University of Benghazi
Faculty of Education Almarj

جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal
المجلة الليبية العالمية

العدد الخامس والأربعون / أكتوبر / 2019

A Study on The Effects of Two Doses of Testosterone on Some Biochemical Parameters in Male Rabbits

Somya A. Aregeb[(1)],Soaad A. M. Moftah[(2)], Ibrahim S. Eldurssi[(1)], Ebtesam M. M. Gheth[(1)] and Abdulsalam M. A. Bolhaj[(1)*]

1- Zoology Department, Science Faculty, Omar Al-mukhtar University, El-Beida-Libya
2- Zoology Department, Faculty of Art and Science, University of Benghazi, Elmarj Campus, Libya

University of Benghazi
Faculty of Education Almarj

جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal

المجلة الليبية العالمية

العدد الخامس والأربعون / أكتوبر / 2019

A Study on The Effects of Two Doses of Testosterone on Some Biochemical Parameters in Male Rabbits

ABSTRACT

The purpose of this paper was to investigate the effects of two doses of testosterone (T) on some biochemical parameters in male rabbits. Rabbits (15) were weighed and divided randomly into 3 groups (5 rabbits in each group): 1- control group (received 100 µl sesame oil), 2- low dose group (received 6 mg testosterone/kg b. w.), 3- high dose group (received 12 mg testosterone/ kg b. w.). The rabbits were injected with testosterone intramuscularly using 1 ml syringe once a week for 6 weeks. After the end of the 6 weeks period, the rabbits were weighed, slaughtered and blood samples were taken from the 3 groups for biochemical analysis. Both doses of T caused reduction in the concentration of glucose in the sera of the treated rabbits. However, only the high dose had a statistically significant effect when compared with the control. The hormone caused an increase in the concentration of serum total protein and cholesterol but had no significant effect on the concentration of serum albumin. The concentration of total cholesterol was found to decrease in a dose dependent manner. Even though the hormone caused a reduction in the concentrations of glucose and cholesterol which could be significant for those who are suffering from diabetes and hypercholesteremia, damage to the liver could not be excluded.

Keywords: Testosterone, Albumin, Total Protein, Glucose, Cholesterol, Rabbits.

الملخص :

الغرض من هذه الدراسة: التحقق من تأثير جرعتين من هرمون التستستيرون على بعض المعايير البيوكيمائية في ذكور الأرانب. لقد تم وزن وتوزيع 15 أرنباً عشوائياً على ثلاث مجاميع: 1- المجموعة الضابطة (تم حقنها بـ 100 ميكروليتر من زيت السمسم)، 2- مجموعة الجرعة المنخفضة (حقنت بـ 6 ملجرام من هرمون التستستيرون/ كجم من وزن الجسم) و 3- مجموعة الجرعة العالية (تم حقنها بـ 12 ملجرام من هرمون التستستيرون/ كجم من وزن الجسم). تم الحقن في العضل بواسطة حقن سعتها 1 مل، مرة في الأسبوع لمدة 6 أسابيع. بعد انتهاء مدة الحقن تم وزن الأرانب وذبحها أخذت عينات دم من المجاميع الثلاث لإجراء التحاليل البيوكيميائية. لقد تسببت الجرعتان في انخفاض تركيز الجلوكوز في أمصال الأرانب المعاملة؛ الجرعة العالية هي التي تسببت في انخفاض معنوي في تركيز الجلوكوز مقارنة بالمجموعة الضابطة. تسبب الهرمون في زيادة تركيز بروتين المصل الكلي، لكن لم يكن له تأثير معنوي على تركيز ألبومين المصل. تركيز الكوليسترول الكلي انخفظ معنويا مع الزيادة في الجرعة. هرمون التستستيرون تسبب في انخفاض تركيز كل من الجلوكوز والكوليسترول الكلي؛ وقد يكون لهذا أهمية صحية بالنسبة للأشخاص الذين يعانون من مرض السكري وأرتفاع معدل الكوليسترول، إلا أنه لا يمكن استبعاد ضر الهرمون بالنسبة للكبد.

