



## HISTOPATHOLOGICAL EFFECTS OF DIFFERENT DOSES OF ANABOLIC ANDROGENIC STEROID (SUSTANON) ON LIVER AND TESTIS OF MALE RATS

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### ABSTRACT

The present pathological study on the male rats aims to investigate the effects on liver and testis tissues induced by repeated weekly administration of two doses of sustanon for two months. The experiment was done on the 60 adult male rats randomly divided into three groups 20 rats in each group. The first group is considered a control treated weekly for 60 days with sesame oil intramuscularly. Second group is considered as therapeutic group was treated with diluted sustanon in sesame oil in concentration of 5 mg/kg body weight intramuscularly weekly for 60 days. Third group is considered as toxic group was treated with diluted sustanon in sesame oil in concentration of 20 mg/kg body weight intramuscularly, weekly for 60 days. After 30 and 60 days, 10 rats from each group were sacrificed, liver and testis was taken for histopathology. All treated groups with sustanon revealed histopathological changes in liver and testis tissues. The liver and testis sections elucidate cellular swelling, vacuolar degeneration in the cytoplasm in addition to fatty change and programmed cell death in the second and third groups during a period 30 and 60 days.

**KEYWORDS:** Anabolic steroid; Sustanon; Liver; Testis.

### INTRODUCTION

Testosterone is the base stone in male sexual differentiation, puberty, sexual behaviour and spermatogenesis (Mitchell *et al.*, 2009; Balthazart and Ball, 2010). Anabolic-androgenic steroids (AASs) are the male-made derivatives of the sex hormone testosterone (Hoffman and Ratamess, 2006). Testosterone is the main androgen (male sex hormone), cholesterol derived hormone (Saladin, 2004; Postlethwait and Hopson, 2006; Parr *et al.*, 2010) that controls male secondary sexual characteristics. Along with follicle-stimulating hormone (FSH), testosterone stimulates sperm production (Mader, 2010). Now days, the intentional misuse of anabolic androgenic drugs especially the testosterone derivatives by athletes especially the body builders have surged rapidly in many countries to become a serious negative phenomenon (Bin Bisher, 2009). Users and many athletes, especially in the power sports like body building and weight lifting, administer illegally high doses of these drugs during sport competitions (Hartgens and Kuipers, 2004). Unfortunately, according to recent report from Iraq, users of anabolic androgenic drugs gradually surged during the past 10 to 15 years (unpublished data). The anabolic androgenic drugs are an important therapeutic target for the cure of diseases such as hypogonadism (Mudali and Dobs, 2004; Seal, 2009), treat senile osteoporosis (Gooren, 2007), in conjunction with other hormones to promote skeletal growth in prepubertal boys with pituitary dwarfism (delay puberty) (Yavari, 2009), and to treat some types of anemia such as Fanconi's anemia (Maravelias *et al.*, 2005). Sustanon is a useful medical drug which possesses multiple clinical therapeutic benefits (Socas *et al.*, 2005). It composed of four different

testosterone esters (testosterone propionate, testosterone phenylpropionate, testosterone isocaproate and testosterone decanoate), which supply a continuous release of testosterone into the blood and producing a stable testosterone level for a long period of time extending from 3-4 weeks (Harvey *et al.*, 2006). The adverse effects caused by misusing anabolic androgenic drugs included cardiovascular disorders (Sader *et al.*, 2001), liver dysfunction (Amsterdam *et al.*, 2010), kidney disease, testicular problems (Socas *et al.*, 2005), psychiatric and behavioural disorders in both sexes (Maravelias *et al.*, 2005) as well as other problems on human body (Mader, 2010). High testosterone rate induces oxidative stress by alteration of the balance between ROS production and antioxidant defenses (Alonso-Alvarez *et al.*, 2007). The aim of the present work was to study the effect of sustanon 250 mg on the testis and sperm count.

