative stress on testes after 90 days, but this effect was absent after 45 days.

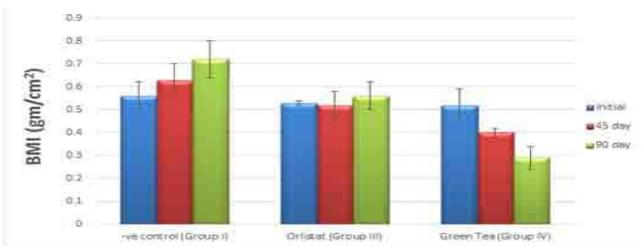


Bar chart for comparison among negative control, orlistat and GTE treated groups all over the period of the study as regard mean values of body weights (gm).

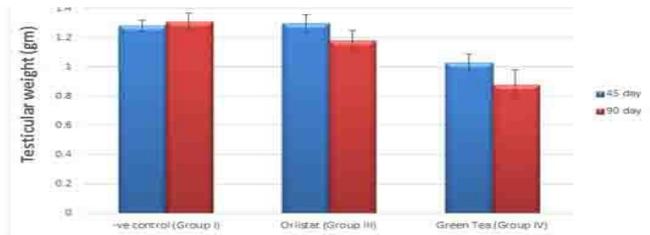


Bar chart for comparison among negative control, orlistat and GTE treated groups all over the period of the study as regard the mean values of body lengths (cm).

(Figure 1): Bar chart for comparison among negative control, orlistat and GTE groups all over the period of the study as regard mean values of body weights (gm) and the mean values of body lengths (cm).



Bar chart for comparison among negative control, orlistat and GTE treated groups all over the period of the study as regard mean values of body mass index (BMI) (gm/cm²).



Bar chart for comparison among negative control, orlistat and GTE treated groups after 45 & 90 days of treatment as regard the mean values of testicular weights (gm).

Figure 2: Bar chart for comparison among negative control, orlistat and GTE groups all over the period of the study as regard mean values of body mass index (BMI) (gm/cm²) and the mean values of testicular weights (gm).

Table 1: Statistical comparison among (-ve) control, orlistat and GTE treated groups regarding mean values of testicular weights, (LH) and testosterone hormone levels all over the period of the study

Parameter	Group Duration	-ve control group (I)	Orlistat group (III)	GTE group (IV)	F	P
		N=6 Mean ± SD	N=6 Mean ± SD	N=6 Mean ± SD		
Testicular weight (gm)	After 45 day	1.28 ± 0.04	1.30 ± 0.06a	1.03 ± 0.06b,c	42.7	<0.001**
	After 90 day	1.31 ± 0.06	1.18 ± 0.07d	0.88 ± 0.10e,f	46.2	<0.001**
	Paired t test	1.35	3.11	2.87		
	P	>0.05 NS	<0.05*	<0.05*		
Serum LH (mIU)	After 45 days	0.06 ± 0.01	0.07 ± 0.01g	0.09 ± 0.02h,i	7.00	<0.05*
	After 90 days	0.07 ± 0.01	0.08 ± 0.01j	0.11 ± 0.02k,l	13.00	<0.05*
	Paired t test	1.37	1.19	2.29		
	P	>0.05 NS	>0.05 NS	<0.05*		
Serum testosterone (ng/ml)	After 45 days	1.43 ± 0.02	1.35 ± 0.07m	1.03 ± 0.19n,o	19.84	<0.001**
	After 90 days	1.42 ± 0.02	1.19 ± 0.09p	0.92± 0.07q,r	84.13	<0.001**
	Paired t test	0.67	5.25	3.16		
	P	>0.05 NS	<0.05*	<0.05*		

LH:Lutenizing hormone; F: ANOVA(Analysis of variance, N: Number of rats in each subgroup=6 rats. NB: All values are expressed as mean± SD, NS: non-significant (p>0.05); P:probability;SD: Standard Deviation;*: Significant (p<0.05) ;**: Highly significant (p<0.001); LSD for repeated measure ANOVA expresses as letters {a(p>0.05) vs control group , b(p<0.001) vs control group , c (p<0.001) vs orlistat group, d(p<0.05) vs control group , e (p<0.001) vs control group , f (p<0.05) vs orlistat group . g(p>0.05) vs control group, h(p<0.05) vs control group , i (p<0.05) vs orlistat group, j (p>0.05) vs control group , k(p<0.05) vs control group , l (p<0.05)vs orlistat group, m (p>0.05) vs control group, n(p<0.001) vs control group, o (p<0.001) vs orlistat, p(p<0.05)vs control group, q(p<0.001) vs control group & r (p<0.001) vs orlistat group }

Table (2) Statistical comparison among negative control, orlistat and GTE treated groups as regard mean values of testicular MDA & GSH all over the period of the study

Parameters	Groups	-ve control (I)	Orlistat (III)	GTE (IV)	F	P
		N=6 Mean ± SD	N=6 Mean ± SD	N=6 Mean ± SD		
Durations						
Testicular MDA (nmol/g.tissue)	After 45 days	35.98 ± 0.35	32.58 ± 0.59a	36 ± 1.62b,c	37.7	<0.05*
	After 90 days	37.12 ± 0.31	34.62 ± 0.89d	227.38± 8.71e,f	275.2	<0.001**
	Paired t test	1.14	2.01	52.62		
	P	>0.05 NS	>0.05 NS	<0.001**		
Testicular GSH (nmol/g.tissue)	After 45 days	155.17±2.98	395.3±36.37g	153.05±21.78h,i	193.3	<0.001**
	After 90 days	154 ± 0.84	382.67±28.51j	19.97 ± 1.93k,L	234.1	<0.001**
	Paired t test	0.76	1.98	22.53		
	P	>0.05 NS	>0.05 NS	<0.001**		

