Pak. J. Biotechnol. Vol. 14 (3) 369-372 (2017) ISSN Print: 1812-1837
www.pjbt.org ISSN Online: 2312-7791

SOME HEPATIC AND RENAL HISTOLOGICAL AND PHYSIOLOGICAL EFFECTS OF THE ARTIFICIAL TESTOSTERONE (SUSTANON) ON FEMALE RATS

Ekhlas Abd Hamza*, Kareem Hameed Rashid**

Veterinary Medicine College, University of Al Qasim Green-Iraq. **College of science-University of Babylon-Iraq. E-mail: *ekhlasalalwany@yahoo.com

Article received 29.8.2017, Revised 8.9.2017, Accepted 15.9. 2017

ABSTRACT

This experimental study was carried out to investigate the effects of three different doses of artificial testosterone (sustanon) on the histological structure and the function of liver and kidney. For this experiment 48 adult female rats divided into four groups received 0.05, 0.1, 0.2 mg/kg respectively in addition to one control group for a period of 42 days. Blood samples were collected at the end of the experiment for liver enzymes levels analysis. Then the animals were sacrificed and dissected out to remove liver and kidneys for histopathological study. The results showed significant changes in liver enzymes compared with control group. All treated groups showed enlargement and congestion in hepatocytes and nephrons. In addition to that, Cellular swelling, vacuolar degeneration, fatty changes and apoptosis were noticed in all treatment groups. It was concluded from this study that artificial testosterone at the above doses had hepatic and renal histological and physiological changes in female rats and these findings suggesting a similar effect in human.

Key words: hepatic; renal histological; Sustanon

INTRODUCTION

In recent years many youth and athletes have been using excessive doses of anabolic steroids (Tahtamouni, 2008; Preeti and Sharma 2017). These steroids are synthetic compounds have similar structure to that of testosterone (Rahwan 1988). Androgens have been used for muscle growth enhancement (Rahwan 1988). High doses of these steroids are used to get a rapid and massive increase in the skeletal muscle size and efficiency during competition. Sustanon is one of these steroids which has many useful uses such as treatment of osteoporosis, male infertility (Harvey and Champe 2002). Regarding sustanon, it is characterized by a special distinguished properties and structure in comparison to other steroids. It is consisted of four different testosterone ester compounds in oily estate ensuring testosterone presence into the blood for a period of 3–4 weeks. These drugs are given to horses and dogs to improve their physical performance (Khedekar, *et al.* 2012), and they could cause a disease called Toxicant Associated Steatohepatitis (TASH) (Schwinge, *et al.*, 2011). They are also able to cause a marked and fast growth in muscle mass and strength, therefore, many athletes prefer to use sustanon to get immediate results. Beside this effect, there were effects of estrogen used to increase breast tissues growth in a condition called gynecomastia (Meriggiola, Costantino *et al.* 2002). Other studies reported serious effects of anabolic drugs abuse such as left ventricle enlargement which may cause death, liver disease and jaundice, kidney and testicular disorder which may lead to infertility, hypertension (Hassan, *et al.*, 2009; Ermawati and Wibisono 2017; Klaewklad, *et al.*, 2017). These drugs can affect libido, baldness, voice in women during the early stages of use and irregular menstrual cycle, and a smaller breast size in longer uses (Hartgens and Kuipers 2004). An early epiphyses closure in adolescence occurs resulting in short stature (Reents 2000). Due to the limited studies on hepatic and renal effects of artificial testosterone, this study was conducted to investigate the possible histological and biochemical alterations in these organs.

MATERIALS AND METHODS

Twenty-four albino rats of three months old weighed 250-350 gm were obtained from laboratory animal house of Sciences College, University of Babylon. Animals had food and water daily, kept on a 12:12 hour light – dark cycle and a temperature of 22-26°C. Ampoules of 5 ml sustanon (250 mg/ml) manufactured by (Organon Oss company, Holland) were used in this study. Sustanon (5 ml) ampoules were bought from local pharmacy. Four groups of 6 rats in each were used in this study. The first group considered as negative control group. The second, third and fourth group were injected intramuscularly (IM) with weekly doses of 0.05, 0.1 and 0.2 mg/kg of body weight respectively of sustanon for 6 weeks. After sacrificing the animals, the Animals were dissected out, liver and kidneys were weighed, fixed in 10% formalin for histological study. blood samples were collected directly from the heart, and serum separated to determine a (ALT) and spartate aminotransferase

(AST) levels in both control and treatment groups by using ELISA technique provided by Elabscience, for statistical analysis.