### MATERIALS & METHODS

#### Experimental Animals

The present study was applied in laboratory house of college of veterinary medicine n Kufa University. It was conducted using 60 mature male albino rats (*Rattus norvegicus*). All rats were healthy, weighing 250 - 300 gm. and 10-12 weeks old at the time when the experiment started. The animals were bred and housed in plastic cages (56 x 39 x 19 cm) bedded with wooden chips in groups of 5 rats per cage in a room with controlled temperature of 24 ± 3°C, in animal house of College of veterinary medicine\ university of Kufa-Iraq. The animals were kept in 12/12 hours light/dark schedule during the experimental study. The rats were fed with standard laboratory chow containing and allowed to drink water.

### Sustanon 250

Sustanon ampoules (manufactured by N.V. Organon Oss Inc. Holland) have been obtained from the local pharmacy in Kufa-Iraq. Each ampoule contains 1mL of oily solution of Sustanon. According to the manufacturer, this 1mL of Sustanon consists of four testosterone ester compounds which include testosterone propionate, testosterone phenylpropionate, testosterone isocaproate and testosterone decanoate. During the present study, two doses of Sustanon have been selected which were 5 and 20 mg/Kg of the animal body weight (b.wt.).

### Experimental Design

The rats were divided randomly into three groups, each group consist of 20 male rats (first group served as control group and the other two groups as the treated groups). Each group consisted of 5 rats per cage. Group 1: Control: was injected once a week with sesame oil intramuscularly (i.m.). Group 2: Sustanon 5 mg/kg b.wt. Sesame oil: injected i.m., once a week with 5 mg/kg b.wt of sustanon-sesame oil suspension. Group 3: Sustanon (20 mg/kg b.wt. in sesame oil): was injected i.m., once a week with 20 mg /kg b.w with sustanon-sesame oil suspension. The duration of the experiment was two months.

### Histological Preparation

#### Paraffin Method

Testicles and livers were removed from the euthanized animals, they immediately fixed in Bouin's fluid for 24 hours, followed by a dehydration using a series of graded ethanol in ascending concentrations (50%, 70%, 95%, and 100%), immersed in xylene for clearing, infiltrated in paraffin wax, and finally embedded in paraffin wax. Four micrometer thick paraffin sections were obtained by using rotary microtome (Bright, MIC) and stained by

hematoxylin and eosin (H&E) (Bancroft *et al.*, 1977). The specimens were examined and photographed under light microscope (digital binocular compound microscope 40x-2000x, built-in 3MP USB camera).

The results of this study showed different effects on body organs and systems. Below we will view these effects in details.

### HISTOPATHOLOGY

Histopathological changes depend largely on the dose of sustanon. The different doses give definitely different effects on body organs. In this study, four groups were depended, so, the histopathological results of each group will be reviewed alone.

#### Control group

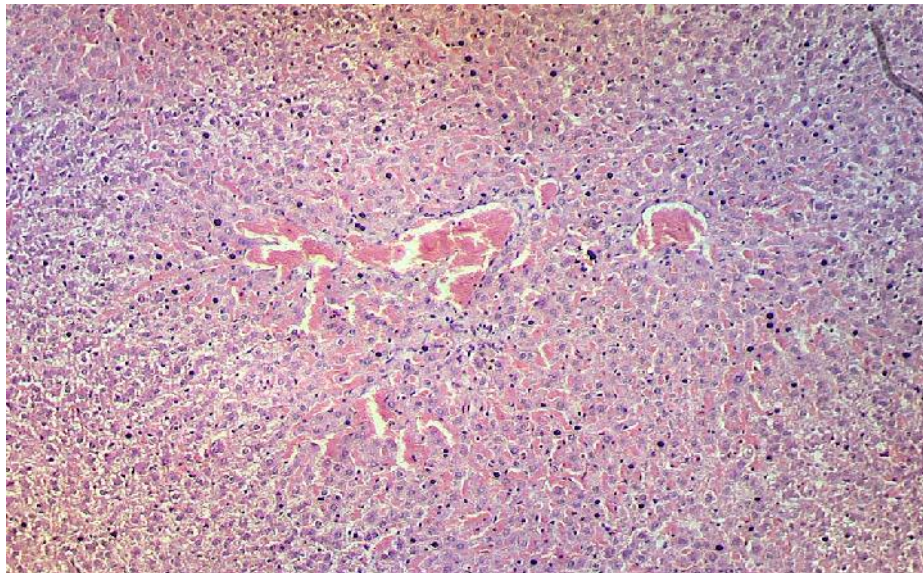
No changes of interesting happened in the control group. The normal histological features of different body organs (liver and testis) remained with no significant changes after the first and second months of treatment.

