ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.5.L211-220

International Journal of Life science and Pharma Research

Research Article

Analytical chemistry for efficient drug analysis

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[1*], Manal Ea Elhalwagy[2] and Najla O Ayaz[3]

[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.

***Corresponding Author**

Sarah T Batais* , University of Jeddah, College of Science, Department of Biochemistry, Jeddah, Saudi Arabia

Recieved On 18 November, 2020
Revised On 16 December, 2020
Accepted On 31 December, 2020
Published On 31 December, 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Sarah T Batais*, Manal EA Elhalwagy, Najla O Ayaz , Influence of Short-Term Supplementation with Anabolic Steroid Drug Oxymetholone And /Or Creatine Widely Used In Ksa on Some Fertility Biomarkers in Albino Rats.(2020).Int. J. Life Sci. Pharma Res.10(5), 211-220 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.5.L211-220

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] Oxymetholone [17b -hydroxy- 2 -hydroxymethylene17a-methyl-5a-androstan-3 one] is a synthetic androgen analogue, and available in the commercial market as Androl. Oxymetholone 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), 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 of 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°C 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-2hydroxymethylene-17a-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 by using equation:

Animal Equivalent Dose (AED) = Human equivalent dose X 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 Carbonyls were measured according to procedure described by Cadenes and Wakeyama[22-23] 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 Masayasu 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 1mm (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 (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

Parameters Groups	MDA (nmol/ml)	PC (µmol/mg)	8HOG (ng/ml)
Control	0.643±0.110	6.52±0.359	3.59±0.216
Oxymetholone (150 Mg/Day)	1.20±0.031[a]	12.26±0.579[a]	8.37±0.296[a]
Creatine (20 Mg/ Day)	1.17±0.067[a]	11.49±0.480[a]	5.77±0.361[ab]
Mixture	1.49±0.127[abc]	13.48±0.686[ac]	9.15±0.243[ac]

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

Parameters/Groups	TAC (µmol/mg)	CAT (Mu/L)	SOD (U/ml)
Control	73.70±2.38	119.16±1.35	180.78±4.94
Oxymetholone (150 Mg/Day)	41.43±1.24[a]	97.51±3.62[a]	100.14±3.72[a]
Creatine (20 Mg/ Day)	43.10±2.07[a]	92.64±4.70[a]	85.53±7.44[a]
Mixture	34.94±3.60[ac]	74.75±10.16[abc]	89.25±4.64[a]

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

Parameters / Groups	Testosterone (ng/dl)	Luteinizing Hormones (IU/L)
Control	450.83±25.08	1.77±0.165
Oxymetholone (150 Mg/Day)	202.33±5.35[a]	0.95±0.191[a]
Creatine (20 Mg/ Day)	355.83±19.56[ab]	1.80±0.122[b]
Mixture	205.50±9.21[ac]	1.01±0.103[abc]

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) affect 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

Groups	Sperm Count (cells/cubic millimeter)	Sperm Motility (%)
Control	13534785.37±741456.66	63.80±2.98776
Oxymetholone	6761111.10±697020.99[a]	45.16±0.872[a]
Creatine	8751053.15±318763.39[ab]	46.43±1.30[a]
Mixture	5492232.00±487908.24[ac]	70.71±2.59[abc]

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

1.5 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 Lumina 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.

