Influence of Short-Term Supplementation with Anabolic Steroid Drug Oxymetholone And /Or Creatine Widely Used In Ksa on Some Fertility Biomarkers in Albino Rats

Sarah T Batais*, Manal EA Elhalwagy and Najla O Ayaz

1 University of Jeddah, College of Science, Department of Biochemistry, Jeddah, Saudi Arabia
2 University of Jeddah, College of Science, Department of Biochemistry, Jeddah, Saudi Arabia
3 University of Jeddah, College of Science, Department of Biochemistry, Jeddah, Saudi Arabia

Abstract: Abuse supplement is becoming a public health problem due to the adverse effects on health and semen quality in Saudi Arabia for the last few years. The present work is aimed to investigates the effect of individual supplementation with oxymetholone as Anabolic Androgenic Steroids (AAS) or creatine protein are the most common nutritional supplement use in the KSA and their mixture on some fertility biomarkers, oxidant-antioxidant activities as well as tissues structure of testis of albino rats. Forty adult male albino rats weighing around 150-200 g were classified into four groups of 10 rats each. Group I: control untreated group. Group II: treated with oxymetholone (150 mg/day). Group III: treated with creatine protein (20 mg/day). Group IV: treated with Oxymetholone (150 mg/day) and creatine protein (20 mg /day) simultaneously. All animals were given orally for 30 days. Individual supplementation with each of oxymetholone or creatine induced significant upregulation in oxidative stress biomarkers Malondialdehyde (MDA), Protein Carbonyls (PC), 8-Hydroxy Guanosine (8OHG). In addition, it also significantly reduced the antioxidants activities Total Antioxidant Capacity (TAC), Catalase (CAT), Superoxide Dismutase (SOD). Remarkable reduction in each of testosterone hormone, luteinizing hormone, sperm counts, and motility was pronounced in oxymetholone treated group than creatine group. The potentiality of oxymetholone was obvious in the mixture treated group. Changes in testis structure architecture follow the same previous trend that confirmed the clinical results. In conclusion; AAS and proteins should be used under supervision of special physicians because it will be very harmful for the reproductive life of users.

Keywords: Oxymetholone, Anabolic Androgenic Steroids, Creatine, Protein Supplements, Testis, Oxidative stress.
1. INTRODUCTION