#### Therapeutic group

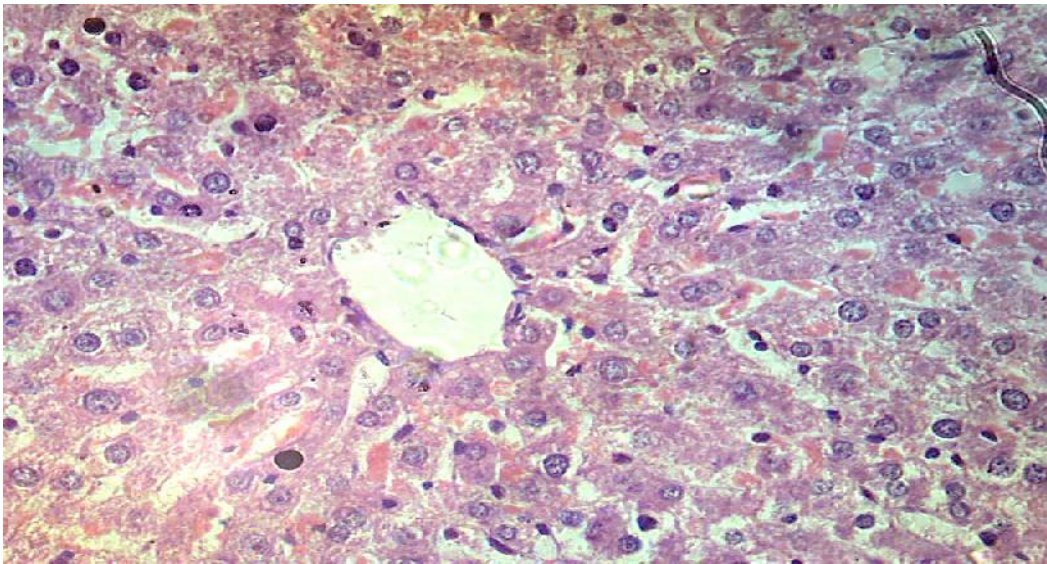
Mild effects of sustanon on different body organs (liver and testis) after the first and second months of treatment were noticed:

Liver: a congestion of central vein and sinusoidal dilatation with mild inflammatory reaction composed of neutrophils an mononuclear cells adjacent to blood vessel together with hydropic degeneration of hepatocytes; some of hepatocytes showed apoptosis and even focal area of coagulative necrosis as in (Fig. 1) and (Fig. 2).

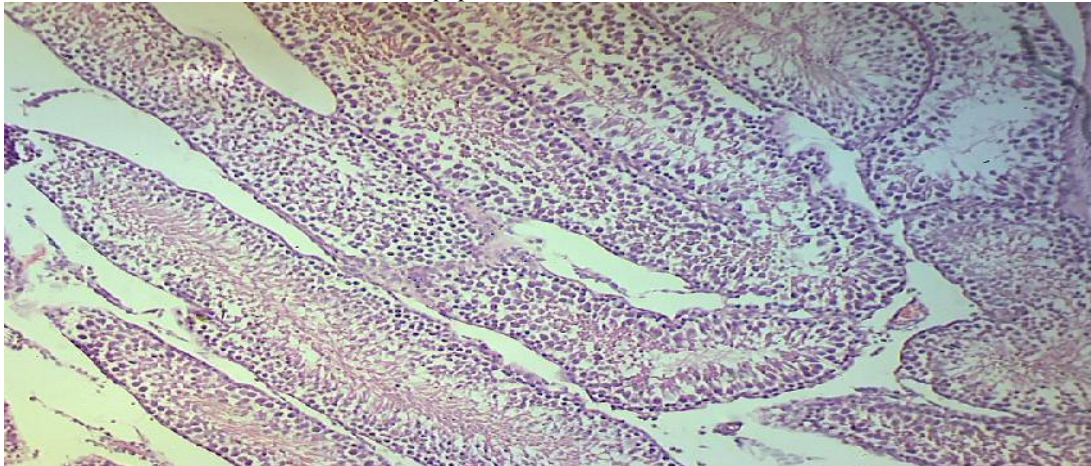
Testis: showed that there was mild loss of spermatogenesis, mild edema in the interstitial tissue, and mild hydropic degeneration as in (Fig. 3) and (Fig. 4).