INTRODUCTION

Testosterone is a steroid hormone from the androgen group and is found in humans and other vertebrates. In humans and other mammals, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. Small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. Testosterone regulates many physiological processes, including muscle protein metabolism, some aspects of sexual and cognitive functions, secondary sex characteristics, erythropoiesis, plasma lipids and bone metabolism[1, 2]. Testosterone is the best known hormone outside professional medicine. This hormone has been abused by some athletes to increase muscle mass and to tolerate stress and exhaustion from hard exercises; and it has also been used by older men with low testosterone concentration to increase the concentration to values that are mid-normal for healthy young men. However, the use of this drug without medical supervision and with supraphysiological doses for long periods of time could have adverse effects. Therefore, this study was carried out to investigate the effects of two doses of testosterone on some biochemical parameters in male rabbits.

MATERIALS AND METHODS

Animals:

Twelve-weeks old, healthy Egyptian male rabbits (total 15) (weighing between 1.2-1.6 kg) were obtained from a local breeder and were maintained in individual cages in the animal care center of the faculty of Veterinary medicine. The animals were maintained under normal temperature and light cycles. The rabbits were given water and food *ad libitum*. The animals were kept and maintained under these conditions for 4 weeks prior to the experiment.

Chemicals:

Testosterone (cidotestone, 250 mg/ml ampoule for intramuscular injection, made by Chemical Industries Development, Egypt) was obtained from a local pharmacy. Sesame oil (Almadina Company, Ajdabia-Libya) was also obtained from a local pharmacy.

Experimental procedure:

The rabbits were weighed and divided randomly into 3 groups (5 rabbits in each group): 1- control group (received 100 µl sesame oil), 2- low dose group (received 6 mg testosterone/kg b. w.), 3- high dose group (received 12 mg testosterone/kg b. w.)[3, 4]. The rabbits were injected with testosterone intramuscularly using 1 ml syringe once a week for 6 weeks[4,5].

After the end of the 6 weeks period, the rabbits were weighed, slaughtered and blood samples were taken from the 3 groups. From each rabbit, blood samples (8 ml) were collected into tubes for biochemical analysis.

Biochemical Parameters:

Blood samples were centrifuged at 4000 rpm for 5 minutes, and the serum was used to measure the levels of the following:

1- Glucose:

The amount of glucose in the serum was measured using a ready to use kit from Archem Diagnostics Industry (Turkey). The enzyme glucose oxidase catalyzes the oxidation of glucose to gluconic acid and H_2O_2. The H_2O_2 reacts with phenol and 4-aminoantipyrine in the presence of peroxidase to form a dye. The intensity of color formed is proportional to the glucose concentration and is measured photometrically by a spectrophotometer between 480 and 520 nm. The sample (10 ul) was mixed with a 1000 ul of the reagent (240 mM phosphate buffer pH 6.5, 15000 U/L GOD, 500 U/L POD, 1mM 4-AAP, and 15 mM phenol) and incubated for 5 minutes at 37^{o}C, and the color change was read by spectrophotometer[6,7].

2- Total Protein:

Total protein was measured using the method of Biuret by a ready to use kit from Archem Diagnostics Industry. Proteins peptidic bonds react with Cu (II) in alkaline solution to form blue-purple complex, the absorbance of which is measured at 520-560 nm. The intensity of color is proportional to the protein concentration. 25 ul of the sample were mixed with 1000 ul of the reagent (8 mM cupric sulphate, 24 mM sodium-potassium tartrate, 8 mM potassium iodide, and 0.80 M NaOH) and incubated for 5 minutes at 37^{o}C. After the incubation period the sample was read spectrophotometrically[8].

3- Albumin:

In citrate buffer albumin forms with green bromoceresol (BCG) a colored compound with color intensity proportional to the albumin concentration present in sample. The absorbance is measured at 580-630 nm. One thousand (1000) ul of the reagent (0.3 mmol/L bromocresol green, buffers, and stabilizers) were mixed with 10 ul of the sample and incubated for 2 minutes and the intensity of color was read spectrophtometrically[9].