Over the last few years, a rise in the amount of gyms in Saudi Arabia was noticed. This rise is attributed to the positive changes in the attitude and opinions of the society towards exercise and bodybuilding. Also, few studies concerned about gym participants’ habits and information about supplement abuse.1 Mostly, the main motive behind exercising for many people is to develop an ideal and proportional body so they use supplements to accomplish it. 2 The supplements are advertised aggressively to teenagers, non-athletes and athletes, which caused its own controversy in this very profitable industry,3 the consumption of the supplements unfortunately does not have any guidance. It is often a result of advice given by websites, magazines, coaches, classmates, and interactions with other gym participants. Additionally, these supplements are available in pharmacies and gyms as an over-the-counter product that does not require a nutritionist prescription or advice. Anabolic Androgenic Steroids (AAS) abuse remains one of the most heavily debated topics in the sport community and is classified as an illegal substance according to the World Anti-Doping Agency. 4 There is a growing use of AAS in Mediterranean region.5 Due to propagation of social clubs and commercial gyms (Saudi Arabia, United Arab Emirates, Iran, Pakistan, Iraq), private clubs (Saudi Arabia), hotel gyms (United Arab Emirates), martial arts clubs (Saudi Arabia), bodybuilding clubs (Kuwait, Iran, Saudi Arabia) and sports centers (Pakistan).6-7 AAS are synthetically derived from the male sex hormone testosterone. They show both androgenic and anabolic effects on the body. Anabolic effects influence the tissue binding effect of these hormones, while androgenic aspects impact the development of masculine characteristics. Two major classes of AAS have been used by athletes based on the route of administration either orally or by injection. Oral AAS are popular and convenient to consume.8 Oxymethalone [17β-hydroxy-2-hydroxymethylene17α-methyl-5α-androstan-3-one] is a synthetic androgen analogue, and available in the commercial market as Androl. Oxymethalone is classified as an edible steroid when mixed with water. It is known to cause an enlargement in muscle size and a bulky appearance in a short time.9 AAS induced damage to the reproductive function of males, it leads to testicular atrophy and reduction in sperm production. Also, it causes water and salt retention which leads to skin puffiness that increases blood pressure and strains the kidneys. Prolonged use of AAS and high-intensity workouts worsen the level of renal damage as a consequence of renal toxicity.10 Also, it induces a drop-in High-Density Lipoprotein (HDL) levels and a noticeable rise in serum Low-Density Lipoprotein (LDL).0 These disturbances may result in a stroke, myocardial infarction, edema, or hypertension. It is known that AAS use is attributed to attitude and psychological changes, and mental issues such as anxiety, irritability, depression, suicide, and schizophrenia are possible results.11 Significant alteration in the activities of antioxidant cellular defenses, such as antioxidant enzymes SOD, CAT and GSH was detected. These changes produce an increase in ROS and induce oxidative stress and lipid peroxidation in cells this imbalance induce significant liver and kidneys damage. Protein powders have a strong market position as dietary supplements. It represents one of the most used supplements among exercising individuals in Fitness Centers. 12 Protein powders are available in various flavors and forms, such as ready-to-drink shakes.13 Creatine is one of the popular sports supplements,14 and is commonly known as ‘magic’ powder among bodybuilders. 15 Easy soluble and stable solution. Therefore, it is used by professional and nonprofessional athletes for improving exercise performance and increasing muscle mass.16 However, if these supplements were taken in high doses for a long time, they can cause various toxic damage, such as a greater increase in testosterone. Oral creatine supplementation increases in oxidative stress markers and impairs antioxidant defenses post-exercise. It may also increase the levels of lipid peroxidation markers after acute exercise.17 However, taking more than the recommended dose causes increased blood creatinine and renal disease. This research work was undertaken to highlight on the effects of short-term supplementation with oxymetholone (AAS) and/or creatine supplement on some fertility markers and on oxidant – antioxidant balance in male albino rats.

2. MATERIALS AND METHODS

40 Adult Male Albino Rats (150-200g) were bought from the Laboratory Animal Production, King Fahed Medical Center (KFMC), KAU, Jeddah, KSA. The animals were allowed to have free access to water and stayed in an air-conditioned room at 25℃ with 12h dark/light cycles. For at least two weeks animals were housed under standard laboratory conditions before and throughout the experimental work and they were maintained on a standard water and diet was available ad libitum. All animal procedures conform the requirements of our institutional Animal Research and Ethics Committee (Approval No 389-19).

2.1 Drugs Creatine

An organic compound, production by Glanbia Performance Nutrition Co (USA) and obtained from Al-Nahdi Pharmacy, the loading dose is (20 mg/day).18

2.2 Oxymetholone

(17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one) A synthetic androgen analogue, was collected from LA Pharma Website, the human recommended dose is (150 mg/day).19 All doses were modified to be suitable for animal treatment according to Food and Drug Administration.20 Human dose can be converted from mg/kg to AED in mg/kg using the equation:

\[ \text{Animal Equivalent Dose (AED)} = \text{Human equivalent dose} \times 6.2 \]

2.3 Experimental Design

After 7 days of acclimatization period, animals were divided into 4 groups. Each group contains 10 rats as follows:

Group I (control group): rats were given 1ml of distilled water daily.

Group II: rats were orally treated with Oxymetholone (150 mg/day).

Group III: rats were orally treated with creatine supplement (20 mg/day).

Group IV: rats were orally treated with Oxymetholone (150 mg/day) and creatine supplement (20 gm / day)
simultaneously. All treatments were performed orally for 30 days.