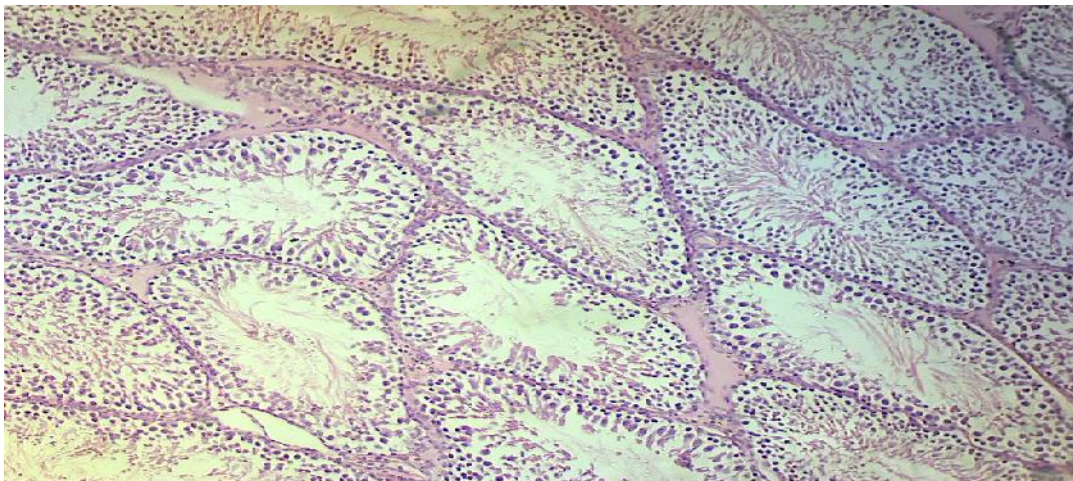
**FIGURE 1:** liver of male rat treated with sustanon 5 mg \kg B.W. B.W.intramuscularly weekly alone(first month), showed a congestion of central vein (black arrow) and sinusoidal dilatation, with mild inflammatory reaction composed of neutrophils and mononuclear cells adjacent to blood vessel (green arrow) together with hydropic degeneration of hepatocytes; some of hepatocytes showed apoptosis (blue arrow) and even focal area of coagulative necrosis (red arrow). X10 H&E



**FIGURE 2:** liver of male rat treated with sustanon 5 mg \kg B.W. B.W.intramuscularly weekly alone(second month), showed a sinusoidal dilatation and congestion, together with hydropic degeneration of hepatocytes; some of hepatocytes showed apoptosis (blue arrow) X10 H&E



**FIGURE 3:** testis of male rat treated with sustanon 5 mg \kg B.W. intramuscularly weekly alone, showed a mild loss of spermatogenesis (black arrow), mild edema in the interstitial tissue (blue arrow), and mild hydropic degeneration. X10 H&E.



**FIGURE 4:** testis of male rat treated with sustanon 5 mg \kg B.W. intramuscularly weekly alone, showed an extensive loss of spermatogenesis (red arrow), hydropic degeneration (blue arrow), with interstitial edema and depletion of Leydig cells (black arrow). X10 H&E

