Egypt. J. Vet. Sci. **Vol. 55,** No. 4, pp. 1095-1108 (2024)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Orlistat and Metformin Ameliorate Testicular Function and Structure with Elevation Efficacy of Antioxidant Enzymes in Experimentally Induced Obesity in Male Rats

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OBESITY represent a real challenge to the human beings and affects greatly the reproductive capacities, metabolic disorders and cardiovascular diseases. Obesity is related greatly with series of oxidative damage and excessive production of free radicals with incidence of inflammation which is considered to be one of the essential factors that affect the reproductive world health. The current study evaluated the effects of obesity (OBS) on the reproductive capacity functions via hormonal measurements (Testosterone hormone level), inflammatory markers [C-reactive protein (CRP) and interleukin-6 (IL-6)] and (CAT , SOD and GPx) which are the vital antioxidant enzymes and final lipid peroxidation marker (MDA) in testis homogenates plus histological structure and caspase-3 staining sections of the testicular tissues. 50 Male rats were used in the present study and they were divided into 5 treated groups: Control group, Experimentally induced obesity group (OBS) , OBS plus Orlistat (ORS) , OBS plus Metformin (MEF) and the last group OBS plus ORS plus MEF. Experimental induced obesity induced significant increment in testicular lipid peroxidation level, both inflammatory markers (CRP and Il-6) , tumor necrosis factor-α and lipid peroxidation marker in concurrent with decline in antioxidant enzymes (SOD, CAT and GPx) levels and damage of testicular main structures with degeneration of spermatogenic layers and detachment of seminiferous tubules with appearance of Azospermia, Meanwhile, restoration of normal testicular structures, and antioxidant levels in both groups treated with either ORS or MEF and the combination group was attained with the best results than each treatment alone .The current study proved the synergistic effect of both ORS and MEF on amelioration of testicular functions, hormonal levels and antioxidant enzyme levels and alleviation of inflammation , tumor necrosis marker and side effects of experimentally induced obesity.

Keywords: Obesity , Orlistat , Metformin , Oxidative stress , Diabetes, Reproductive toxicity

Introduction

Obesity is increasing gradually and become more common, it is associated with serious and high healthy concerns. Overfeeding and wrong life habits may lead to metabolic disturbances [1]. The most common types of obesity is abdominal obesity and the major risk that is commonly caused by obesity is hypertension accompanied by a decline in high density lipoprotein (HDL) with elevation of low density lipoprotein (LDL) , triglycerides, and glucose intolerance [2].

Infertility is a major public health concern around the world. Between 1990 and 2010, the expected number of infertile persons grew from 42 million to 48.5 million [3,4]. Male infertility accounts for 40-50% of these cases [5,6].Obesity has emerged as a key linked factor in the causes of male infertility. Obesity has been shown in studies to have a deleterious impact on the hormonal level and spermatozoa function [7,8]. Furthermore, the hypogonadism, which is defined as a case having hormonal level of testosterone ≤ 300 ng/dL, this case was observed to be higher by 2.74 folds in the obese men [9].

There is a high negative relationship between sperm count and body mass index [10]. Previous

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(Received 24/11/2023, accepted 27/12/2023)
DOI: 10.21608/EJVS.2023.249994.1672

studies reported that, obese men are characterized by the decreased semen quality and sperm normal morphology, thus altering their fertility potential [11].

Overweight and obese males had higher prevalence of oligospermia [12], accompanied by lower levels of androgens and sex hormone-binding globulin (SHBG) [13]. Defects in the gonadotropic releasing hormone either (LH) and/or (F.S.H) may disrupt Leydig and Sertoli cell activities, resulting in decreased sperm maturation [14]. Increased scrotal temperature and oxidation, inflammation and apoptosis, as well as an altered endocrine profile have all been linked to lower fertility in obese males [11]. Obesity has been attributed to a lot of medical disorders, with some reported evidenced for reduced fertility in obese men [15]. Oxidative stress has been identified as the primary cause of reproductive problems in healthy and obese males, and its presence has been shown to trigger pro-cytotoxic pathways as apoptosis and inflammation [16]. Oxidative stress is a condition associated with severe cellular damage produced by oxygen and oxygen-derived oxidants. Due to the high polyunsaturated fatty acid composition of sperm membranes, testicular tissues and sperms are the extremely vulnerable to reactive oxygen species attack and lipid peroxidation. Several studies have found a link between sperm motility, characteristics and sperm-oocyte contact with male obesity [17].

A three-year multicenter study found that infertile , obese males had much more percentage of DNA damage of spermatozoa, which could be caused by severe oxidative damage [18]. Regarding the male fertility, obese men had higher levels of reactive oxygen species and more DNA breakage in their sperm [19]. Apoptosis, in addition to oxidative injury, have key roles in induction of DNA damage of sperm and male infertility and inflammation which reduce sperm production, produces (Reactive oxygen species) ROS, and causes apoptosis [20-23].

There are various therapeutic drugs which are used to treat obesity, and their main mechanism of action is to decline the energy intake, elevate energy expenditure, and reduce the process of nutrients' absorption. One of the orally common anti-obesity therapeutic drug is Orlistat (ORS), which is a semi-synthetic derivative of lipstatin. It works in the gastrointestinal tract directly by partially blocking triglyceride hydrolysis, limiting subsequent absorption of monoglycerides and free fatty acids [24].

ORS inhibits pancreatic lipase enzyme while having no effect on amylase, trypsin or even chymotrypsin **[25]**. Some authors [26], discovered that ORS therapy of high-fat diet-fed rats resulted in significant elevations in glutathione levels (GSH) and superoxide dismutase enzymatic level.