F: ANOVA (Analysis of variance);N: Number of rats in each subgroup=6 rats.;NB: All values are expressed as mean± SD ;NS: non-significant (p>0.05); P: propensity;SD: Standard Deviation *: Significant (p<0.05); **: Highly significant (p<0.001); LSD for repeated measure ANOVA expresses as letters; { a p<0.05 vs -ve control group, b p>0.05 vs -ve control group, c p<0.05vs orlistat group , d(p<0.05)vs -ve control group , e (p<0.001) vs -ve control, f (p<0.001) vs orlistat group, g (p<0.001) vs -ve control group, h(p>0.05)vs -ve control group, i(p<0.001) vs orlistat group, j(p<0.001) vs -ve control group, k (p<0.001) vs -ve control group , L(p<0.001) vs orlistat group }

III.4. Seminal analysis :

Orlistat induced significant reduction in sperm motility percentage and sperm count (p<0.05) together with significant increase in the sperm shape abnormalities percentage (p<0.05) after 90 days. Semen analysis of GTE-treated group showed significantly high decrease in sperm motility percentage and sperm count together with high significant increase (p<0.001) in sperm shape abnormalities percentage after 45 and 90 days when compared with control group. There were significantly high decreases in sperm motility percentage and sperm count with a significantly high increase in sperm shape abnormality percentage in the GTE-treated group after 45 days and 90 days,

compared with the orlistat-treated group (p<0.001) (table 3).

After 45 days, orlistat showed nonsignificant change as regard all studied parameters except for some histopathological changes, while GTE caused more histopathological changes than orlistat

Characteristics of sperm shape abnormalities: Control group showed normal sperm shape with hook head and long tail all over the period of the study (Figure 3A). Orlistat-treated group showed normal sperm shape with hook head and long tail after 45 days (Figure 3B), but after 90 days some abnormal sperm shapes in the form of detached head and bent neck were apparent (Figure 3C). GTE-treated group showed

abnormal sperm shapes in the form of abnormal hookless head, coiled tail and bent tail after 45 days (Figure 3D). After 90 days, abnormal sperm shapes in the form of broken

head, tail without head, abnormal thick middle piece of the tail and bent neck were apparent (figure 3E,F)

Table (3) Statistical comparison among negative (-ve), orlistat and GTE treated groups as regard mean values of sperm motility percentage, sperm count and sperm shape abnormalities percentage all over the period of the study

Parameter	Group Duration	-ve control group (I)	Orlistat group (III)	GTE group (IV)	F	p
		N=6 Mean ± SD	N=6 Mean ± SD	N=6 Mean ± SD		
Sperm motility percentage (%)	After 45 day	90 ± 4.47	87.5 ± 5.24 ^a	57.5 ± 5.24 ^{b,c}	78.5	<0.001**
	After 90 day	89.17±3.76	74.17± 8.61 ^d	32.5 ± 5.24 ^{e,f}	134.1	<0.001**
	Paired t test	1.00	5.39	7.32		
	P	>0.05 NS	<0.05*	<0.001**		
Sperm count (Sp.c.c/ml×125×10 ⁴)	After 45 days	139 ± 5.76	136.67± 4.32 ^g	115 ± 3.74 ^{h,i}	47.9	<0.001**
	After 90 days	137.5 ± 3.45	129.83 ± 3.71 ^j	86.83± 5.88 ^{k,L}	222.9	<0.001**
	Paired t test	0.45	3.12	7.56		
	P	>0.05 NS	<0.05*	<0.001**		
Sperm shape abnormalities percentage (%)	After 45 days	9.17 ± 1.47	12.17 ± 3.06 ^m	31.33 ± 7.42 ^{n,o}	39.1	<0.001**
	After 90 days	9.67 ± 1.75	17.17 ± 3.49 ^p	56.83 ± 7.68 ^{q,r}	155.7	<0.001**
	Paired t test	0.50	3.12	6.38		
	P	>0.05 NS	<0.05*	<0.001**		

F: ANOVA test(Analysis of variance); N: Number of rats in each group=6 rats; NB: All values are expressed as mean± SD; NS: non-significant (p>0.05); P: probability; SD: Standard deviation; Sp.c.c: Sperm cell count.; **: highly significant (p<0.001); *: significant (p<0.05);LSD for repeated measure ANOVA expresses as letters:a (p>0.05)vs -ve control group, b(p<0.001) vs control group, c (p<0.001) vs orlistat, d(p<0.05) vs-ve control group, e (p<0.001) vs -ve control group, f (p<0.001) vs orlistat group, g (p>0.05) vs-ve control group , h (p<0.001) vs -ve control group, i (p<0.001) vs orlistat group, j(p<0.05) vs-ve control group,k (p<0.001) vs -ve control group, L(p<0.001) vs orlistat group , m (p>0.05) vs -ve control group, n (p<0.001) vs -ve control group, o (p<0.001) vs orlistat group , p (p<0.05) vs-ve control group, q (p<0.001) vs -ve control group, r (p<0.001) vs orlistat group