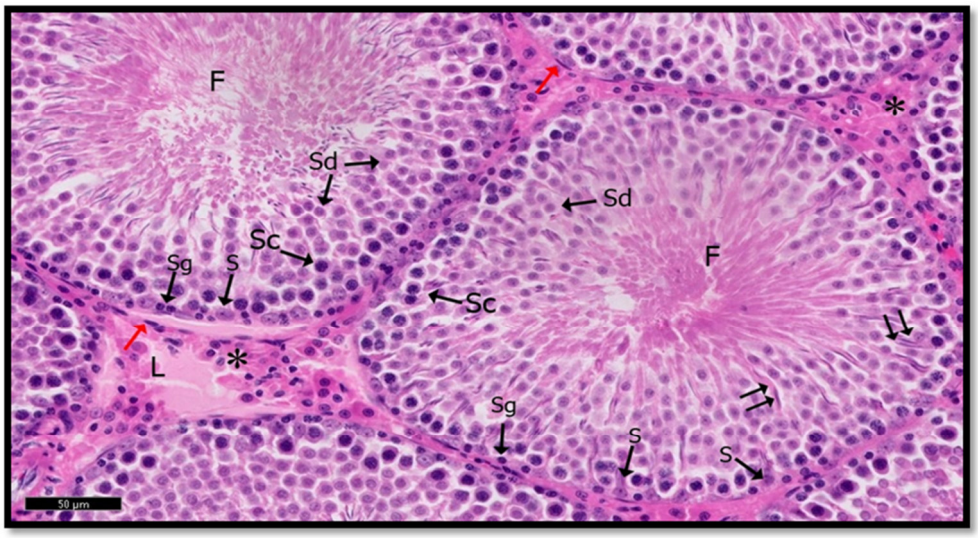

Fig1: A section of a control rat testis demonstrating seminiferous tubules resting on a thin basement membrane and myoid cell (red↑). The spermatogenic epithelium is formed of spermatogonia (Sg), the largest cells; primary spermatocytes (Sc), early spermatids (Sd), late spermatids (↑↑), flagella of mature sperms (F), and Sertoli cell (S) are seen. The interstitial tissue (*) in between the seminiferous tubules. The Leydig cells (L) appear with vesicular nuclei. (H&E x20)

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.

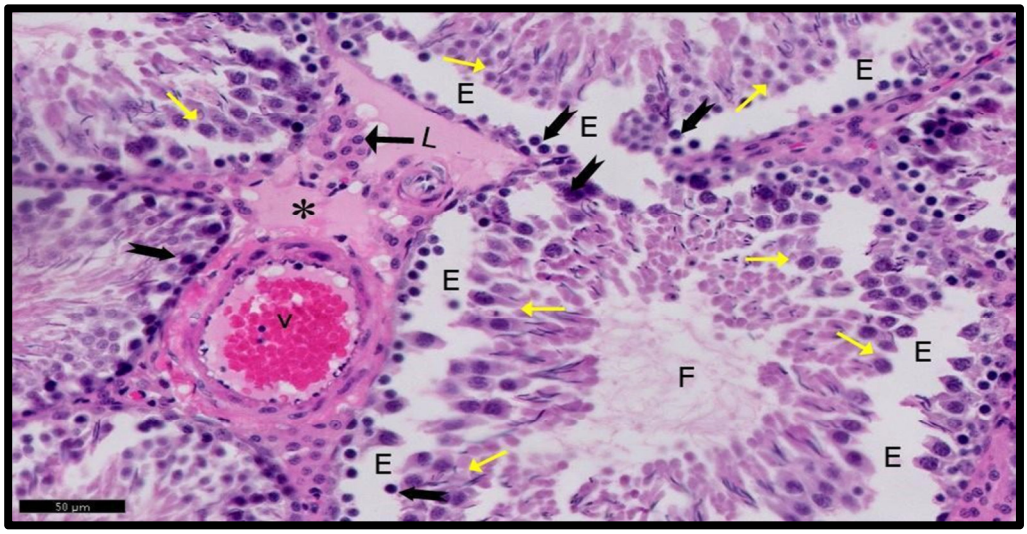

Fig2: A section of rat testis of group 2 (received Oxymetholone 150 mg/kg for 28 days) showing distorted tubules, detached spermatogenic cells (yellow↑) with empty spaces (E), darkly stained nuclei of spermatogenic cells (bifid↑), and the lumen of the tubule appeared empty from flagella of mature sperms (F). Wide edematous interstitial space (*) with acidophilic homogenous material showed dilated congested blood vessel (v) and pyknotic nuclei of Leydig cells (L). (H&E x20)

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

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.

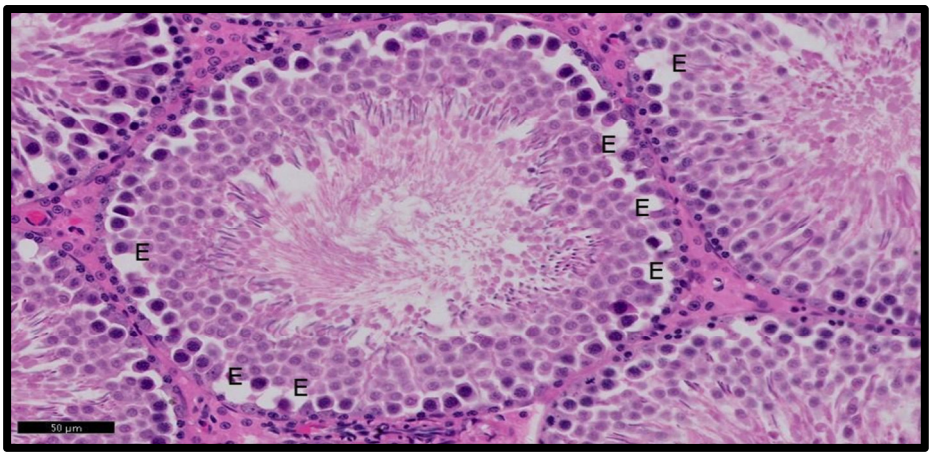

Fig 3: A section showing seminiferous tubules full of spermatogenic epithelium and apparent increase of empty spaces (E) in-between the spermatogenic cells. (H&E x20)

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.