Metformin (MEF) is a commonly used medicinal therapeutic agent used to treat diabetes mellitus (type II). MEF works by declining the hepatic glucose synthesis while raising insulin resistance and elevating the glucose uptake in the muscles [27]. MEF has been shown to help with weight loss, cardiovascular disease, and polycystic ovarian syndrome in much recent studies [27]. In terms of male fertility, MEF therapy of obese male rats resulted in improved sperm quality [28]. In a clinical trial of infertile patients with metabolic syndrome, MEF was found to elevate the testosterone hormonal concentrations with elevation of the sperm quality [29].

Thus, the current experimental study was an attempt to assess the effects of either ORS and/or MEF against experimentally induced obesity and mitigating it's metabolic and reproductive disturbances. We hypothesized that the combination of both treatment (ORS and MEF) could improve the obesity induced spermatogenesis deficiency and metabolic disturbances by suppressing the potential related processes including oxidative stress and inflammation.

Material and Methods

Ethical statement and experimental Animals

A total of 50 male albino rats " 8 weeks age" and weighing 150-180 g. The male rats were all housed in hygienic metal cages with controlled all environmental conditions and free regular standard feed and water for the normal control group. Meanwhile, the obese groups were given high fat diets and marked as (OBS) animals. This experimental work was carried out in accordance with European animal care and was approved by Zagazig University's ethical committee (ZU-IACUC/2/F/62/2022). Based on the obtained ethical approval, anesthesia was obtained via administration of Ketamine-Xylazine at very low dosage to avoid any possible pain.

Experimental groups

Fifty Male rats were divided randomly into 5 groups, 10 male rats/each group , experimental design as shown in Fig.(1).

Treatment groups were divided into 5 groups as follows:

I-Control group: was fed by standard diet of normal equally balanced diet with addition of water *ad libitum*; (II-OBS group): High fat diet (more than "26500.00" Kcal Kg^{-1} calories /day)

(For two times/day) successively for 30 days. The composition of the diets were: 34% Fats , 25 % Fibers , 22 % proteins , 7 % salts , 2% Calcium , and some trace minerals in minute quantities as follows: Cu , I_2 , Se and Zn; (III-OBS + ORS group): The male rats were fed and induced OBS and then successive administration of ORS at a dosage of (2 mg Kg^{-1}) based on previous study [30], for successive 30 days; (IV-OBS + MEF group): Male rats were fed and induced OBS and then concurrent treatment of MEF at a dosage of (70 mg Kg^{-1}) [30]; (V-OBS+ ORS+ MEF group): Male rats were OBS and then subsequent treatment of both ORS and MEF (1/2 hr after the 1st dose) at a dose for successive 30 days.

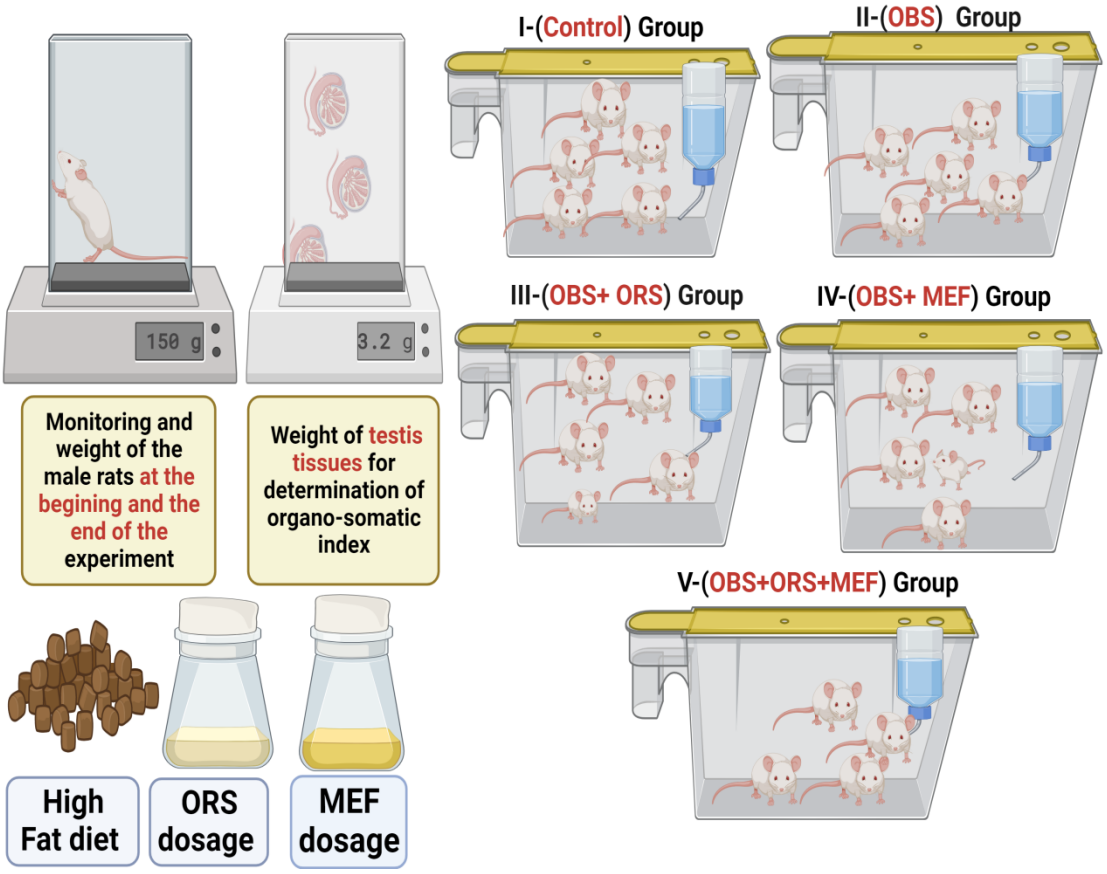

Fig. 1. Experimental design

Samples collection

Blood samples were centrifuged at ~ 3000 r.p.m for about 5 minutes to get the serum samples, the serum samples were used and were immediately persevered at -20°C. The experimental animals were immediately decapitated after the anesthesia with xylene/ketamine (I.P), and the testicular tissues were suddenly removed, two parts were used for both histological and immune-testing, and part was used for determination of the antioxidant enzymes' capacities after homogenization. The tissue homogenates' supernatants were collected and used at -20 °C for.