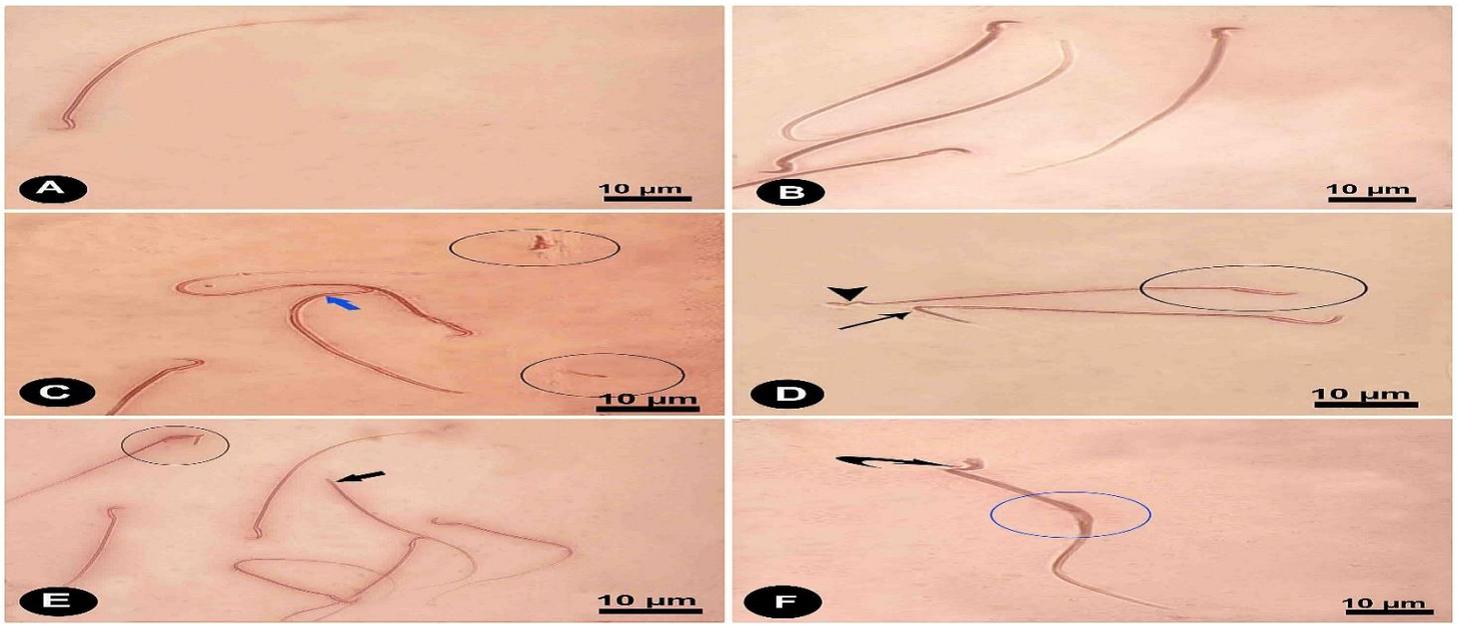


Figure 3: Eosin and Negrosin stained sections in testes of albino rats of the study groups. A :control group with normal hook head and long tail. B: orlistat group after 45 days with normal hook heads and long tails. C: orlistat group after 90 days showing detached head (black circle) and bent neck (blue arrow). D: green tea group after 45 days showing abnormal hookless head (black circle), coiled tail (arrow head) and bent tail (thin arrow). E, F: green tea group after 90 days showing broken head (black circle) and tail without head (thick arrow), abnormal thick middle piece (blue circle) and bent neck (curved arrow).

III.5. Histopathological results

III.5.1. Haematoxylin and eosin (H&E) stain

In the orlistat-treated group, some histopathological changes in the form of wide separation of germ cells of seminiferous tubules with dark stained nuclei appeared after 45 days. Some tubules appeared with empty lumina. Mildly congested blood vessels were seen (figure 4 C, D). Orlistat after 90 days induced many histopathological changes in the form of distortion of seminiferous tubules and their germinal cells. Sparse sperms were presented in the lumina of seminiferous tubules. The interstitial tissue showed dilated markedly congested blood vessels with thick wall and some interstitial cells with dark nuclei (figure 4 E, F). The histopathological examination of testes of GTE treated group after 45 days showed distortion of seminiferous tubules together with degeneration of spermatogenic and Sertoli cells and appearance of acidophilic

hyaline material in the interstitium (figure 4 G, H). Marked deterioration of testes was observed after 90 days in the form of disorganized architecture of seminiferous tubules. Some lumina showed scanty sperms, while others appeared empty. Markedly dilated congested blood vessels with thick wall were clearly apparent (figure 4 I, J, K, L). Regarding Johnsen's testicular score, where the values were represented as the mean \pm SE, A significant difference in green tea group when compared with control after 45 days was found. Also, there were significant testicular histopathological changes in both orlistat and green tea groups when compared with control group after 90 days as well (figure 5).