2.4 Blood sample collection

After 30 days of the experiment, all rats were anaesthetized using ether inhalation. Blood samples were gathered by heparinized capillary glass tube and collected in a centrifuge tube, and centrifuged at 3500 rpm for 10 min. The serum sample was immediately kept frozen at -20°C in Eppendorf tubes for further biochemical analysis. Animals were dissected for separation of testis for histopathological examination.

2.5 Biochemical analysis

Lipid peroxidation assay Malondialdehyde (MDA) is the final product of fatty acids peroxidation was measured by using the thiobarbituric acids test (TBA) employing the methods of Yoshioka et al.21 Protein Carboxyls were measured according to procedure described by Cadenes and Wakeyama22-23 using DNPH reagent and spectrophotometric methods. Determination of 8-Hydroxy Guanosine (8OHG) was performed using the method of Desouza et al.24 by double-sandwich Elisa technique and the ELISA Kit provided is typical. Serum Total Antioxidant Capacity (TAC) was assayed using colorimetric assay kit according to Koracevic et al.25 Catalase activity (CAT) is calculated by the process of measuring the decomposition of hydrogen peroxide as proposed for Aebi.26-27 Superoxide Dismutase (SOD) determination was carried out using the method of Masayas and Hiroshi.28 Determination of Testosterone and Luteinizing Hormone utilizing immunoassay technique were performed by Elisa.

2.6 Sperm count

Sperm count was determined by the Hemocytometer chamber. The suspension of Testicular sperm was measured as million sperm cells per ml of suspension under 200X magnification using phase contrast microscope and sperm calculation was carried out manually.

2.7 Sperm motility

Sperm samples were collected as fast as possible after the rat dissection ended. The cauda epididymis was placed in 1 ml of 37 °C phosphate buffer saline solution (pH 7.4). The cut was performed by surgical blades into approximately 1 mm (3 pieces). The solution was pipetted multiple times to homogenize the suspension of sperm. One drop of the suspension was placed on a slide, covered by a cover slip. The sperms were categorized based on their level of motility as “motile” or “immobile”. Then results were written as percentage.29

2.8 Histopathological Studies

At the end of experimental periods, animals were sacrificed, testis was quickly dissected and washed in normal saline. After fixing in 10% formalin, tissues were embedded in paraffin, sectioned at 4-µm thickness and then section were stained by hematoxylin and eosin (H&E).30 All sections were examined by light microscopes.

3. STATISTICAL ANALYSIS

The data will be analyzed using the Statistical Package for Social Sciences (SPSS for Windows, version 23). Values are expressed as means ± Standard Error of ten rats in each group (n=10) and values will be analyzed using One-way Analysis of Variance (ANOFA). The level of significance was set at P<0.05.

4. RESULTS

1.1 Markers of Oxidative Stress

Serum oxidant biomarkers of lipids malondialdehyde (MDA), proteins as protein carbonyls (PC) and oxidized DNA as 8 hydroxyl guanosine (8OHG) were expressed in Table 1. Data showed that rats treated individually with oxymetholone 150 mg/ day and Creatine 20 mg/day for 4 weeks had a remarkable significant increase in the abovementioned oxidant markers compared to the respect untreated group at P<0.05. Pronounced elevation was noticed in the mixture treated group in comparison to the control and other non-mixed groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MDA (nmol/ml)</th>
<th>PC (µmol/mg)</th>
<th>8HOG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.643±0.110</td>
<td>6.52±0.359</td>
<td>3.59±0.216</td>
</tr>
<tr>
<td>Oxymetholone (150 Mg/Day)</td>
<td>1.20±0.031*</td>
<td>12.26±0.579*</td>
<td>8.37±0.296*</td>
</tr>
<tr>
<td>Creatine (20 Mg/Day)</td>
<td>1.17±0.067*</td>
<td>11.49±0.480*</td>
<td>5.77±0.361*</td>
</tr>
<tr>
<td>Mixture</td>
<td>1.49±0.127**</td>
<td>13.48±0.686*</td>
<td>9.15±0.243**</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE (n=10)
a significance difference versus control at P < 0.05  
b significance difference versus Oxymetholone group at P < 0.05  
c significance difference versus Creatine group at P < 0.05  
d significance difference versus Mixture group at P < 0.05