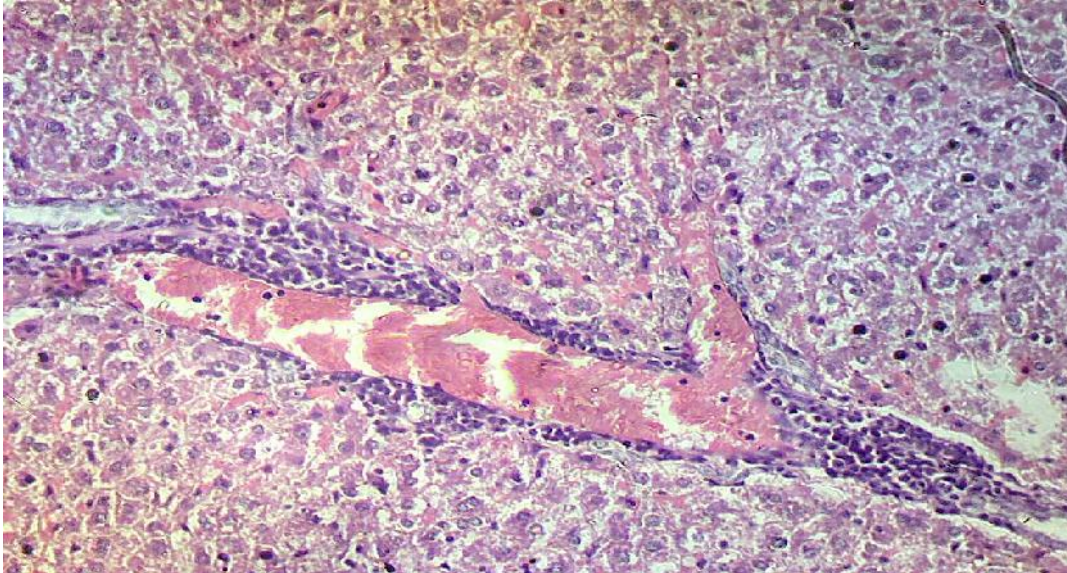
**Toxic group**

Different extensive lesions in different body organs (liver and testis) after the first and second months of treatment occurred:

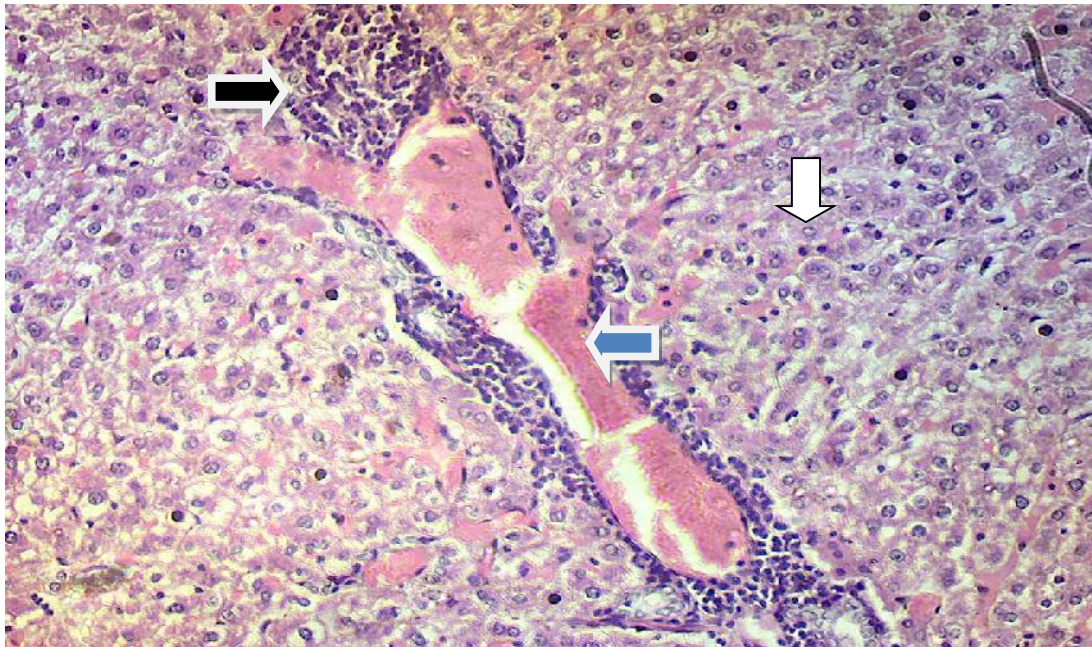
Testis: there is an extensive hydropic deg. of germinal layer of seminiferous tubule together with diffuse edema and extensive loss of spermatogenesis, also there was

depletion in Leydig cells with very extensive interstitial edema as in (Fig. 5) and (Fig. 6).