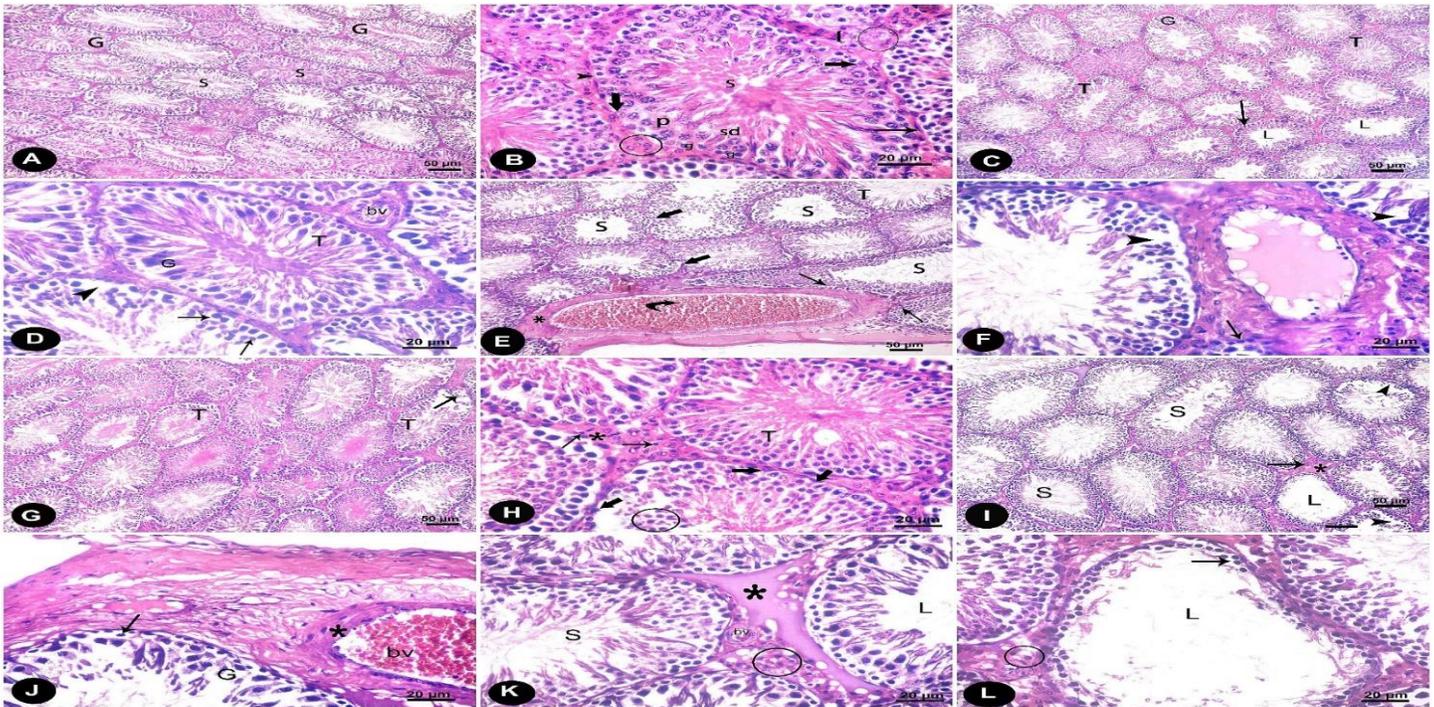
III.5.2. PAS-stained sections

Regarding PAS slides, no changes appeared in basement membrane thickness in the orlistat-treated group at 45 and 90 days. It appeared thin and regular periodic acid schiff (PAS) positive membrane as seen in the

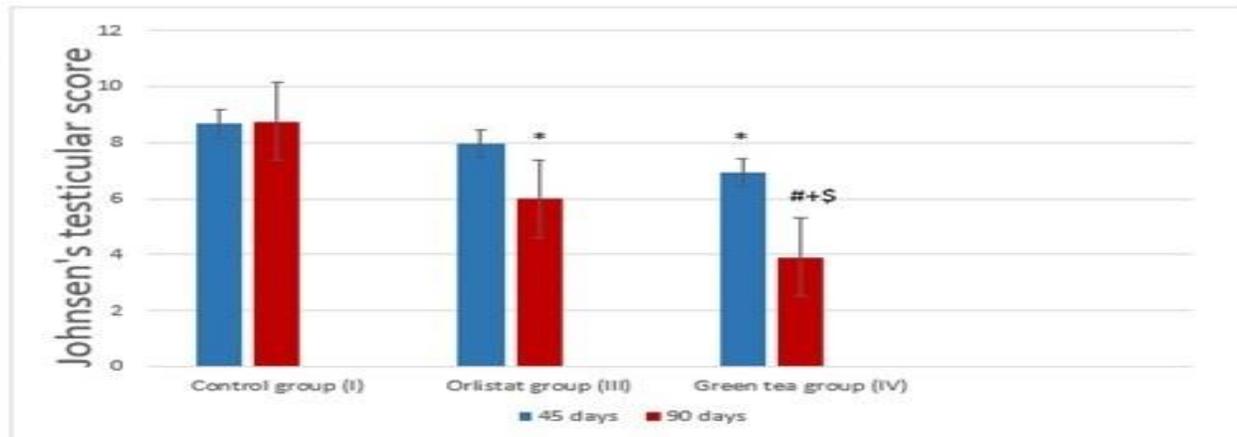
control group (figure 6 B, C). Examination of PAS stained sections after 45 days of GTE treatment showed thickening in basement membrane of seminiferous tubules corresponding to proliferation of glycosaminoglycans and glycoproteins contents of basement membrane (figure 6 D). These changes appear more clearly after 90 days (figure 6 E, F).

III.5.3. Immuno-histochemical results

The orlistat-treated group after 45 and 90 days showed moderately positive cytoplasmic and nuclear caspase-3 reaction (figure 7 B, C). Green tea extract-treated group after 45 and 90 days showed strong positive cytoplasmic and nuclear reaction (figure 7 D, E).