1.2 Markers of Defense System

Current data depicted in Table 2 declared that treatment with each of the oxymetholone (150 mg/day) and creatine (20mg/day) induced remarkable significant reduction in the measured antioxidant biomarkers in serum; Catalase (CAT), Superoxide dismutase enzymes (SOD) and Total antioxidant capacity (TAC) as compared to the control group at p<0.05. On other hands; synergistic effect of both supplements was obvious in mixture treated group where the percentage of reduction was pronounced in TAC (-52.59%), CAT (-37.26%) and SOD (-50.63%) compared to the control group and, significant compared to control and individually treated groups at P<0.05.

Table (1): Effect of Treatment with Oxymetholone 150 Mg/Day And /Or Creatine 20 Mg/ Day For 4 Weeks on Serum Oxidative Stress Biomarkers of Albino Rats
### Table (2): Effect of Treatment with Oxymetholone 150 Mg/Day And /Or Creatine 20 Mg/ Day For 4 Weeks on Antioxidant Biomarkers of Albino Rats

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>TAC (µmol/mg)</th>
<th>CAT (µ/L)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.70±2.38</td>
<td>119.16±1.35</td>
<td>180.78±4.94</td>
</tr>
<tr>
<td>Oxymetholone (150 Mg/Day)</td>
<td>41.43±1.24a</td>
<td>97.51±3.62a</td>
<td>100.14±3.72a</td>
</tr>
<tr>
<td>Creatine (20 Mg/ Day)</td>
<td>43.10±2.07a</td>
<td>92.64±4.70a</td>
<td>85.53±7.44a</td>
</tr>
<tr>
<td>Mixture</td>
<td>34.94±3.60abc</td>
<td>74.75±10.16abc</td>
<td>89.25±4.64a</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE (n= 10)
- a significance difference versus control at P < 0.05
- b significance difference versus Oxymetholone group at P < 0.05
- c significance difference versus Creatine group at P < 0.05
- d significance difference versus Mixture group at P < 0.05

### 1.3 Testis Biomarker

Considering the effects of each of the oxymetholone (150 mg/day) or creatine (20mg/day) and their mixture on some testis biomarkers in serum of albino rats. Results demonstrated in Table3 indicated remarkable significant drop in the testosterone level among the oxymetholone treated group (-55.12%) and mixture (-54.41%) treated groups. However, Creatine treated group showed mild decrease with (-21.07%) the potentially of the oxymetholone was obvious in combined group. On the other hand, the status of luteinizing hormone in serum of treated groups showed pronounced reduction in oxymetholone treated group (-46.33%) versus control at P<0.05. Creatine administration showed slight non noticeable changes versus control but significant difference versus oxymetholone group at P<0.05. On the other hand, mixture treated groups depicted significant decrease (-42.93%) in comparison control and non-mixed groups at P<0.05.

### Table (3): Effect of Treatment with Oxymetholone 150 Mg/Day And /Or Creatine 20 Mg/ Day For 4 Weeks on Testosterone and Luteinizing Hormones in Serum of Albino Rats

<table>
<thead>
<tr>
<th>Parameters / Groups</th>
<th>Testosterone (ng/dl)</th>
<th>Luteinizing Hormones (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>450.83±25.08</td>
<td>1.77±0.165</td>
</tr>
<tr>
<td>Oxymetholone (150 Mg/Day)</td>
<td>202.33±5.35a</td>
<td>0.95±0.191a</td>
</tr>
<tr>
<td>Creatine (20 Mg/ Day)</td>
<td>355.83±19.56ab</td>
<td>1.80±0.122b</td>
</tr>
<tr>
<td>Mixture</td>
<td>205.50±9.21abc</td>
<td>1.01±0.103abc</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE (n= 10)
- a significance difference versus control at P < 0.05
- b significance difference versus Oxymetholone group at P < 0.05
- c significance difference versus Creatine group at P < 0.05
- d significance difference versus Mixture group at P < 0.05