4- Cholesterol:

All cholesterol esters present in plasma are hydrolyzed quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, free cholesterol is then oxidized by cholesterol oxidase to cholesten-4-ene-3-one and H_2O_2. The H_2O_2 . reacts with p-chlorophenol and 4-aminoantipyrine in the presence of peroxidase to form a quinoneimine dye. The intensity of color formed is proportional to the cholesterol concentration and can be measured photometrically between 480 and 520 nm. Ten (10) ul of the sample were mixed with 1000 ul of the reagent (Good's buffer, pH 7.20, 8.3 mM sodium cholate, 400 U/L cholesterol esterase, 400 U/L cholesterol oxidase, 500 U/L peroxidase, 0.8 mM 4-aminoantipyrine, and 2.4 mM 4-chlorophenol) and incubated for exactly 5 minutes at 37^{o}C, then the sample was read photometrically[10].

Statistical analysis:

Statistical analysis was performed using a computer run package (Graph Pad Prism 7). One way ANOVA followed by Tukey's HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard error of the mean (SEM), N = 5. P-value below 0.05 was considered to be statistically significant.

RESULTS

None of the rabbits in this study exhibited overt clinical signs of toxicity in response to treatment with testosterone.

The results of the effect of testosterone on the level of glucose in the serum of rabbits are shown in **figure 1**. From this figure the mean glucose concentration in the serum of the control group was 86.8 ± 4.91 mg/dl. In the 6 mg T treated group the glucose level was slightly reduced to 84 ± 4.15 mg/dl. This reduction, however, was not statisticallysignificant when compared with the value of the control group. The 12 mg T, on the other hand, had a significant effect on the glucose level. It reduced the glucose level to 68.4 ± 4.06 mg/dl, and this level was statistically significant ($p = 0.029$) when compared with that of the control group and not significant ($p = 0.066$) when compared with the level of the 6 mg T treated group.

The relationship between the dose of testosterone and total protein is represented in **figure 2**. The concentration of total protein in the serum of the control group was 6.58 ± 0.073 g/dl. This is in the normal range (5-7.1 g/dl) reported by[11]. The 6 mg T increased the baseline total protein to 7.0 ± 0.084 g/dl, and this increase was statistically significant ($p = 0.0152$). However, the 12 mg T increased the total protein even further to 7.12 ± 0.11 ($p = 0.003$). There was no significant difference between the means of the 6 mg T and 12 mg T treated groups.

Mean serum albumin **(figure 3)** of the control group was 4.04 ± 0.11 g/dl, which is within the normal range (3.3 ± 5 g/dl)[11]. The concentration of albumin was reduced to 3.76 ± 0.129 g/dl in the 6 mg T treated group, and to 3.9 ± 0.230 g/dl in the 12 mg T treated group, when compared with the control. However, these reductions were not statistically different from each other and from the control group.

The testosterone had a significant effect on total cholesterol concentration in the serum of rabbits (figure 4). Testosterone at 6 mg/kg reduced the cholesterol level to 62.6 ± 3.56 mg/dl when compared with the level found in the control group (74 ± 3.15 mg/dl). This difference was statistically significant ($p = 0.035$). The 12 mg T treated group had their cholesterol level reduced to 49 ± 1.0 mg/dl. This was statistically lower than that of the control group ($p = 0.0001$) and that of the 6 mg T treated group ($p = 0.013$). It should be noted that the concentration of cholesterol in the control group was within the normal range (4-77 mg/dl) reported by[11]. These results indicate that the effect of testosterone on total cholesterol level is dose dependent.