RESULTS

Biochemical analysis: The results of biochemical analysis for blood serum in (figure 1) showed an increase in ALT enzyme in all injected groups in comparison to control group and significantly increased levels of AST compared to control group were also observed .

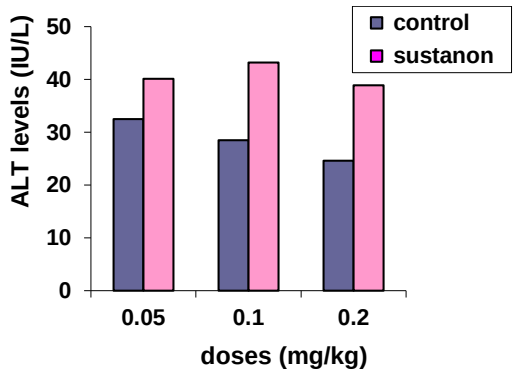

Figure 1: Comparison of ALT levels in sustanon injected groups and control.

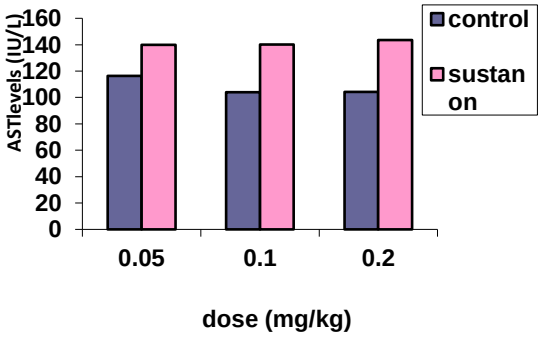

Figure 2: Comparison of AST levels in sustanon injected groups and control.

Histological study

A- Hepatic effect: The histopathological study of females rats liver tissue revealed mild to severe changes in liver histology at 0.05mg/kg of sustanon showed mild increasing number and cytoplasm of hepatocytes (figure 4). In 0.1 mg/kg sustanon injected group, hyperplasia in liver lobules , increasing in nuclear density with increasing in hepatocyte eosinophilic granules were oberved (figure 5), while at concentration of 0.2mg/kg of sustanon, hyperplasia of hepatocyte in addition to increase in nuclear density with increase in hepatocyte eosinophilic granules (figure 6) as compared with control group (figure 3) were noticed.

B- Renal effect: The histological study of females rats kidney showed histopathological changes of the kidney at different concentrations of sustanon represented by mild to severe changes at 0.05 mg/kg of sustanon compared with control group (figure 8). In concentration 0.1mg/kg of sustanon few changes in tubular lining epithelium were noticed (figure 9). Meanwhile, at 0.2mg/kg of sustanon group,distortion in renal tubules, orange-red amorphous material in some of renal tubules (fig. 10), compared with control group (figure 7) were observed .

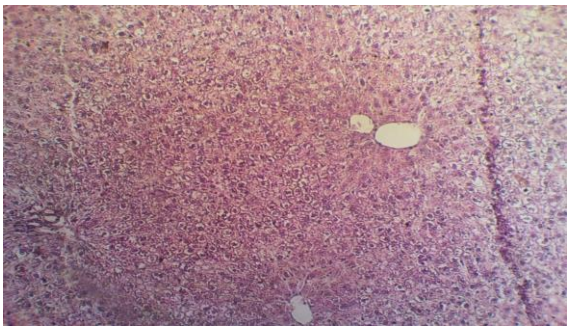

Figure 3: Liver of control group stained with Haematoxylin and eosin 100X.