Organo-somatic index of testis

The body weight of each animal was noted before treatment and also on the last day of the experiment. After the male rats were sacrificed, the testis weight of each male rat was recorded. Then, the organo-somatic index (OSI) of testis tissues were calculated based on the following formula**:** Organo-somatic index = [Weight of the testis (g) / Day 30 total body weight (g)] X100 [31].

Biochemical investigation

Measurement of inflammation markers

-TNF-α and IL-6 levels in the testicular homogenates

Cytokines levels of (IL-6 and TNF-α) were evaluated in the hepatic tissues homogenate by using (ELISA) kit that were obtained from Immuno-Biological Laboratories, USA.

- Inflammation marker (CRP) activity:

CRP levels were evaluated as by Wener *et al.,* [32] by using SEA821- ELISA Kit For C Reactive Protein (CRP).

Assessment of the oxidative stress markers

Small piece of the testicular tissues (~0.20 g) were homogenized with cold ice alkaline buffer and then get the supernatant for performing the antioxidant assays. Malondialdehyde (MDA) levels were measured according to Ohkawa *et al.* [33].

Superoxide dismutase enzyme activity (SOD) was assessed according to Marklund and Marklund *et al.* [34]. CAT activity was estimated according to Aebi and the breakdown rate of hydrogen peroxide was at 240 nm (Spectrophotometer SP-2200, Bioespectro) [35], it was expressed in (U/g). Glutathione peroxidase (GPx) activity was evaluated [36].

Determination the testosterone hormone level

Testosterone hormone levels were measured by automata (Elecsys, Roche Diagnostics) according to the method of Wheeler [37].

Histological and immunohistochemically assessment of testis tissues

Fixed testicular samples were processed by using hematoxylin/eosin staining. Photomicrographs of the testicular tissue samples were taken by light microscope to view the stained slides. The excessed testis slices (4 mm thickness) were suddenly blocked with 0.1% mix of water and methanol for 1/4 h to study apoptosis-related markers. Both tissue sections were treated at 4°C overnight with caspase-3 antibody. The color intensity of caspase-3 in the immunohistochemical sections was used to classify the intensity of immunostaining.

Statistical analysis

The statistical data were expressed as (mean ± SE).By using One-Way ANOVA. Significant value at $P \leq 0.05$. CAT levels were assumed to be 2.17 ± 0.56 in OBS induced group versus OBS+ ORS+MEF with value 3.11 ± 0.75. At the power of 80% and the confidence level of 95%, sample size is 50 (10/each group). This statistical ratio was calculated by using OPEN EPI software [**38**].

Results

Organo-somatic index

The organo-somatic index of the testicular tissues in the OBS experimentally induced group was declined significantly as compared to the normal control group (Fig.2). There was a marked elevation of the organo-somatic index of the testicular tissues in the groups administrated either ORS or MEF and their combination showing the best improvement ration.

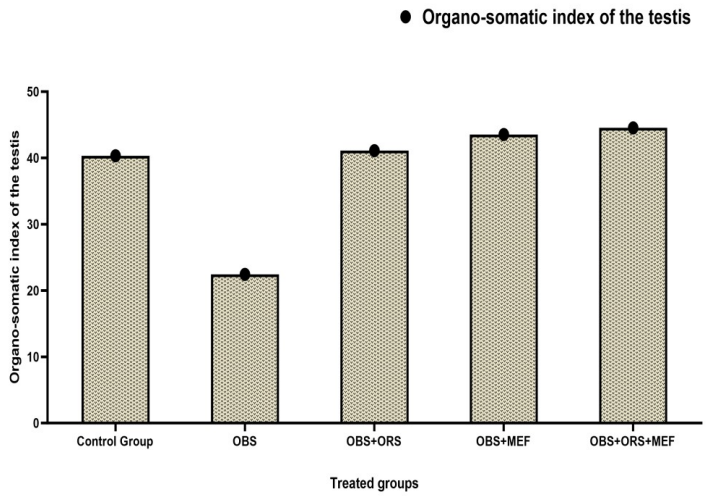

Fig. 2. Organo-somatic index of the testicular tissues of different treated groups

Testosterone hormonal level

The level of testosterone hormone of control, ORS and/or MEF treated groups was significantly increased as compared with OBS experimentally induced group. The best level of testosterone hormone level was the group treated with combination of both ORS and MEF after induction of experimental obesity. The testosterone level decreased only in experimentally induced obese animals (Fig.3).