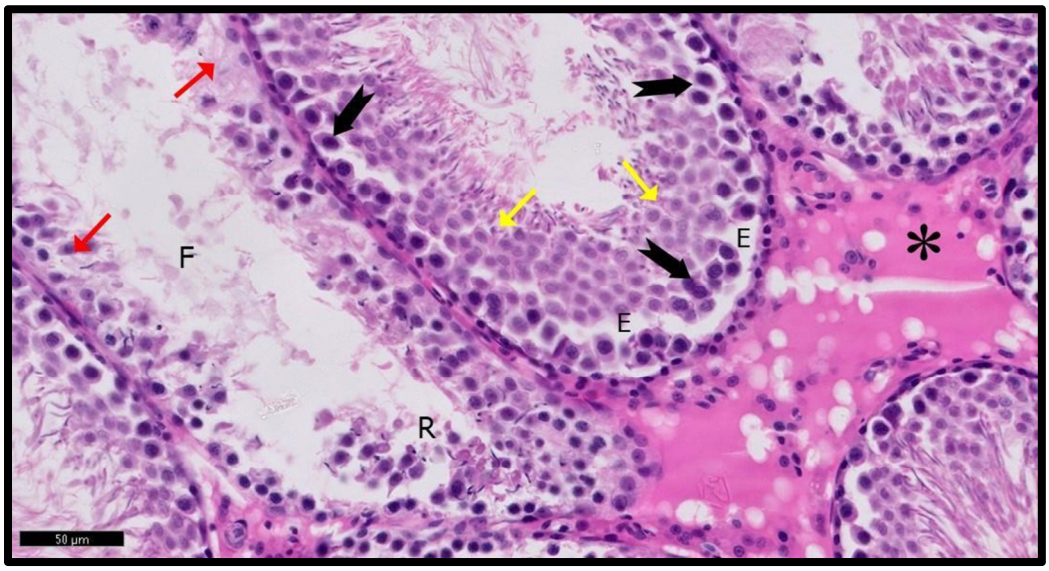

Fig 4: marked distorted histological architecture of the seminiferous tubules (St). Some tubules appeared filled with remnants of spermatogenic cells (R) and absent flagella of mature sperms (F) in the lumen. Detached spermatogenic cells (yellow↑) with empty spaces (E) and some areas with arrest spermatogenesis (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.[31] Particularly amongst gym users[32] 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.[33] 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 supplementations become common