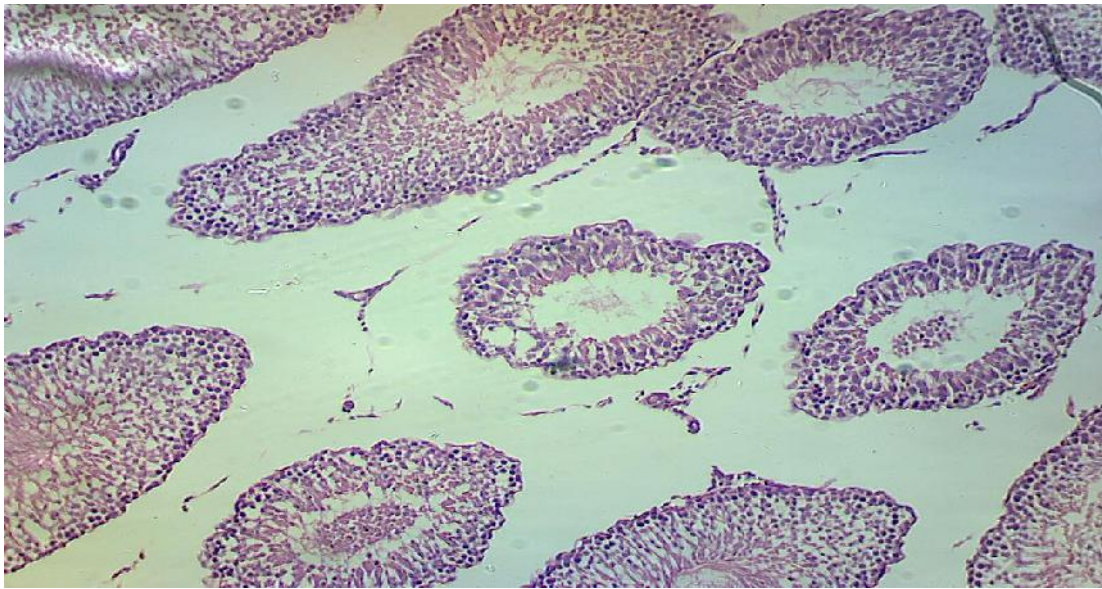
Liver: there was an extensive inflammatory reaction composed of mononuclear cells (macrophages and lymphocytes ) and neutrophils together with extensive congestion in the portal area; also there was extensive hydropic degeneration as in (Fig. 7) and (Fig. 8).



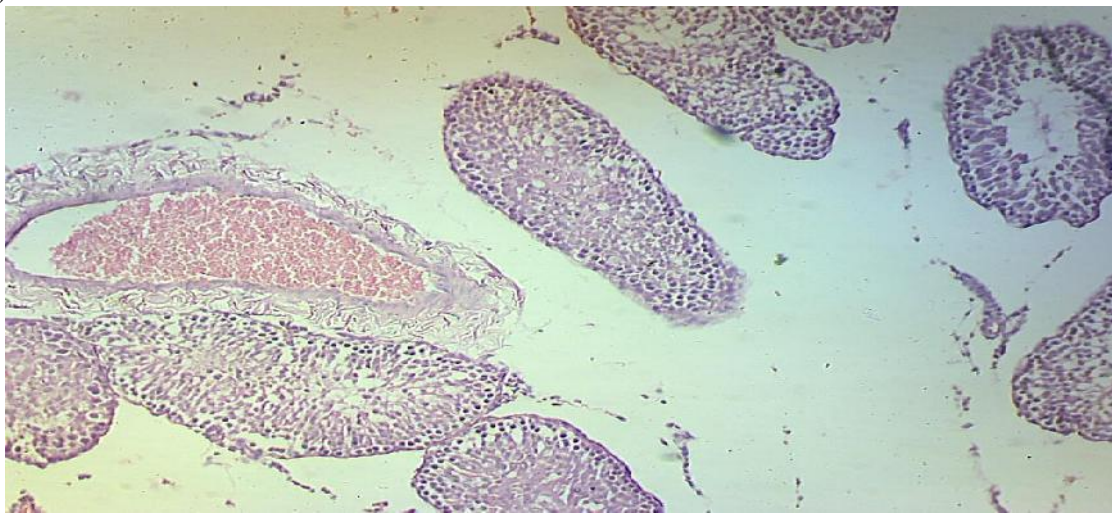
**FIGURE 5:** liver of male rat treated with sustanon 20 mg\kg B.W. intramuscularly weekly alone(first month), showed an extensive inflammatory reaction composed of mononuclear cells (macrophages and lymphocytes ) and neutrophils (black arrow) together with extensive congestion in the portal area (blue arrow); also there was extensive hydropic degeneration (white arrow). X20 H&E.