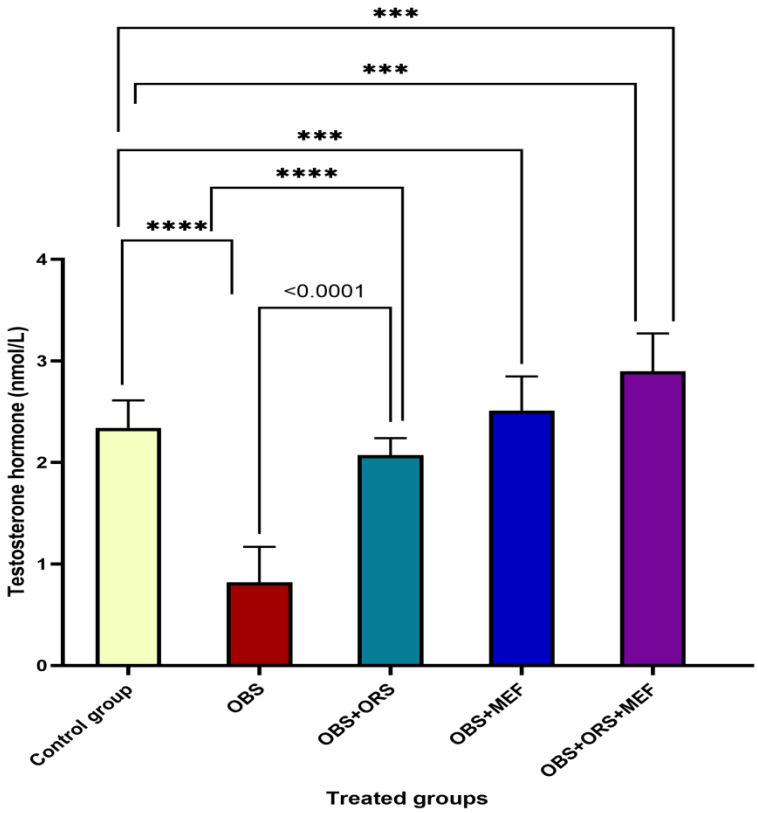

Fig. 3.Testosterone hormonal levels of different treated groups

Oxidative damage marker

The testicular oxidative parameters of Malondialdehyde (MDA) level , which is the final marker of lipid peroxidation was significantly elevated in the experimentally induced obesity group after 30 days of treatment (Fig. 4). Both ORS and MEF declined this oxidative parameter marker as compared to the OBS and control groups, especially MDA level was significantly decreased in group treated with combination of both ORS and MEF.

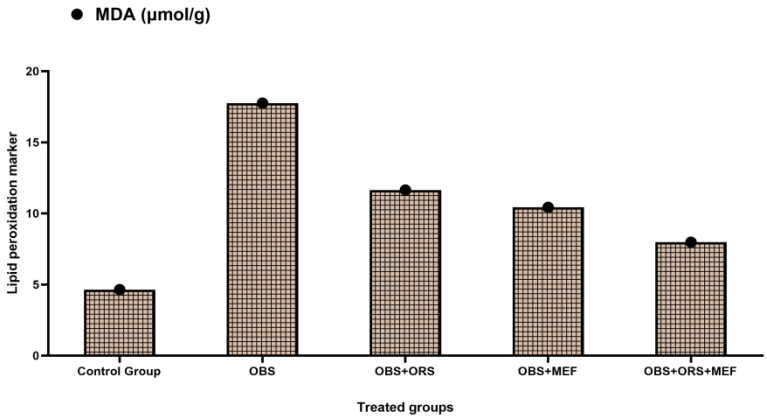

Fig. 4 . Lipid peroxidation marker level of different treated groups

Antioxidant enzyme levels

Data on (Fig.5) revealed that there is a remarkable improvement in SOD level of induced experimental obesity and treated with ORS. Meanwhile, there was slight improvement in other OBS plus MEF and OBS plus ORS and MEF treated groups but with less percentage than OBS plus ORS treated group only. The same improvement was recorded in OBS group treated with combination of ORS and MEF in both CAT and Glutathione peroxidase (GPx) levels. In contrast, decrease all the previous mentioned antioxidant enzyme activity and reducing superoxide dismutase (SOD), catalase (CAT) and GPx levels were recorded in experimentally induced obese animals

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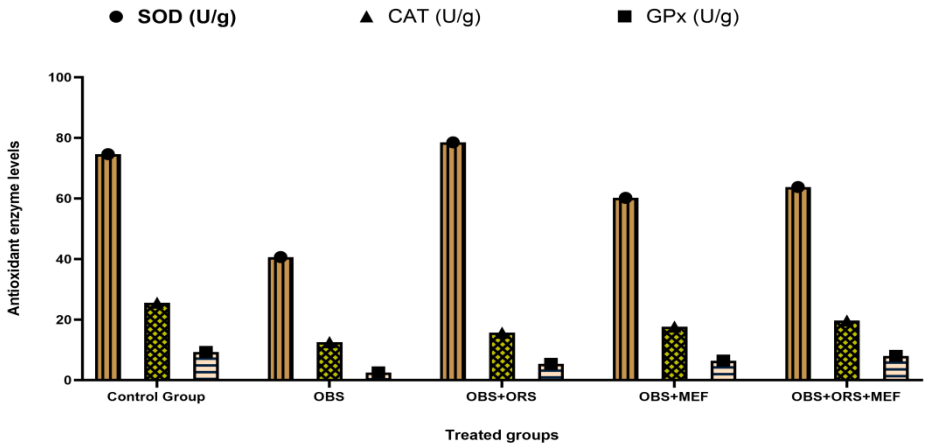

Fig. 5. Antioxidant enzyme levels of different treated groups

Inflammatory markers and tumor necrosis factor

The inflammatory markers including (CRP and IL-6) levels, these levels were significantly elevated in the experimentally induced obesity group after 30 days of treatment (Table 1). Both ORS and MEF combination declined these inflammatory markers as compared to the control group.

TABLE 1.Inflammatory markers of different treated groups

Treated groups	CRP (mg/L)	IL-6 (Pg/ml)	TNF-α (Pg/ml)
Control group	3.87±0.87^{e}	170.58±9.55^{c}	60.02±4.25^{e}
OBS	12.58±2.52^{a}	260.87±12.58^{a}	110.87±6.87^{a}
OBS+ORS	7.84±1.54^{b}	179.45±14.25^{b}	89.58±7.85^{b}
OBS+MEF	6.25±1.65^{c}	174.85±13.57^{bc}	82.68±6.98^{bc}
OBS+ORS+MEF	5.47±1.58^{d}	164.58±8.58^{d}	71.05±9.58^{d}

Means within each column category (mean ± SE) with different letters are considered as significant, where the highest value has the symbol (a) and declining in values were assigned alphabetically.