(Figure 4): H&E-stained sections in the testes of the study groups. A, B: control group showing seminiferous tubules with normal architecture lined by germinal epithelium (G). Sperms (S) are seen in their lumina. Regular basement membrane (arrow head) separated by narrow interstitium (I) containing interstitial cells (circle). Myoid cells (thin arrow) are seen surrounding the tubules. The stratified germinal epithelium is formed of spermatogonia (g), primary spermatocytes (p) and spermatids (sd). Sertoli cells (thick arrow) are observed between the spermatogenic cells. C, D: orlistat group after 45 days showing seminiferous tubules (T) lined with spermatogenic cells (G). Some tubules appeared with empty lumina (L). Few widely separated germ cell (arrow head); with dark nuclei (black arrow) are observed. Also mild congested blood vessel (bv) could be seen in the interstitium. E, F: orlistat group after 90 days showing seminiferous tubules (T) with irregular outlines (thick arrow) containing widely separated germ cells (arrow head) with dark nuclei (thin arrow) and scanty amount of sperms (S). The interstitium shows dilated markedly congested blood vessel (curved arrow) with thick wall (*). G, H: green tea group after 45 days showing some distorted seminiferous tubules (T) with darkly stained germ cells (black arrow). Some seminiferous tubules appear with irregular distributed germ cells (black circle) and Sertoli cells with dark nuclei (thick arrow). The interstitium contains acidophilic hyaline material (*). I, J, K, L: green tea group after 90 days showing disorganized germ cells (G) with wide separation (arrow head), darkly stained nuclei (black arrow). Some tubules appeared with scanty amount of sperms in their lumina (S); others appeared with empty lumina (L). Interstitial tissue contains acidophilic hyaline material (*) and darkly stained interstitial cells (black circle). Interstitial tissue contains markedly dilated congested blood vessel (bv) with thick wall (*).



Bar chart for comparison among negative control, orlistat and GTE groups after 45 & 90 days of treatment as regard the mean values of Johnsen's testicular score.

* $p < 0.05$ vs control

$p < 0.001$ vs control

\$ $p < 0.001$ vs orlistat

Figure 5: Bar chart for comparison among negative control, orlistat and GTE groups after 45 & 90 days of treatment as regard the mean values of Johnsen's testicular score.

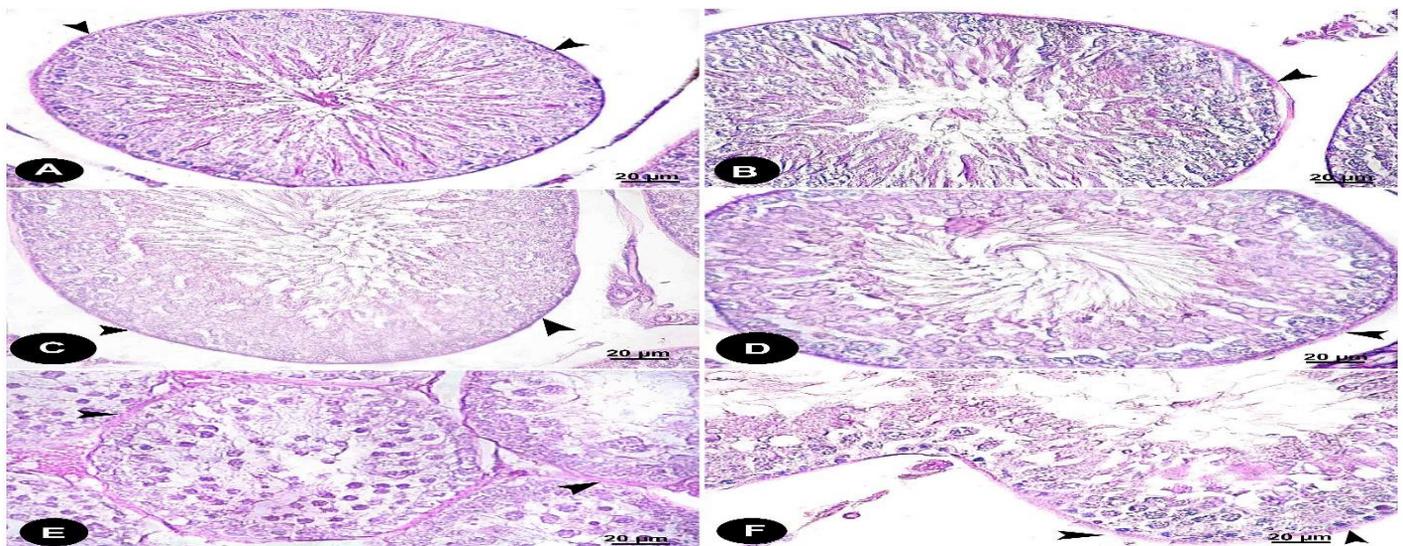
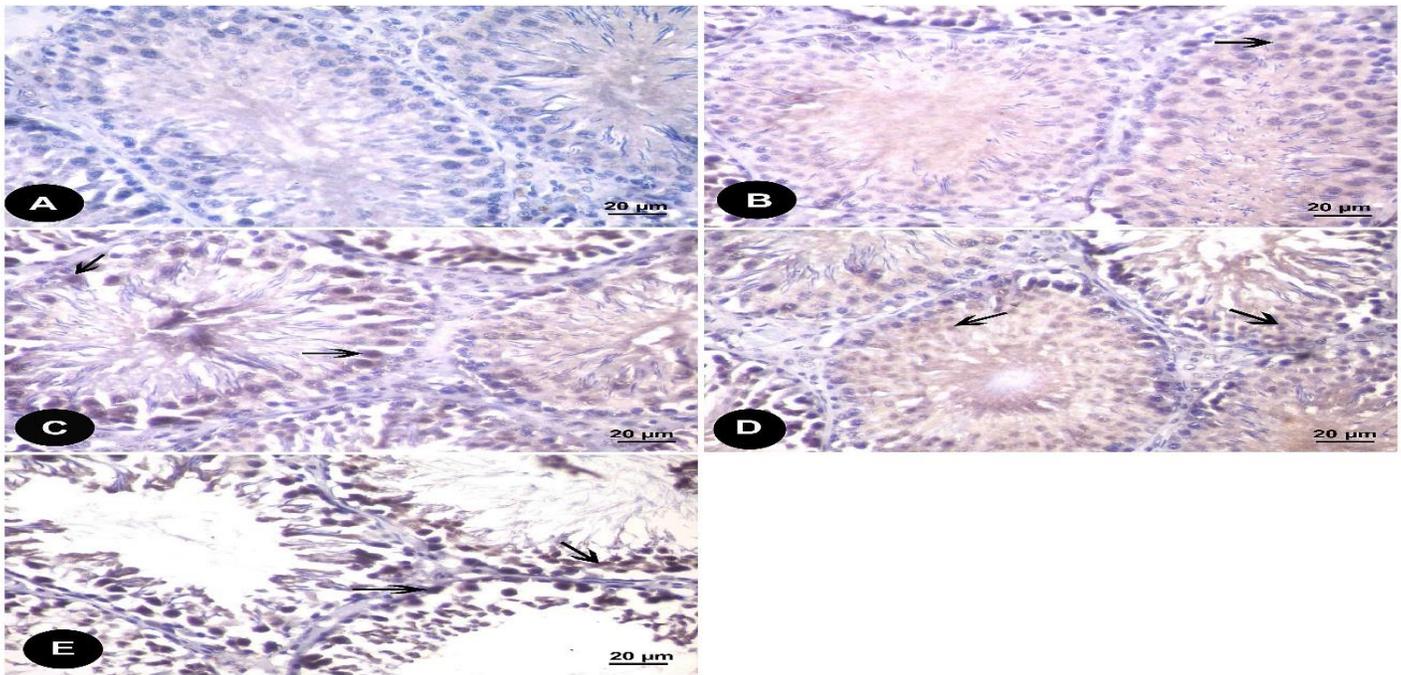