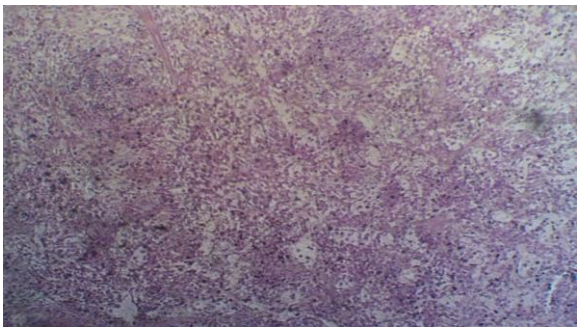

Figure 4: liver of rat injected with 0.05 mg/kg sustanon. Haematoxylin and eosin 100X.

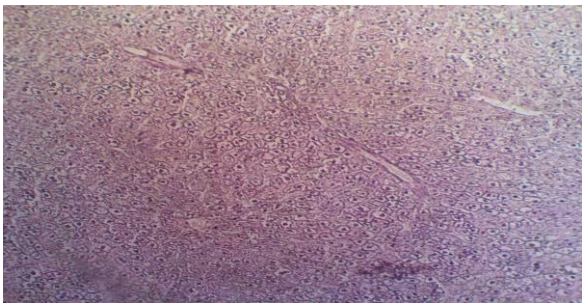

Figure 5: Liver of rat injected with 0.1 mg/kg of sustanon. H&E 100X.

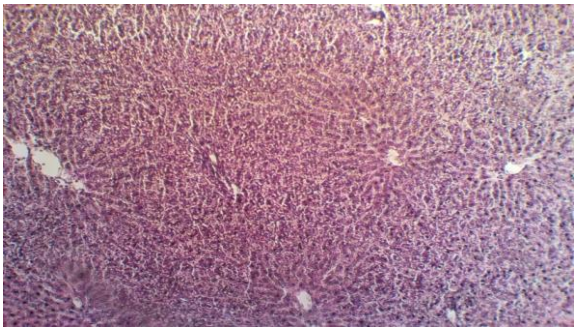

Figure 6: Liver of rat injected with 0.2 mg/kg sustanon, H & E stain 100X.

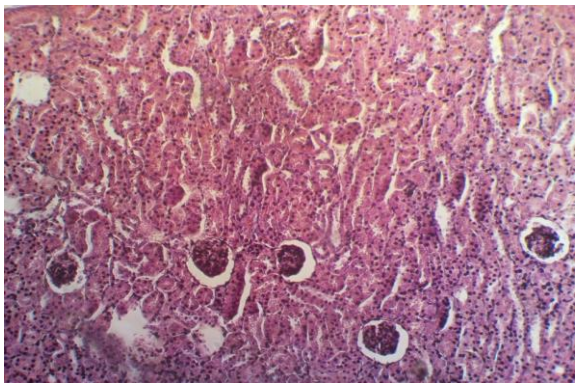

Figure 7: Kidney of control group. H & E stain 100X.

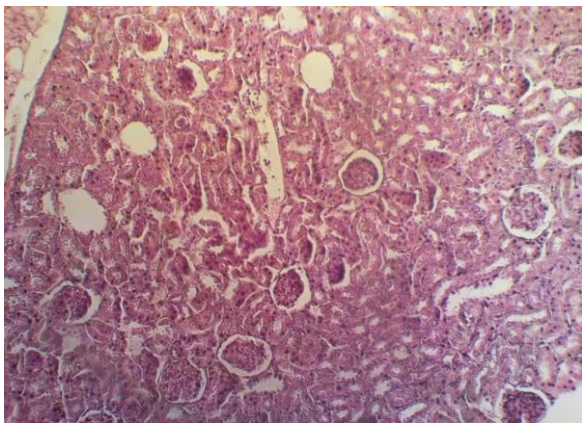

Figure 8: Kidney of 0.05 mg/kg injected group. H & E stain 100X.