Histological evaluation

The testicular tissues were normal in appearance in the normal control group (Fig. 6A), with complete normal seminiferous tubules (ST) normal cells lined with layers of spermatogenic cells (Sg) and filled with sperms. Also, both experimentally induced obesity and treated with either ORS and/or MEF-treated male rats showed normal seminiferous tubules (ST) with normal process of spermatogenesis (Sg) (Fig. 5B) as shown in OBS plus ORS treated group showed normal somatic sperms in the tubular lumen , but with marked loss of interstitial tissue and peritubularmyoid cells, resulting in expanding of the interstitial spaces (Fig.6C). Meanwhile, OBS+MEF group showing almost normal structure with slight toxicity in the form of loss of some spermatogenic cells in some of the seminiferous tubules with detachment of the spermatogenic cells, resulting in expansion of the interstitial space (Fig.6D).The last group treated

with combination of both ORS and MEF showing normal seminiferous tubules which are thinned and no detected per tubular myoid cells (Fig.6E).

Histological activity index was assessed based on the degrees of the microscopic lesions in the testicular tissues due to administration of either ORS and/or MEF (Fig.7). A reduction in microscopic lesion's scoring was observed in the OBS induced animals based on the dosage. An improvement in scoring index was noted in the male rats that received both combination of ORS and MEF in all the spermatogenic cells either normal seminiferous tubules, several layers of sperms with absence of azospermia, thus showing its ameliorative action.

Immunoassay evaluation:

The immunohistological investigation of the testicular tissues (Fig.8), for caspase-3 sections of male rats which showed highly positive staining in experimentally induced obesity (Fig. 8B), but showed mild reactivity in both groups treated with either ORS and/or MEF (Fig.8C,D). Meanwhile, control group showed negative caspase-3 staining (Fig.8A) and mild caspase-3 staining in the treated group with both ORS and MEF (Fig. 8E)**.**

Discussion

All age categories are affected by obesity, which has serious consequences for both men and women [39], Male subfertility, is one of the consequences of obesity [40]. Several studies have demonstrated elevated levels of oxidative injury in the many organs of obese male rats [41]. The current study showed the potent efficacy and synergistic action of both ORS and MEF in alleviation of toxic effects of Obesity in male rats.

There are signs that testicular oxidative stress brought on by obesity contributes significantly to male reproductive dysfunction. There are also hints that male subfertility in obesity may be caused by steroidogenesis decline on its own. Thus, we looked at how ORS influences steroidogenesis and how sperm parameters are impacted by this on the majority of the spermatozoa by evaluation of the oxidative stress parameters [42].

The current study is in great accordance with the previous results of [42] who observed increases in BMI and obesity index after high fat diet feeding, as compared to control rats, these observations confirmed the finding of low organo-somatic index of testicular tissues in experimentally induced obesity in male rats , but this index was improved in OBS group treated with both ORS and MEF that showed a marginal decline in organo-somatic index which are affected due to its direct action on the body and its adipocyte diameter and fat deposits, as confirmed previously [43].

ORS act by inhibition of the lipase enzymes of the pancreas with successive prevention of ingested fats' hydrolysis. In previous study, there was elevation in the epididymal fat diameter and size in the OBS experimentally induced group. This may be due to the triglycerides' accumulations, which are then leading to the hypertrophy of the adipocytes. The current study revealed ORS treatment induced decline in the diameter and size of the adipocytes in obese animals treated with either ORS and/or MEF groups and consistent with the previous studies [44].

The complete spermatogenesis process is the vital process in determination of normal sperm quality. Briefly, spermatogenic cells at the base of the seminiferous tubules start division and generate 1^{ry} spermatozoa, which then process other cellular divisions. Then differentiate into spermatozoa (Mature) [45].

Testosterone hormone is necessary for spermatogenesis to be maintained. Testosterone is in charge of the development of secondary sexual traits [46]. Additionally, the maintenance of all the body function depends on appropriate testosterone levels [47]. The hypothalamic-pituitary-gonadal axis controls the secretion of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and gonadotropin-releasing hormone (GnRH) at the proper amounts to regulate the generation of testosterone. The steroidogenic acute regulatory protein (StAR) is responsible for delivering cholesterol into Leydig cells throughout the testosterone production process [48]. All these findings confirmed the current concept and the great role of both ORS and MEF in attenuation of the reproductive toxicity induced by obesity in male rats and the improvement in the testicular structures and function as observed in different treated groups.

MEF as a therapeutic treatment for the obesity, the effect of MEF on obesity which induced male infertility was investigated. In accordance and complete agreement with the present study, a high-fat diet resulted in lowering the weight of the reproductive organs and induced imbalance in the level of testosterone. Additionally, testicular damage and Azospermia case were also noted clearly in the animals by experimental induction of obesity. In agreement with the previous studies [49-51], after the treatment with ORS or MEF, the level of testosterone hormone, concentration of the sperm production, and semen quality were restored.

The appropriate level of testosterone hormone is vital for maintenance of the male reproductive

capacity. Testosterone hormone levels play a vital role in the process of spermatogenesis [**52**], Similarly, previous studies revealed that a higher dietary cholesterol intake that mimic OBS induced experimentally, led to high abnormalities in the testicular Leydig cells and may disrupt the testosterone level production [53].