Figure 6: PAS stained sections in the testes of the study groups. **A:** control group showing thin and regular PAS positive basement membranes of the seminiferous tubules (arrow). **B,C:** orlistat group after 45 and 90 days showing thin and regular PAS positive basement membranes of the seminiferous tubules (arrow). **D:** green tea group after 45 days showing thick PAS positive basement membranes of the seminiferous tubules. **E,F:** green tea group after 90 days showing marked thickened and irregular PAS positive basement membranes of the seminiferous tubules.



(Figure 7): Immunohistochemical stained sections in the testes of the study groups. **A:** control group, faint cytoplasmic immunoreaction for Caspase-3. **B,C:** orlistat group after 45 and 90 days showing moderately positive cytoplasmic and nuclear reaction. **D,E:** green tea group after 45 and 90 days showing strong positive cytoplasmic and nuclear reaction.

IV. DISCUSSION

Obesity has become a worldwide epidemic due to dietary habits, use of too many drugs and stressful or sedentary life style. It causes impaired quality of life (Kovesdy et al., 2017), documented as a risk factor for various diseases such as hypertension and stroke and has contributed to fertility problems in both sex (Medina-Remón et al., 2018). Obesity has been reported to affect the testicular function through endocrinal disruption. It reduces androgen and sex hormone-binding globulin (SHBG) levels, affect the GnRH-LH/FSH pulse that may impair Leydig and Sertoli cell functions and interfere with the release of sex hormones with consequent effect on sperm maturation and parameters (Hofny et al., 2010). However, Multiple antiobesity drugs in markets have significant health risks and should be prescribed after diagnosis of the cause of obesity in patients who do not respond to diet and exercise (Malipeddi,

2016) in order not to increase the health problems of obesity. The present study has been conducted to evaluate and compare the chronic toxic effects of orlistat, as an antiobesity drug and green tea extract (GTE), as an antiobesity herbal compound on testes of adult albino rats

The significant decrease in the mean values of total body weights, BMI and testicular weight in orlistat and green tea groups suggests that with regular diet, orlistat and green tea hindered the expected body weight gain. Chandra et al. (2011) and Alhashem et al. (2014) found similar results. When comparing orlistat and green tea in weight reduction, Ellrichmann et al. (2008) and Amin et al. (2014) found that orlistat is able to decrease body weight by only reducing dietary fat intake as a consequence of reducing both the consumption and absorption of fat. While, green tea can reduce body weight by several mechanisms including, increased energy expenditure, fat

oxidation, up-regulation of the adiponectin level (Murase et al. (2002) , Ikeda et al. (2005) and Xu et al. (2015)), the increase of peroxisome proliferator-activated receptor γ (PPAR- γ) phosphorylation, and expression Tian et al. (2013), inhibits catechol orthomethyltransferase (COMT) enzyme that induces norepinephrine (NE) degradation, leads to prolonged NE effect. This, in turn, stimulates thermogenesis and fat oxidation which are responsible for weight reduction Chantre and Lairon. (2002) and affects the olfactory system so it diminishes an animal's sense of smell, and consequently, taste and finding food with the resultant weight loss Chan et al. (2010)

As the quantity of differentiated spermatogenic cells determine the weight of testis; so, the drop in testicular weight may be explained by degeneration occurred in spermatogenic cells as detected by our histopathological studies. Also, the change in the weight of an organ after administration a drug or chemical is considered a sign of the lethal consequence of that chemical (Hijazi et al. 2015).