between young men. Creatine as one of these supplements. Endogenously synthesized from arginine, glycine and methionine in the kidney and liver.[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_2O_2 detoxification which gives rise to H_2O directly, while superoxide dismutase (SOD) promotes the transformation of superoxide to H_2O_2. Malondialdehyde, a result of the reaction of polyunsaturated fatty acids and superoxide anion (O_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.[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.[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 oligozoospermia, azoospermia 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.[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 azoospermia. 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.[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, spermiation), 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.[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.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Althobiti SD, Alqurashi NM, Alotaibi AS, Alharthi TF, Alswat KA. Prevalence, attitude, knowledge, and practice of anabolic androgenic steroid (AAS) use among gym participants. Mater Socio Med. 2018 Mar;30(1):49-52. doi: 10.5455/msm.2018.30.49-52, PMID 29670477.
2. Alves C, Lima RV. Dietary supplement use by adolescents. J pediatr. 2009;85(4):287-94. doi: 10.2223/JPED.1907, PMID 19585056.
3. Juhn MS. Popular sports supplements and ergogenic aids. Sports Med. 2003;33(12):921-39. doi: 10.2165/00007256-200333120-00004, PMID 12974658.
4. Madrakian T, Afkhami A, Rahimi M, Ahmadi M, Soleimani M. Preconcentration and spectrophotometric determination of oxymetholone in the presence of its main metabolite (mestanolone) using modified maghemite nanoparticles in urine sample. Talanta. 2013 Oct;115:468-73. doi: 10.1016/j.talanta.2013.05.056, PMID 24054620.
5. Hearne E, Wazaify M, Van Hout MC, Atkinson A, McVeigh J. Anabolic-androgenic steroid use in the eastern Mediterranean region: a scoping review of extant empirical literature. Int J Ment Health Addict. 2020 Jan 15:1-28. doi: 10.1007/s11469-019-00217-8.
6. Al Bishi KA, Afify A. Prevalence and awareness of anabolic androgenic steroids (AAS) among gymnasts in the western province of Riyadh, Saudi Arabia. Electron Phys. 2017 Dec 25;9(12):6050-7. doi: 10.19082/6050, PMID 29560159.
7. Alharbi FF, Gamaleddin I, Alharbi SF, Almodayfer O, Allohidan F, Alghobain M, Arafah A, Al-Surimi K. Knowledge, attitudes and use of anabolic-androgenic steroids among male gym users: A community based survey in Riyadh, Saudi Arabia. Saudi Pharm J. 2019;27(2):254-63. doi: 10.1016/j.jsps.2018.11.007, PMID 30766438.
8. O'Hagan A, Bigger WH. Bigger, Faster, stronger! An overview of anabolic androgenic steroids and their use and impact on the sport industry. Forensic Res Criminol Int J. 2015 Nov 9;1(3):1-18. doi: 10.15406/frcij.2015.01.00018.
9. Vadi M, Noshadi Y. Investigation of adsorption isotherm of oxymetholone as a kind of steroid drug by multi-wall carbon nanotube. Orientjchem. 2012;28(1):297-301. doi: 10.13005/OJC/280136.
10. Cerretani D, Neri M, Cantatore S, Ciallella C, Riezzo I, Turillazzi E, Fineschi V. Looking for organ damages due to anabolic-androgenic steroids (AAS): is oxidative stress the culprit? Mini Rev Org Chem. 2013 Dec;10(4):393-9. doi: 10.2174/1570193X113106660025.
11. Mazzeo F, Ascione A. Anabolic androgenic steroids and doping in sport. Sports Medicine [journal]. Med Sportivâ. 2013;9(1).
12. Sánchez Oliver A, Miranda León MT, Guerra-Hernández E. Prevalence of protein supplement use at gyms. Nutr hosp. 2011;26(5):1168-74. doi: 10.1590/S0212-16112011000500037, PMID 22072369.
13. Kalman DS. Amino acid composition of an organic brown rice protein concentrate and isolate compared to soy and whey concentrates and isolates. Foods. 2014 Jun 30;3(3):394-402. doi: 10.3390/foods3030394, PMID 28234326.
14. Rawson ES, Stec MJ, Frederickson SJ, Miles MP. Low-dose creatine supplementation enhances fatigue resistance in the absence of weight gain. Nutrition. 2011 Apr;27(4):451-5. doi: 10.1016/j.nut.2010.04.001, PMID 20591625.
15. Almukhtar SE, Abbas AA, Muhealdeen DN, Hughson MD. Acute kidney injury associated with androgenic steroids and nutritional supplements in bodybuilders(†). Clin Kidney J. 2015;8(4):415-9. doi: 10.1093/ckj/sfv032, PMID 26251708.
16. Omar Aljaloud S. Dietary Supplements for Professional Athletes: A Great Potential for Saudi Arabia. JNHFE;3(1). doi: 10.15406/jnhfe.2015.03.00097.
17. Deminice R, Rosa FT, Franco GS, Jordao AA, de Freitas EC. Effects of creatine supplementation on oxidative stress and inflammatory markers after repeated-sprint exercise in humans. Nutrition. 2013 Sep;29(9):1127-32. doi: 10.1016/j.nut.2013.03.003, PMID 23800565.
18. Deldicque L, Décombaz J, Zbinden Foncea H, Vuichoud J, Poortmans JR, Francaux M. Kinetics of creatine ingested as a food ingredient. Eur J Appl Physiol. 2008 Jan;102(2):133-43. doi: 10.1007/s00421-007-0558-9, PMID 17851680.
19. A. Pagonis T, P. Givissis P. Contemporary patterns of Anabolic Steroids abuse and associated short term side effects in athletes. Int J Orthop. 2015 Aug 23;2(4):347-52. doi: 10.17554/j.issn.2311-5106.2015.02.89.
20. Food U, Administration D. Guidance for industry: estimating the maximum safe starting dose in adult healthy volunteer; 2005.
21. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol. 1979;135(3):372-6. doi: 10.1016/0002-9378(79)90708-7, PMID 484629.
22. Cadenas E, Boveris A, Ragan CI, Stoppani AO. Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. Arch Biochem Biophys. 1977;180(2):248-57. doi: 10.1016/0003-9861(77)90035-2, PMID 195520.
23. Wakeyama H, Takeshige K, Takayanagi R, Minakami S. Superoxide-forming NADPH oxidase preparation of pig polymorphonuclear leucocyte. Biochem J. 1982;205(3):593-601. doi: 10.1042/bj2050593, PMID 6293459.
24. De Souza-Pinto NC, Eide L, Hogue BA, Thybo T, Stevnsner T, Seeberg E, Klungland A, Bohr VA. Repair of 8-oxodeoxyguanosine lesions in mitochondrial dna