Confirming the strength of the present study, as that infertility is essentially correlated with severe oxidative stress, inflammation and apoptosis in the testicular tissues and spermatozoa [54], MEF has a great effect on the oxidative pathway regarding the testicular tissues. On the same line, previous studies confirmed the current results showed that MEF improved the imbalance of the antioxidant enzymes and declined the testicular marker of oxidative damage (MDA) in the obese animals. Beside elevation of the enzymes SOD and GSH-Px and declined MDA in the testicular tissues [55].

Additionally, on the same concept and give strength point to the current study, MEF activate the AMPK pathway, and promote the antioxidant capacities in the testis [56]. MEF also enhanced the series of obesity by the downstream of AMPK [57].

Providing a new strength of the current results, previous study proved that in obese animals due to the increment of either apoptosis and/or inflammation mediators, lead to increasing of TNF-α and this may be the cause for induction of apoptosis of the sperms [58].

It is well-known that fat accumulation is one of the main inducers for oxidative damage [59-62] which may adversely effect on the reproductive capacities. Confirming the current results, it is apparent that ORS and/or MEF influence effectively steroidogenesis, spermatogenesis and fertility potentials of obese rats. Additionally, from the current results, a decline in weight and fat accumulation is a requirement for saving health reproductive capacities and the beneficial effects of both ORS and MEF against serious consequences of obesity.

Conclusion

In conclusion, Experimental induction of obesity suspiciously affects the testicular structure and function of the male rats via organosomatic index and testosterone hormonal level, as well as to its effects on biomarkers of the antioxidant enzymes activities (SOD, CAT, and GPx), non-enzymatic antioxidant marker of lipid peroxidation (MDA) and structure of the testicular tissues via histological structure and immunostaining against caspase-3. OBS caused testicular degeneration supported by histopathological, and immunostaining alteration. The results revealed the mechanisms through which stress may lead to cellular oxidative status in the testis by experimental induction of obesity. Therefore, both ORS and MEF were effective synergistic agents in the drug delivery in the testicular diseases medications and achieved the high antioxidant potency and elevate testosterone levels with amelioration of the testicular structure and functions.

Acknowledgements: The researchers would like to acknowledge Deanship of Scientific Research, Taif University for funding this work.

Conflict of interest: Authors declare that there is no conflict of interest.

Funding statement: This work did not receive any external funding.

Author`s contributions: Reham Z. Hamza , Khadeejah Alsolami: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