The nonsignificant statistical difference ($p > 0.05$) in serum testosterone hormone level after 45 days of orlistat administration in the present work is in parallel with Banakar et al. (2013). However, the decrease in the hormone level after 90 days could be explained by De Krester. (2004) to be secondary to disrupted seminiferous tubular function. The non-significant change in serum LH level in orlistat-treated group denotes that its effect was not reflected at central level. This coincides with Ghandi et al. (2011). They attributed that to the ability of orlistat to affect serum LH only indirectly through body weight reduction and longer treatment with orlistat is needed to reach a significant reduction in serum LH level.

Regarding green tea, Figueiroa et al. (2009) stated that GTE and its main catechin (EGCG) have direct inhibitory effects on

Leydig cell testosterone production. They attributed this to some changes in cell signalling pathway. Moreover, Das and Karmakar (2015) stated that GTE decreased activity of steroidogenic enzymes such as Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 3 β HSD) and 17 β HSD which are required for production of testosterone.

In orlistat-treated group the decrease in MDA and increase in GSH levels in this study are mirror to Galaly et al. (2014) who reported that plasma MDA levels after six months' treatment with orlistat in obese subjects were significantly reduced, indicating that orlistat acts as antioxidant. The protective effect of orlistat on studied testicular oxidative stress parameters (MDA & GSH) denoted that there is another possible mechanism for such orlistat-induced testicular toxicity.

Green tea extract (GTE) caused no significant difference of testicular MDA and GSH levels when compared with control group after 45 days, indicating absence of oxidative stress at this period. The antioxidant effect of green tea occurs directly by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. The indirect antioxidant action occurs through the inhibition of 'pro-oxidant' enzymes and inactivation of antioxidant enzymes. (Huang et al., 2005). Moreover, Zanchi et al. (2015) found that green tea significantly lowered lipid peroxidation and DNA damage but reestablished glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activity in testes. So, this work suggests that oxidative stress is not the explaining mechanism of GTE induced testicular toxicity after 45 days.

However, it showed high significant increase in testicular MDA with high significant decrease in testicular GSH when compared with control group after 90 days indicating oxidative stress process. Thus a possible mechanism may be responsible for

such toxicity which could be explained by Figueiroa et al. (2009), who found that inhibition of testosterone production from rat spermatogenic cells induced by GTE and its major catechin EGCG resulted from inhibition of the PKA/PKC (protein kinase A and protein kinase C, signalling pathways in testicular androgen production) as well as direct or indirect inhibition of both P450_{scc} (mitochondrial P450 side-chain cleavage enzyme) and 17 β -HSD (17 β hydroxysteroid dehydrogenase activity), which are required for testosterone hormone synthesis. These results are concomitant with Salminen et al. (2012) who attributes the depletion in GSH to its reaction with epigallocatechin gallate (EGCG) component.

The change in sperm number and motility in orlistat- and GTE-treated groups could be explained by Luo et al. (2006) who reported that the decrease in testosterone hormone level, as occurred in this study, is an obstacle to spermatogenesis which led to appearance of degenerated dark cells and reduction in sperm number and motility. In addition, Türk et al. (2008) state that germinal epithelial damage in the testes results in oligo- or azoospermia. Sakr (2010) also reported that orlistat caused alteration or inhibition of the process of spermatogenesis.

Regarding histopathological changes in the orlistat-treated group, there were degenerative changes in seminiferous tubules with signs of pyknosis in spermatocytes. These results were in accordance with Galaly et al. (2014) who added that some of seminiferous tubules appeared devoid of sperm most probably due to drug inhibited spermatogenesis. Elbakary and Bayomy (2011) found expressed inducible nitric oxide synthase (iNOS) in pancreatic acini treated with orlistat and suggested that to play an important role in cellular damage.

Abdelwahab et al. (2017) also found expression of tumour necrosis factor α (TNF- α) in tissue macrophage after orlistat

administration for eight consecutive weeks. TNF- α is capable of stimulating cell death through necrosis and/or apoptosis as reported by Kempuraj et al. (2013).

The changes in histo-architecture of testes after GTE administration in the present study coincide with Chandra et al. (2011), who observed reduced spermatozoal mass and disorganized cellular orientation along with decrease in somatic indices. Yamasaki et al. (2010) attributed these changes to decreased testosterone level that can enhance the Sertoli cells to secrete proteins that initiate the apoptosis signalling cascade in germ cells which are expressed as dark nuclei and the resultant decrease or absence of sperms in the lumen of some tubules. Also, Leon et al. (2005) attributed some of the changes occurred in histoarchitecture due to mitochondrial dysfunction induced by oxidative stress and led to the release of cytochrome c and then caspase activation, which results in apoptotic cell death in germ and Sertoli cells. Moreover, dysfunctioning Sertoli cells cannot secrete stem cell factor (SCF) and consequently decrease the expression of c-Kit protein receptors which are normally expressed by developing germ cells and considered as a strong marker of the germ cell population (Sá et al., 2013) which may be linked with the etiology of infertility (Figueira et al., 2014).

The interstitial cells in this study also appeared with pyknotic nuclei. Mesbah et al. (2015) clarified that Leydig cells, which is known to have LH receptors stimulating them to produce testosterone, have a high risk for the exogenous toxicants due to the close relationship between them and the blood vessels.

Congested vessels and hyaline material seen in the interstitial spaces in GTE treated group could be caused by increase in vascular permeability according to Salama et al. (2003) or due to increased breakage of blood capillaries and their leakage to interstitial

tissue and then formation of the acidophilic hyaline material according to Mohamed and Mohamed. (2015), who added that this damage in the interstitial tissue may also affect the Leydig cells and the production of testosterone.