depends on the oxoguanine dna glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial dna of OGG1-defective mice. Cancer Res. 2001;61(14):5378-81. PMID 11454679.
25. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54(5):356-61. doi: 10.1136/jcp.54.5.356, PMID 11328833.
26. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. doi: 10.1016/S0076-6879(84)05016-3, PMID 6727660.
27. Fossati P, Prencipe L, Berti G. Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem. 1980;26(2):227-31. doi: 10.1093/clinchem/26.2.227, PMID 7353268.
28. Masayasu M, Hiroshi Y. A simplified assay method of superoxide dismutase activity for clinical use. Clin Chim Acta. 1979;92(3):337-42. doi: 10.1016/0009-8981(79)90211-0.
29. Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A, Buyukkomurcu F. Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pestic Biochem Physiol. 2007;87(2):115-22. doi: 10.1016/j.pestbp.2006.06.010.
30. Drury R, Wallington E, Cameron S. Carleton's histological techniques. 4th ed. Oxford: Oxford University Press; 1967.
31. Evans-Brown M, McVeigh J, Perkins C, Bellis MA. Human enhancement drugs: the emerging challenges to public health. North West Public Health Observatory; 2012.
32. Kimergård A, McVeigh J. Environments, risk and health harms: a qualitative investigation into the illicit use of anabolic steroids among people using harm reduction services in the UK. BMJ Open. 2014;4(6):e005275. doi: 10.1136/bmjopen-2014-005275, PMID 24898090.
33. Sjöqvist F, Garle M, Rane A. Use of doping agents, particularly anabolic steroids, in sports and society. Lancet. 2008;371(9627):1872-82. doi: 10.1016/S0140-6736(08)60801-6, PMID 18514731.
34. Bonilla DA, Moreno Y. Molecular and metabolic insights of creatine supplementation on resistance training. Rev Colomb Quím. 2015;44(1):11-8. doi: 10.15446/rev.colomb.quim.v44n1.53978.
35. Arazi H, Mohammadjafari H, Asadi A. Use of anabolic androgenic steroids produces greater oxidative stress responses to resistance exercise in strength-trained men. Toxicol Rep. 2017 Jun 08;4:282-6. doi: 10.1016/j.toxrep.2017.05.005, PMID 28959650.
36. Najafi G, Nejati V, Shalizar Jalali A, Zahmatkesh E. Protective role of royal jelly in oxymetholone-induced oxidative injury in mouse testis. Iran J Toxicol. 2014;8(25):1073-80.
37. Moss JL, Crosnoe LE, Kim ED. Effect of rejuvenation hormones on spermatogenesis. Fertil Steril. 2013 May 10;99(7):1814-20. doi: 10.1016/j.fertnstert.2013.04.003, PMID 23663992.
38. Fernández-Landa J, Fernández-Lázaro D, Calleja-González J, Caballero-García A, Córdova A, León-Guereño P, Mielgo-Ayuso J. Long-term Effect of Combination of creatine monohydrate Plus β-hydroxy β-Methylbutyrate (HMB) on Exercise-Induced Muscle Damage and Anabolic/Catabolic Hormones in Elite Male Endurance Athletes. Biomolecules. 2020 Jan 15;10(1):140. doi: 10.3390/biom10010140, PMID 31952174.
39. Shalizar Jalali AS, Najafi G, Hosseinchi M, Sedighnia A. Royal jelly alleviates sperm toxicity and improves in vitro fertilization outcome in stanozolol-treated mice. Iran J Reprod Med. 2015 Jan;13(1):15-22. PMID 25653671.