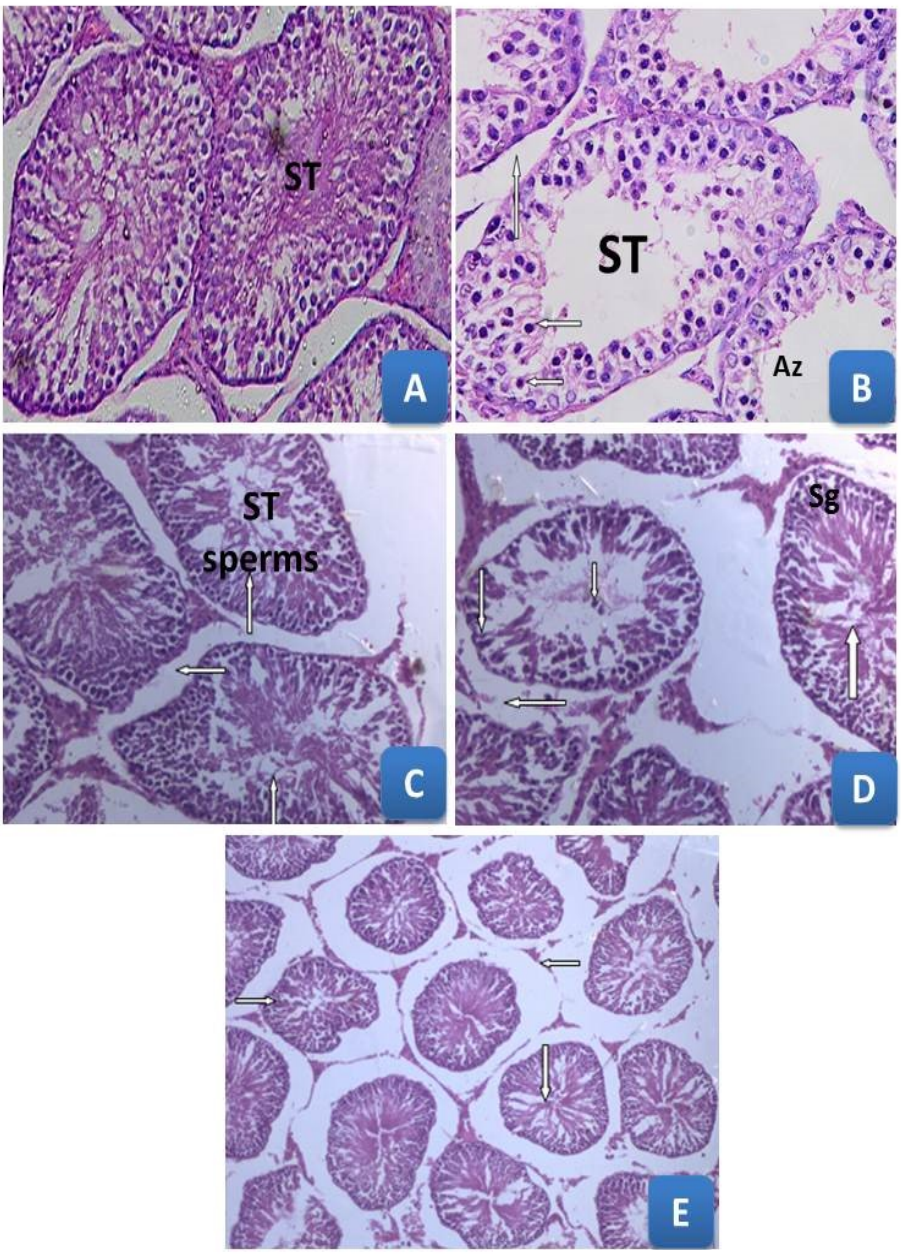

Fig. 6. Histological sections of different treated groups (A) Control group: A cross-section of the testicular tissues showed normal appearance with normal seminiferous tubules (ST) and spermatogenic cells (H&Ex400). (B): OBS group showed showing high toxicity in the form of disarrangement of spermatogenic cells with clearing of their cytoplasm, vesicular nuclei and fibrosis around them (Up-headed arrow), absence of somatic sperms (Az), loss of interstitial tissue and peritubularmyoid cells resulting in expansion of the interstitial space (Left headed arrow) (H&Ex400). (C) OBS plus ORS treated group showing normal seminiferous tubules (ST) with normal spermatogenic cells and normal somatic sperms in the tubular lumen (Up-headed arrow), marked loss of interstitial tissue and loss of peritubularmyoid cells , resulting in expanding of the interstitial space (Left headed arrow) (H&EX400). (D) OBS plus MEF treated group showed showing mild toxicity in the form of loss of some spermatogenic cells in some of the seminiferous tubules with detachment of the cells from the basement membrane and cellular debris (Low headed arrow), presence of normal somatic sperms in some tubules (Upper –headed arrow), loss of interstitial tissue and loss of peritubularmyoid cells, resulting in expansion of the interstitial space (Left headed arrow). (H&Ex400). (H&EX400). (E) OBS plus ORS and MEF showing normal seminiferous tubules and interstitial tissue which is thinned (Right headed arrow). The seminiferous tubule consists of normal somatic sperms (Low headed arrow), and spermatogenic cells (Left headed arrow). No detected marked peritubular myoid cell (H&Ex100).