### 1.4 Semen Quality

Regarding semen quality parameters individual supplementation with each of the oxymetholone (150 mg/day) or creatine (20mg/day) and their mixture on some semen quality by pronounced reduction in the sperm count and motility. Synergistic effect was declared in combined treated group where sperm counts were recorded remarkable significant decrease as compared to control and individually treated groups at P<0.05 surprisingly, statistical analysis recorded slight elevation in sperm motility in mixture treated groups more or less near to the control level and significant versus all other treated groups Table 4.

### Table (4): Effect of Treatment with Oxymetholone 150 Mg/Day And /Or Creatine 20 Mg/ Day For 4 Weeks on Semen Analysis of Albino Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm Count (cells/cubic millimeter)</th>
<th>Sperm Motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13534785.37±741456.66</td>
<td>63.80±2.98776</td>
</tr>
<tr>
<td>Oxymetholone</td>
<td>6761111.10±697020.99a</td>
<td>45.16±0.872a</td>
</tr>
<tr>
<td>Creatine</td>
<td>8751053.15±318763.39ab</td>
<td>46.43±1.30a</td>
</tr>
<tr>
<td>Mixture</td>
<td>5492232.00±487908.24ac</td>
<td>70.71±2.59abc</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE (n= 10)
- a significance difference versus control at P < 0.05
- b significance difference versus Oxymetholone group at P < 0.05
1.5 Histopathological results

Testis of rats in the control group demonstrated testicular tissue was formed of a group of small sized rounded or oval seminiferous tubules (STs) with patent Lumen containing spermatozoa. The Leydig were situated in clusters surrounding the blood vessels. They showed large polygonal cells, acidophilic cytoplasm, pale vesicular nuclei, and many small lipid droplets filling in the cytoplasm. Each STs was lined by a regular basement membrane that is surrounded by myoid cells containing flattened nuclei shown in Fig 1.

However, rats received Oxymetholone 150 mg / day alone showed that STs showed absence of basement membranes and detached spermatogenic cells leaving large empty spaces between the germ cells. Apoptotic changes in the form of pyknotic or irregular darkly stained nuclei and deeply acidophilic cytoplasm were observed in most spermatogenic cells. In addition, some STs showed remnants of spermatogenic cells. Others showed apparent decrease of the flagella of mature sperm and some with empty lumen without sperms, shown in Fig 2.

On the other hand, testicular sections of rats received protein as creatine 20mg/day alone showed that some STs appeared remarkably nearly similar to the histological appearance of the control. They appeared lined with layers of stratified germinal epithelium at various stages of spermatogenesis and Sertoli cells. In addition, empty spaces

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c significance difference versus Creatine group at \( P < 0.05 \)
d significance difference versus Mixture group at \( P < 0.05 \)
were still seen among spermatogenic epithelium and darkly stained nuclei of spermatogenic cells with deeply acidophilic cytoplasm. Also, in some sections exhibited few primary spermatocytes, marked early spermatids, and few late spermatids. While other STs appeared with absent sperms in the lumen, shown in Fig 3.

In contrast, testis of rats received Oxymetholone 150 mg / day and protein as creatine 20 mg / day for 28 days showed severely distorted seminiferous tubules with loss of their normal histological architecture in many sections in comparison to the control group. The testicular capsule appeared thickened irregular with marked congested dilated blood vessels; many seminiferous tubules were shrunken, atrophied, and completely devoid of spermatogenic epithelium. Other tubules showed arrested spermatogenesis and detached spermatogenic cells surrounded with wide empty spaces. No sperms could be detected in the Lumina of the seminiferous tubules. The seminiferous tubules were widely separated from each other with areas filled with edematous interstitial spaces filled with acidophilic hyalinized structure and areas with marked depletion of the Leydig cells, shown in Fig 4.