**FIGURE 6:** liver of male rat treated with sustanon 20 mg\kg B.W. intramuscularly weekly alone(second month), showed an extensive inflammatory reaction composed of mononuclear cells (macrophages and lymphocytes ) and neutrophils (black arrow) together with extensive congestion in the portal area (blue arrow); also there was extensive hydropic degeneration(white arrow). X20 H&E.



**FIGURE 7:** testis of male rat treated with sustanon 20 mg/kg B.W. intramuscularly weekly alone (first month), showed an extensive hydropic deg. of germinal layer of seminiferous tubule (black arrow) together with diffuse edema and extensive loss of spermatogenesis (blue arrow), also there was depletion in Leydig cells with very extensive interstitial edema (white arrow). X10 H&E.



**FIGURE 8:** testis of male rat treated with sustanon 20 mg/kg B.W. intramuscularly weekly alone (second month), showed a very extensive interstitial edema between seminiferous tubules. X10 H&E.

## DISCUSSION

Two different doses of sustanon, which have been chosen regarding the doses used by athletes, were administered to the rats in the current investigation. Administration of testosterone propionate led to a significant elevation of oxidative stress (Aydilek *et al.*, 2004). Subsequently, the targeted molecule becomes a free radical itself and initiates a cascade of events that can ultimately lead to cellular damage. The pathological roles of free radicals include lipid peroxidation, DNA damage and apoptosis (Kothari *et al.*, 2010). Free radicals have the ability to directly damage sperm DNA by attacking the purine, pyrimidine bases and deoxyribose backbone as well as they can damage the sperm membrane (Tremellen, 2008). It is also possible that the Sertoli cells might have been affected due to steroid injection and the other possibility might be due to its effect on the epididymal function (Bairy *et al.*, 2010). The reduction in Sertoli cell number

in treated rats with anabolic steroids could have resulted in a subsequent reduction in the number of spermatogonia leading eventually to a decrease in sperm count and testicular atrophy (Tahtamouni *et al.*, 2010). Within the testes, the main target cells for toxicants that disrupt spermatogenesis are the somatic cells (Leydig and Sertoli cells) and the germ cells. In animal models, each of these cell types can be selectively targeted by specific toxicants, resulting in apoptosis (Reddy *et al.*, 2009). Anabolic steroids such as nandrolone decanoate may cause loss of AR activity from Sertoli cells would lead to spermatogenic failure resulting in incomplete meiosis and collapse transition of spermatocytes to haploid round spermatids (Holdcraft and Braun, 2004).

O'donnell *et al.* (2001) revealed that exogenous testosterone administration caused significant decrease in sperm count and intratesticular testosterone concentration. Studies have reported that AASs use is strongly related to

decreased sperm count, decreased sperm motility, abnormal sperm morphology (Ciocca, 2005).