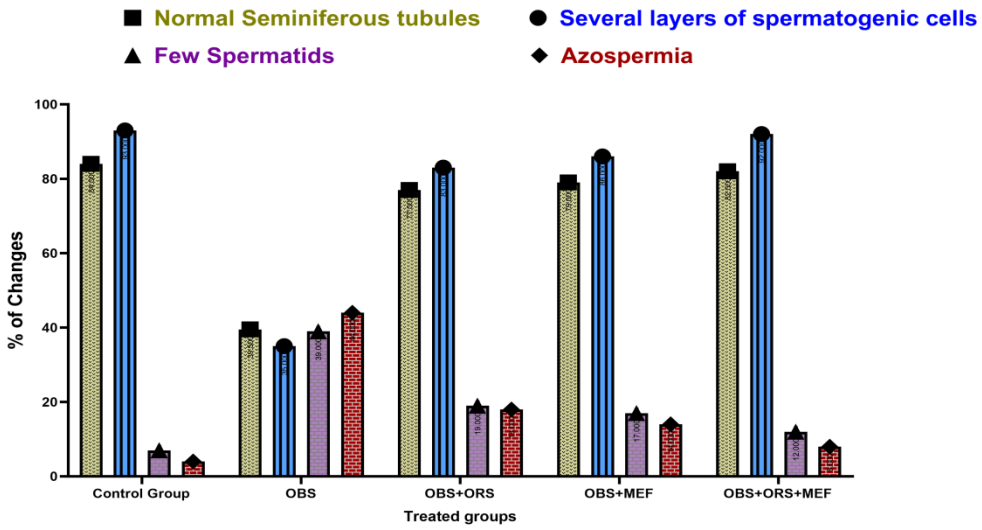

Fig. 7. Histological activity index of the testicular tissues

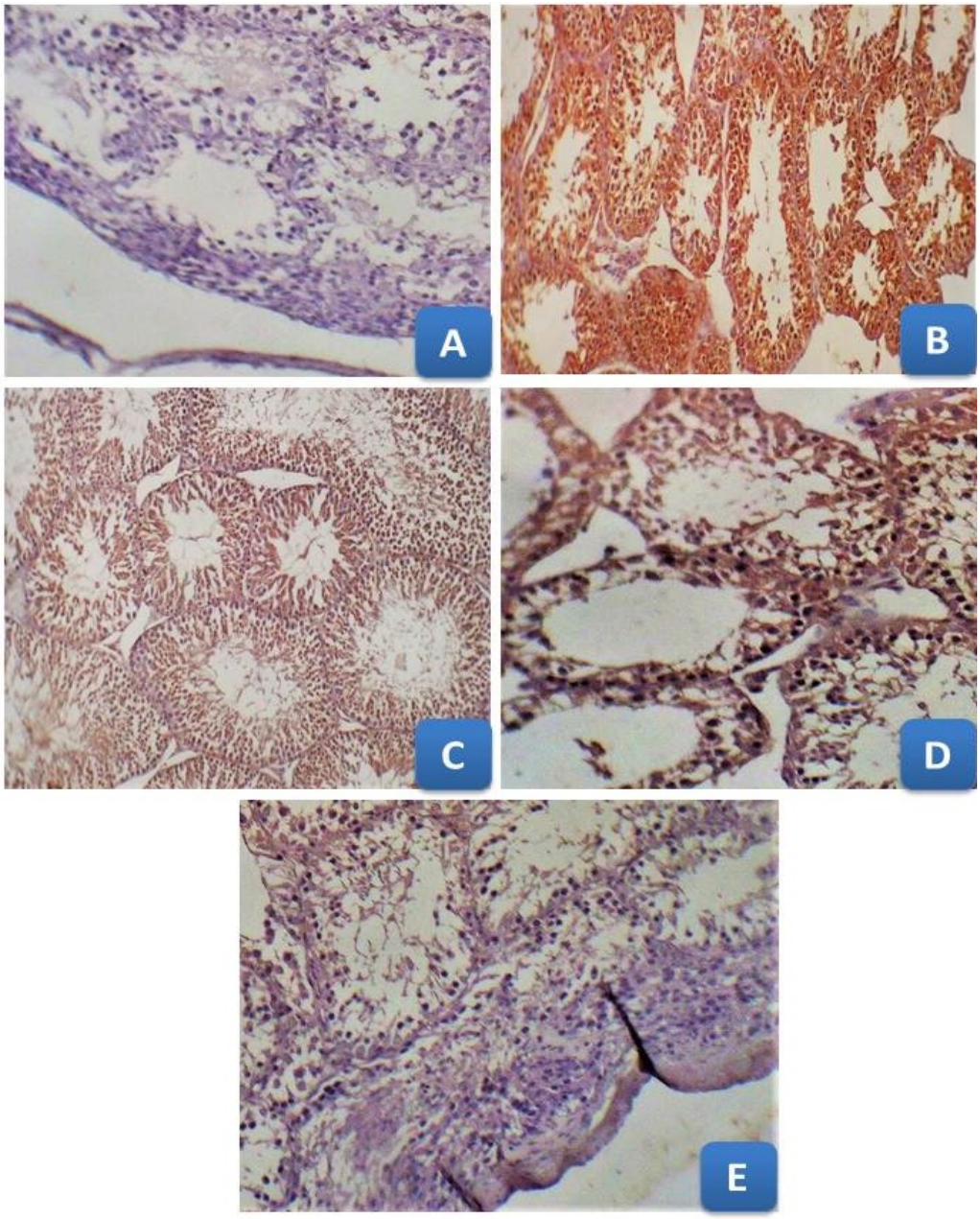

Fig. 8. (A) Control group: A cross-section of the testicular tissues of the control group showing (-ve) immunostaining of caspase-3 (160 µm). (B): A cross-section of the testicular tissues of experimentally induced OBS clarified the seminiferous tubules with marked caspase-3 staining (cytoplasmic and nuclear) especially in spermatogenic cells marked and heavy staining of caspase-3 (++++) (very strong immunoreactivity) (160 µm). (C) A cross-section of the testicular tissues and treatment with ORS after induction of OBS showing moderate immune-staining (++--) (160 µm). (D) A cross-section of the testicular tissues after induction of OBS and treatment with MEF treated group showed mild immune-staining and absence of toxicity (++--) (160 µm). (E) A cross-section of the testicular tissues after administration of ORS and MEF after induction of OBS showed very mild caspase-3 immunostaining (+---) (160 µm).

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أورليستات والميتفورمين يعملان على تحسين وظيفة الخصية وبنيتها مع زيادة فعالية الإنزيمات المضادة للأكسدة في حالة السمنة المحدثة تجريبياً في ذكور الجرذان

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تمثل السمنة تحدياً حقيقياً للإنسان وتؤثر بشكل كبير على القدرات الإنجابية، والاضطرابات الأيضية، وأمراض القلب والأوعية الدموية. ترتبط السمنة بشكل كبير بسلسلة من الأضرار التأكسدية والإفراط في إنتاج الشوارد الحرة مع حدوث الالتهابات التي تعتبر من العوامل الأساسية التي تؤثر على الصحة الإنجابية في العالم. قامت الدراسة الحالية بتقييم تأثيرات السمنة على وظائف القدرات الإنجابية من خلال القياسات الهرمونية (مستوى هرمون التستوستيرون)، وعلامة الالتهابات، والبروتين التفاعلي والإنترلوكين-6، والإنزيمات المضادة للأكسدة(السوبر أكسيد ديسميوتيز ، الكاتليز، الجلوتاثيون بيرأوكسيداز) وعلامة التأكسد النهائية للدهون (المالون داى الدهيد) في مستخلص نسيج الخصية بجانب التركيب النسيجي والنشاط المناعى ضد كاسباس -3 فى أنسجة الخصية. تم استخدام 50 فأراً ذكراً في هذه الدراسة وتم تقسيمهم إلى 5 مجموعات معالجة: مجموعة ضابطة، مجموعة السمنة المحدثة تجريبياً، مجموعة محدث بها السمنة ومعالجة بعقار أورليستات، مجموعة محدث بها السمنة ومعالجة بعقار ال ميتفورمين والمجموعة الأخيرة معالجة بعقارين من الأورليستات والميتفورمين .أظهرت النتائج أنه تسببت السمنة المستحثة تجريبيًا في زيادة كبيرة في مستوى الأكسدة الفوقية للدهون في الخصية، وكل من علامات الالتهاب البروتين التفاعلي والإنترلوكين -6وعامل نخز الأورام-ألفا وزيادة علامات التأكسد الفوقي للدهون بالتزامن مع انخفاض مستويات الإنزيمات المضادة للأكسدة وتلف تركيب الخلايا النسيجية. وتلف الطبقات الرئيسية المنبتة للحيوانات المنوية للخصية مع ضمور الطبقات المنوية وانفصال الأنابيب المنوية مع ظهور فقد النطاف، وفي الوقت نفسه، تم استعادة التركيب الطبيعي الخصية ومستويات مضادات الأكسدة في كلا المجموعتين المعالجتين إما بالأورليستات أو الميتفورمين وحققت هذه المجموعة أفضل النتائج من العلاج كل منهما على حده. أثبتت الدراسة الحالية التأثير التآزري لكل من الأورليستات أو الميتفورمين على تحسين وظائف الخصية، والمستويات الهرمونية ومستويات الإنزيمات المضادة للأكسدة وتخفيف الالتهاب، وعلامة نخز الأورام والآثار الجانبية للسمنة المحدثة تجريبيا.

كلمات مفتاحية: السمنة، أورليستات، الميتفورمين، الإجهاد التأكسدي، مرض السكري ،السمية الإنجابية.