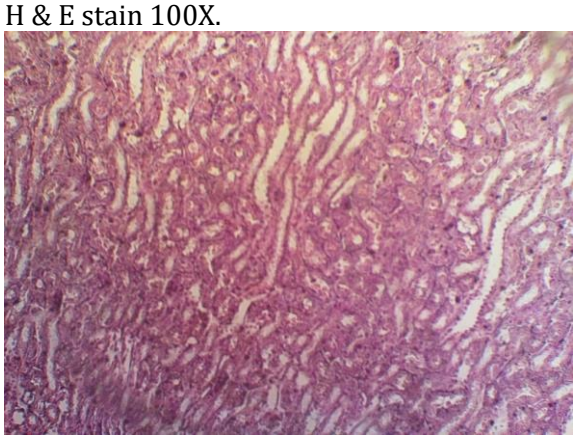

Figure 9: Kidney of 0.1 mg/kg injected group. H & E stain 100X.

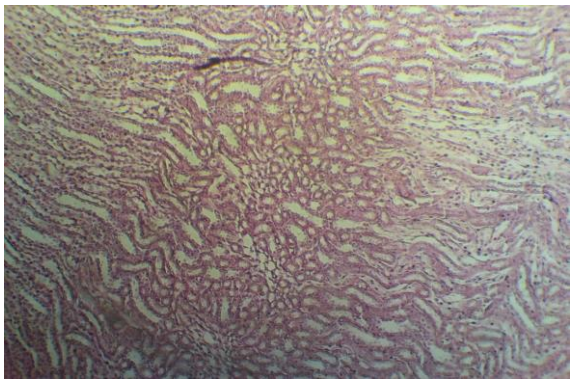

Figure 10: Kidney of 0.2 mg/kg injected group. H & E stain 100X.

DISCUSSION

The result of this study revealed increases of serum AST and ALT enzymes in female rats injected with the various doses of sustanon. The increase in ALT and AST levels as the dose was increased means that the damage of hepatocytes was maybe due to the destruction of mitochondria and therefore the release of these enzymes outside the hepatocytes. ALT increase is more indicative of liver damage than AST (Yang Lee, *et al.,* 2011). AST increase in treated animals is indicative of muscle and hepatocytes damage because this enzyme is specific for muscles (Gragera, *et al.,* 1993). This result disagree with other results when serum parameters usually used for testing hepatic function did not change considerably after the injection with these steroids (Bin-Bisher 2009). Another finding in this study was the occurrence of hepatocytes apoptosis similar to what happened in the use of blodenon (mention the generic name of the drug) which is another anabolic androgenic steroid (Hild, *et al.,* 2010). This result could suggest that sustanon may have caused apoptosis through the destruction of mitochondria. The unplanned use of these steroid for long periods could lead to morphological changes in liver cells. On the other hand, alkylation of the molecule may cause decreased liver function (Hall 2005).

In regard to the results of the kidney function, it is clear that high doses of sustanon resulted in dramatic changes in the histology of nephrons during the 6 weeks of exposure to sustanon which means that these results reflect kidney disorder and less ability to excrete nitrogenous compounds. The increased potassium and sodium ions observed by in sustanon treated rats indicated a lower kidney functional ability which is another sign of sustanon effects on kidney function. The kidney results seem to support results obtained by Habscheid (1999) who recorded a kidney dysfunction in a youth athlete who had taken high doses of anabolic drugs for months and he concluded that high doses of these drugs for long periods can lead to renal failure and liver disorders. The result of this study was similar to the observations obtained by Hoseini (2008) who found marked abnormalities in the kidney structure caused by high doses of steroids (Hoseini, *et al.,* 2009). The results of this study were different from results found by Conway (2000) who suggested that sustanon had no or very rare effects on kidneys. In regard to the mechanism of action of these abnormal effects on the kidney which are induced by high doses of sustanon in particular is still unknown (Conway, *et al.,* 2000). Nevertheless, a few hypotheses are available. Welder (1995) for example supposed that testosterone toxic metaboli-

tes may be responsible for these effects(Welder, *et al.*, 1995).

Conclusions

The results showed significant changes in liver enzymes compared with control group. All treated groups showed enlargement and congestion in heaptocytes and nephrons. In addition to that, Cellular swelling, vacuolar degeneration, fatty changes and apoptosis were noticed in all treatment groups. It was concluded from this study that artificial testosterone at the above doses had hepatic and renal histological and physiological changes in female rats and these findings suggesting a similar effect in human.