**Fig 3:** A section showing seminiferous tubules full of spermatogenic epithelium and apparent increase of empty spaces (E) in-between the spermatogenic cells. (H&E x20)

**Fig 4:** marked distorted histological architecture of the seminiferous tubules (St). Some tubules appeared filled with remnants of spermatogenic cells (R) and absent flagella of mature sperms (F) in the lumen. Detached spermatogenic cells (yellow↑) with empty spaces (E) and some areas with arrest spermatogenesis (red↑) are seen. Notice wide edematous interstitial spaces with acidophilic hyalinized structure (*). Apparent increase of spermatogenic cells with darkly stained nuclei with deeply stained cytoplasm. (H&E x20)

5. DISCUSSION

The rise in the numbers of individuals they use of performance enhancing medication to improve physique all over the world. Particularly amongst gym users and in gymnastic clubs in KSA. Gym attendees are eager to take dietary supplements, without the advice provided by health professionals, in order to increase lean body mass quickly. Among these supplements anabolic androgenic steroids (AAS) derivatives from testosterone. The anabolic state can be defined as a state in which retention of nitrogen in lean body mass occurs due to an increase in protein synthesis and/or a decreased protein breakdown. It includes growth promotion, collagen and protein synthesis and an increase in bone metabolism and muscle size. The physiological changes occurring in the body of a male are known as the androgenic state. These changes include the appearance of secondary male characteristics, such as sebaceous gland activity, maturation of sperm and libido, hair growth pattern. Moreover, Protein supplementation becomes common
between young men. Creatine as one of these supplements. Endogenously synthesized from arginine, glycine and methionine in the kidney and liver.\textsuperscript{34} Exogenous creatine is obtained from the diet and is also readily available as a commercial dietary supplement advertised it has beneficial effects on muscle, increase exercise training capacity and intensity. Several side effects were recorded as a result of abuse of these supplements. This study corroborates the relation between the infertility and abuse of oxymetholone in presence or absence of creatine supplement. The obtained results from this study declared induction of oxidative stress in all groups treated with each of oxymetholone or creatine and their mixture represented by elevation of specific markers MDA, PC and 8OHG. Concomitant with the reduction in superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase (CAT) antioxidant markers assessed with the present study. Oxidative stress is begin by lopsidedness between the activity of endogenous peroxidative enzymes, such as xanthine oxidase, NADPH oxidase, and antioxidative enzymes such as SOD, CAT, and paraoxonase. Antioxidant systems help to keep Intracellular reactive oxygen species (ROS) at suitable levels. These systems react with these molecules producing lower reactive compounds. Catalase helps in H\textsubscript{2}O\textsubscript{2} detoxification which gives rise to H\textsubscript{2}O directly, while superoxide dismutase (SOD) promotes the transformation of superoxide to H\textsubscript{2}O, Malondialdehyde, a result of the reaction of polyunsaturated fatty acids and superoxide anion (O\textsubscript{2}). Changes in oxidative stress biomarkers, such as 8-OHG, helps in indicating levels of oxidative DNA damage. Trainers supplemented with AAS recorded an enhancement in oxidative stress levels due to raise the lipid peroxidation.\textsuperscript{35} Also, rats treated with oxymetholone 5mg/kg/day showed elevation in MDA and reduction in CAT enzyme. Other study reported that oral creatine supplementation increases in oxidative stress markers and antioxidant defenses post-exercise, it may also increase levels of lipid peroxidation markers. The outcomes can be understood through the fact that oxidative damage may target the mitochondria because its membrane has large amounts of polyunsaturated fatty acids. Changes that occur in the lipid environment in the complexes of the respiratory chain may contribute to lowering their activity causing a perturbation of metabolism. Additionally, aldehydes, generated from lipid peroxidation, are stable and can easily diffuse intracellularly. This explains the fact that they are able to attack targets far from the original free radical event. This contributes to the expansion of oxidative damage to a variety of molecules, proteins, DNA, enzymes, or hormones. We hypothesized that combination between AAS and creatine induced pronounce enhancement in the above descriptive status. Najafi et al.