Apoptosis which occurs in testis for regulation of germ cell population (Giampietri *et al.*, 2005) may be related to lowering of the level of testicular testosterone (Richburg *et al.*, 2000). The suppressing of testicular testosterone production, as mentioned previously, may be due to administration of exogenous testosterone (Thabet *et al.*, 2010) and this may lead to the formation of more apoptotic germ cells. This may explain the dose dependent appearance of the multinucleated giant cells. Depending on histological and ultrastructural study, the present work reported that intramuscular injection of male rats with different doses of sustanon for four and eight weeks respectively is deleterious to the structure of rat testes. After analyzing different sections of testes of control and treated animals by light microscope, clear differences were noted. The testes of sustanon injected groups showed histological changes including severe damage of the seminiferous tubules, degeneration of germinal epithelia, appearance of apoptotic nuclei, presence of multinucleated giant cells having an apoptotic nuclei, lack of spermatids in the seminiferous tubules and appearance of vacuoles which increase in size and number with elevated doses of sustanon.

The present investigation showed a step-like stages of seminiferous tubules cells degeneration starting with the appearance of few apoptotic nuclei and vacuoles with small size, then followed by the appearance of multinucleated giant cells containing these apoptotic nuclei accompanied by larger size and number vacuoles and depletion of the germinal epithelium. Approximately similar stages were suggested by Anton (2003) after ligation of the efferent duct of the rat testis and he suggested time dependence for this stepwise changes. Since the present work has a fixed duration for all groups, we propose a dose relation rather than time relation for these stages.

The result of this study showed that IM injection of sustanon at 5 and 20 mg/Kg BW for 30 and 60 days induce hepatotoxicity in male rats of groups II and III as verified by histological finding, suggesting that these changes reflected the occurrence of liver injury.

The elevation of ALT and AST in blood using anabolic steroid randomly for long time can induce hepatotoxicity and morphological alterations of the liver cells. The uses of anabolic androgenic steroids particularly sustanon cause alteration in liver function due to alteration in morphological and histological structure of hepatocytes. The pathological changes revealed hypertrophy, congestion in central vein and sinusoid (particularly in 20 mg/kg Bwt for 30 to 60 days) indication for chronic venous congestion takeplac, and these changes reflect to injury of liver cell occurrence due to toxic hypoxia producing from different doses of sustanon like other anabolic steroid (Legros *et al.*, 2000). Congestion and hemorrhage take place due to increase hydrostatic pressure in blood cause vessels accumulation of red blood cell in the lumen of blood vessels, which allow blood cells to diapedesis and leakage out the blood vessels to cause bleeding. Moreover, sustanon may have ability to cause lipid peroxidation via reactive oxygen species which lead to fatty changes in addition to hypoxia.

Histopathological lesions also observed in this study started in 5 mg/kg Bwt, and reach to 20 mg/kg B.wt. in 30 and 60 days, this means that sustanon have ability to induce progress liver cell injury because its considered as a first organ responsible for metabolism of sustanon compound. So, this injury induced by accumulation of toxic metabolite (Xenobiotic) from sustanon metabolism that includes 17, -19-Nortestosterone, 17- testosterone (Pertusi *et al.*, 2001). On the other hand, figures showed cell swelling, sinusoid dilation and congestion in addition to centrolobular necrosis, these lesions occurs due to that sustanon like other anabolic androgenic steroid have ability to cause injury of hepatocytes through the effect on mitochondria particularly mitochondrial membrane and inhibit mitochondrial respiration which lead to swelling of mitochondria.

An interesting finding in this study was a presence of programmed cell death in hepatocytes, like other anabolic androgenic steroid for example blodenone undecyleante (Hild *et al.*, 2010) they illustrated that this drug have ability to cause programmed cell death by using immunohistochemistry through increasing in a protein P53 which responsible for programmed cell death (so this observation need more studying in future). Also, our study suggested that sustanon have ability to cause programmed cell death through the ability to damage cell DNA and mitochondria.

## CONCLUSIONS

The results of this study concluded that:

1. Mild effects of therapeutic doses of sustanon on body organs of rats (liver and testis).
2. Extensive effects of toxic doses of sustanon on body organs of rats (liver and testis).
3. Study the therapeutic doses effects of sustanon on other laboratory animals like rabbits and hamsters.
4. Study the toxic doses effects of sustanon on other laboratory animals like rabbits and hamsters.
5. Study the biochemical parameters accompanied with therapeutic and toxic doses effects on the body organs.
6. Study the effects of therapeutic doses of sustanon under electron microscope and immunohistochemistry effects of therapeutic dose of sustanon.

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