Absent PAS changes in the orlistat-treated group could be attributed to its ability to affect fat only not protein or carbohydrate present in basement membranes of seminiferous tubules. However, thick PAS positive membranes in GTE treated group in this work was similar to findings by Chan et al. (2010), who demonstrated accumulation of positive PAS glycoprotein pigments in olfactory epithelium of nasal cavity of male rats after administration of GTE orally by a dose of 1000 mg/kg daily 5 times/week for 14 weeks. These results were explained by Elharoun and Bashandy (2014), who mentioned that myoid cells produce more extracellular matrix of the basement membrane in response to toxic stimulus. Also, Elshennawy and Elwafa (2011) mentioned that the pathological thickening process of basement membrane of seminiferous tubules affects the Sertoli cell functions that gradually altered and finally suppressed. It also disturbs the movement of germ cells and arrests the process of spermatogenesis.

The moderately positive cytoplasmic and nuclear caspase-3 immune reactions in the orlistat-treated group in this work clarified that the apoptosis one of the cause of histopathological and biochemical changes detected in this group and not oxidative stress as mentioned before. Peng et al. (2018) found similar results in orlistat treatment in cases of cancer ovary and they reported that orlistat was used to induce apoptosis through activation of caspase. However, Green tea extract-treated group showed strong positive cytoplasmic and nuclear immune. These findings reflected that mechanism of toxicity

of GTE attributed to both oxidative stress and apoptosis.

Wei et al. (2019) approved the role of green tea in inhibiting cell growth by inhibiting glycolysis and inducing caspase; all these changes occur in dose dependent manner like what happened in this study

For oxidative stress markers, De Amicis et al. (2012) stated that green tea polyphenols (GTP) may act as either antioxidants or pro-oxidants depending on several factors such as dose, duration, concentration, structure and interactions of polyphenols. Also, cell type and experimental conditions; but orlistat act as antioxidant only.

V. CONCLUSION AND RECOMMENDATION

It is concluded that weight reduction and testicular toxicity were prominent in GTE compared with orlistat-treated groups and was evidenced by synchronization between the reduction of testicular weight and testosterone hormone together with histopathological destruction that coincide with semen analysis abnormalities. Apoptosis considered a possible mechanism of testicular toxicity for both GTE and orlistat. However, oxidative stress was another mechanism for GTE only after 90 days. So it is recommended that orlistat and GTE should be taken under prescription and not at high dosage or for long duration to prevent health hazards.

Conflict of interest

The authors declare that they have no conflict of interest.

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تقييم التأثيرات السامة لكل من عقار الأورليستات ومستخلص الشاي الأخضر علي خصي الجرذان البيضاء البالغة: (دراسه مقارنة)

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مقدمه : تعتبر السمنة مشكلة صحية طبية خطيرة في العالم. عقار أورليستات وعشب الشاي الأخضر لهما تأثير مهم في مكافحة السمنة. هدف العمل: قيمت هذه الدراسة التأثيرات السمية المزمنة لأورليستات ومستخلص الشاي الأخضر على خصي ذكور الجرذان البيضاء البالغين بعد خمسة واربعين و تسعين يوماً من استخدامهم. المواد والطرق: أجريت الدراسة لمدة 90 يوماً على ستة وثلاثين من ذكور الجرذان البيضاء البالغين ، مقسمة إلى ثلاث مجموعات. (كل واحدة مقسمة إلى مجموعتين فرعيتين متساويتين). المجموعة الأولى: المجموعة الضابطة ، المجموعة الثانية: عولجت عن طريق الفم بأورليستات بجرعة 500 مجم / كجم / يوم. المجموعة الثالثة: عولجت بالفم بمستخلص الشاي الأخضر بجرعة 700 مجم / كجم / يوم. في بداية التجربة ، بعد 45 و 90 يوماً تم حساب مؤشرات الجسم لجميع الجرذان قبل الذبح ، ثم تم إخضاع الدم وأنسجة الخصية لتحليل الكيمياء الحيوية والسائل المنوي ، بينما تم صبغ أنسجة الخصية المتبقية للدراسات النسيجية المرضية والدراسات المناعية الكيميائية. **النتائج:** أظهر اعطاء اورليستات لمدة 45 يوماً تغيرات نسيجية مرضية خفيفة. بعد 90 يوماً ، كان هناك انخفاض ذو دلالة احصائية في هرمون التستوستيرون مع تدهور في حركة الحيوانات المنوية ونسبة تشوهات في الشكل وعدد الحيوانات المنوية دون تأثير على LH ، تفاعل إيجابي caspase 3 الموجود داخل النواة والسيتوبلازم وعدم وجود تغييرات في بقع حمض شيفف الدورية (PAS) بينما أظهر اعطاء مستخلص الشاي الأخضر انخفاض هرمون التستوستيرون والذي زاد انخفاضه بزيادة مدة التعاطي ، وزيادة هرمون LH في الدم ، وتدهور معايير السائل المنوي ، وتغيرات نسيجية مرضية ملحوظة وسماكة في الغشاء القاعدي للأنايبب المنوية مع تفاعل إيجابي caspase 3 الموجود داخل النواة والسيتوبلازم ووجود تغييرات في بقع حمض شيفف الدورية (PAS). كما أظهرت الدراسة حدوث إجهاد تأكسدي على الخصيتين بعد 90 يوماً فقط في مجموعة مستخلص الشاي الأخضر **الخلاصة:** الاستهلاك المزمن لمستخلص الشاي الأخضر تسبب في فقدان الوزن وتسمم الخصيتين مقارنة بأورليستات. التوصية: يجب تجنب إساءة استخدام أورليستات و مستخلص الشاي الأخضر وتقييدها بروشته طبيه لتجنب سميتها.