\textsuperscript{36} recorded that rats treated with oxymetholone (5mg/day) for 28 days showed marked reduction in testosterone as well as considerable damage in testis tissue architecture affect semen quality inducing oligozoosperma, azoosperma and spermatogenic arrest. Exogenous testosterone such as AAS leads to a negative feedback on the HPG axis which eventually leads to the suppression of spermatogenesis. It greatly lowers the production of gonadotropin releasing hormone (GnRH). Therefore, it inhibits the secretion of Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Likewise, Moss et al.\textsuperscript{37} reported that the reduction of intratesticular testosterone levels (ITT) and overall testosterone creation is due to suppression of gonadotropins. Exogenous testosterone therapies can greatly damage and suppress intratesticular testosterone. Intratesticular testosterone is needed for normal spermatogenesis, if it is inhibited, it can lead to azoosperma. These findings support our results treatment with oxymetholone induced pronounced reduction in both testosterone and luteinizing hormone. Moreover, treatment with creatine caused a mild reduction in testosterone hormone however luteinizing hormone (LH) did not show any changes from control. Fernandez-Lenda et al.\textsuperscript{38} recorded nonsignificant decrease in testosterone hormone in trainers supplemented with creatine 0.04 g/kg/day for ten weeks. The effects of acute (few days) creatine supplementation can be explained by three mechanisms: quicker regeneration of phosphocreatine (PCr) during the recovery period, an increase in PCr storage in muscle, and an enhanced ATP production from glycolysis, increasing the quantity, density of muscle cell mitochondria, fat oxidation, and angiogenesis. On the other word, our hypothesis explains the reduction in each of the luteinizing hormone and testosterone hormone recorded in mixture treated group proves the potential effect of oxymetholone. Histopathological studies showed severe damage in testis tissue architecture in oxymetholone treated rats expressed by empty spaces between the germ cells, remnant of spermatogenic cells and spermatogenic arrest. However, creatine treatment induced non pronounced changes in testis cells structure. On the other hand, mixture treated groups showed shrunken in many seminiferous tubules, atrophy, and completely devoid of spermatogenic epithelium. Other tubules showed arrested spermatogenesis and detached spermatogenic cells surrounded with wide empty spaces; these findings confirm the clinical results and Proven the potential effect of oxymetholone. Najafi et al. reported alteration in some histological parameters (tubule differentiation, Sertoli cell, spermatiation), depletion in seminiferous epithelium of testis rats treated with oxymetholone 5mg/kg for 28 days were signs about the testicular damage. It is important to note that over-oxidation may contribute to sperm dysfunction by various mechanisms: lipid peroxidation in the plasma membranes of sperm, the production of large amounts of double and single breaks in the strands of DNA, impairment of sperm morphology and motility. Additionally, current literature notes that the DNA damage caused by oxidative stress can accelerate the apoptosis of germ cell leading to a reduction in sperm concentrations.\textsuperscript{39}

6. CONCLUSION
Abuse of some supplements like oxymetholone as AAS increased ROS generation resulting in oxidative stress expressed by elevation of MDA and PC and 8OHG concurrent with reduction of some antioxidant parameters (TAC,CAT and SOD) in serum in the present study that cause severe damage in testis tissue architecture affect spermatogenesis process, sperm quality as well as disturbance in testosterone and luteinizing hormone secretion. However, supplementation with creatine as a protein supplement induced mild effects on the previous mentioned parameters. Synergistic effects were recorded in combination between the two supplements. These results confirm that all supplements should be used under supervision of specialist physicians especially between young adults.

7. AUTHORS CONTRIBUTION STATEMENT
S. Batais conceptualized, designed and gathered the data related to this work. Dr. M. Elhalwagy and Dr. N. Ayaz
reviewed, analyzed these data and gave the necessary inputs towards the scheming of the manuscript. All authors discussed the results and contributed to the final manuscript.

9. REFERENCES


8. CONFLICT OF INTEREST
Conflict of interest declared none.