REFERENCES

Hoseini, L., J. Roozbeb, M. Saqheb and A. Noorafshan, Nandrolone decanoate increases the volume but not the length of the proximal and distal convoluted tubules of mouse kidney. Micron. J. 40: 226-230 (2009).

Bin-Bisher, A.S., The physiological effects on hormones levels and kidneys functions induced by the Anabolic androgenic Drug) in male Guinea pigs. Amer. J. App. Sci. 6: 1036-1042 (2009).

Conway, A., D. Handelsman, et al., Use and abuse of androgenic drugs. Med. J. Aust. 172: 220-226 (2000).

Ermawati, N. and Y. Wibisono, Early isolation of cell cycle-associated protein kinase (Oswee) gene in rice (Oryza Sativa L.). Pak. J. Biotechnol. 14: 71-76 (2017).

Gragera, R., A. Sabrido, et al., Ultrastructural changes induced by anabolic steroids in liver of trained rats. Histopath. 8: 449-455 (1993).

Hall, R., Abuse of supraphysiologic doses of anabolic steroids. South Med.J. 98:550-555 (2005).

Hartgens, F. and H. Kuipers, Effects of androgenic-anabolic steroids in athletes. Sports Med 34: 513-554 (2004).

Harvey, R.A. and P.C. Champe, The Lippincotts Illustrated Reviews of Pharmacology New York, USA., JB Lippincott Co. (2000)

Hassan, N.A., M.F. Slem, et al., Doping anf effects of anabolic androgenic effects on the heart: histological, Utrastructural, and echocardiographic assessment in strength athletes. Hum Exp Toxicol. 28: 273-283 (2009).

Hild, S.A., B.J. Attadi, et al., Effects of synthetic androgens on liver function using the rabbit as a model. J Androl. 31: 427-481 (2010).

Hoseini, L., J. Roozbeb, et al., Nandrolone decano ate increases the volume but not the length of the proximal and distal convoluted tubules of mouse kidney. Micron. J. 40: 226-230 (2009).

Khedekar, S., B.J. Patgiri, et al., Antihyperglycemic effect of Makaradhwaja on streptozotacin induced diabetes in rats. Journal of Global Pharma Technology 4: 16-24 (2012).

Klaewklad, A., K. Nakkanong, et al., Rubber elongation factor and small rubber particle protein (SRPP) gene expression responses to variation of seasonal change in four selected rubber clones. Pak.J. Biotechnol. 14: 115-120 (2017).

Meriggiola, M., A. Costantino, et al., Higher testosterone dose impairs sperm suppression induced by a combined androgen-progestin regimen. J. Androl. 23: 684-690 (2002).

Preeti, K. and R.A. Sharma, Isolation and identification of steroids from different parts of Prosopis cineraria. Journal of Global Pharma Technology 9: 1-4 (2017).

Rahwan, R.G., The Pharmacology of androgenic anabolic steroids. Amj Pharm Edue. 52: 167-177 (1988).

Reents, S., Androgenic-anabolic steroids. In Sport and Exercise Pharmacology. Champaign: Human Kinetics 4: 161-181 (2000).

Schwinge, l.P.A., H.P.Cotrim, et al., Anabolic-androgenic steroids :a possible new risk factor of toxicant-associated fatty liver disease. Liver Internat. 31: 348-353 (2011).

Tahtamouni L.N., M.A. Al-Muthana, I. Hassan, M. Yasin, S., Prevalence and risk factors for anabolic androgenic steroid abuse among Jordanian collegiate students and athletes. Eur. J. Public Health 28: 661-665 (2008).

Welder, A., J. Robertson, et al., Toxic effects of anabolic-androgenic steroids in primary rat hepatic cell culture. J. Pharmacol. Toxicol. Methods 335: 187-195 (1995).

Yang L.G., H. Lee, et al., Rhabdomyolysis recognized after elevation of liver enzyme following prolonged decubitus position - A case report. Korean J. Anesthesiol. 61: 341-343 (2011)