DISCUSSION

In this study the testosterone concentrations used significantly reduced the glucose levels in the serum of treated rabbits. It is well known that testosterone deficiency is associated with reduced insulin sensitivity and impaired glucose tolerance[12]. Testosterone administration was first shown to improve glucose disposal and lower plasma insulin levels over a 3-month period in obese men[13]. Injection of testosterone into hypogonadal men with type 2 diabetes over 3 months resulted in significant improvement in insulin sensitivity and glycemic control[14]. It has been long known that castration is followed by decreased muscle glycogen levels in rats and that administration of testosterone induces a considerable increase in glycogen content[15]. An equal increase in skeletal muscle glycogen synthesis is apparent in

castrated male rats supplemented with testosterone, diminishing the elevated blood glucose levels seen in untreated controls[16]. A testosterone-induced increase in glycogen synthase activity was implicated for the alteration in the rate of glycogen synthesis from blood glucose. Insulin-stimulated glucose uptake into muscle and adipose tissue is largely mediated by the glucose transporter Glut4. In agreement with androgen action on glucose control, Glut4 was up-regulated in cultured adipocytes and skeletal muscle cells following testosterone treatment[17].

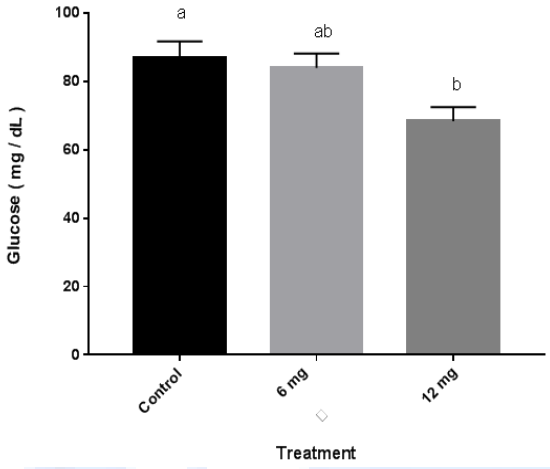

Figure 1: The concentration of glucose in the serum of control and testosterone (6 mg/kg and 12 mg/kg) treated rabbits after 6 weeks treatment period. Results are mean ± SEM (n = 5). Different letters indicate significant differences between the means. Similar letters indicate no significant differences.

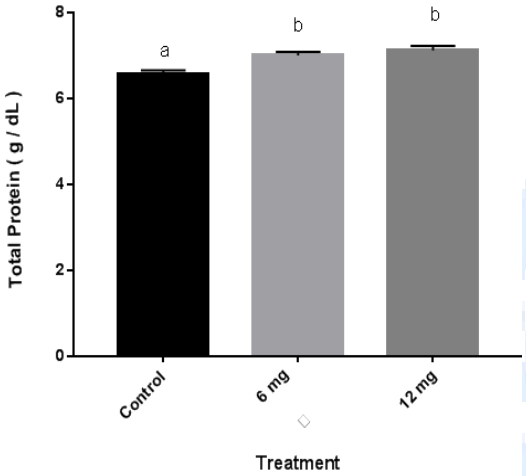

Figure 2: Total protein concentration in the serum of control and testosterone (6 mg/kg and 12 mg/kg) treated rabbits after 6 weeks treatment period. Results are mean ± SEM (n = 5). Similar letters indicate no significant differences between the means. Different letters indicate significant difference.

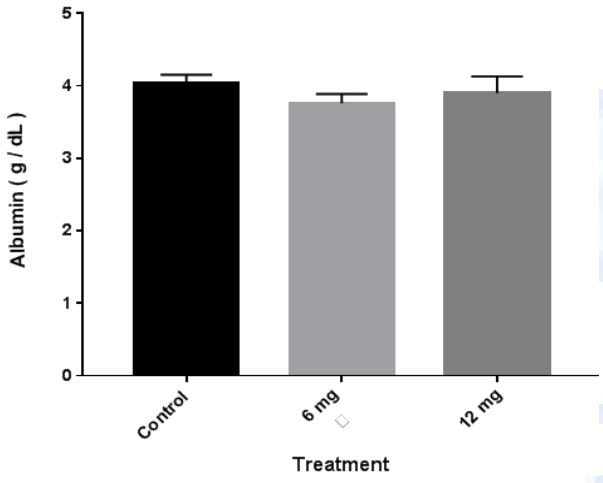

Figure 3: Albumin concentration in the serum of control and testosterone (6 mg/kg and 12 mg/kg) treated rabbits after 6 weeks treatment period. Results are mean ± SEM (n = 5). No significant differences between the means.

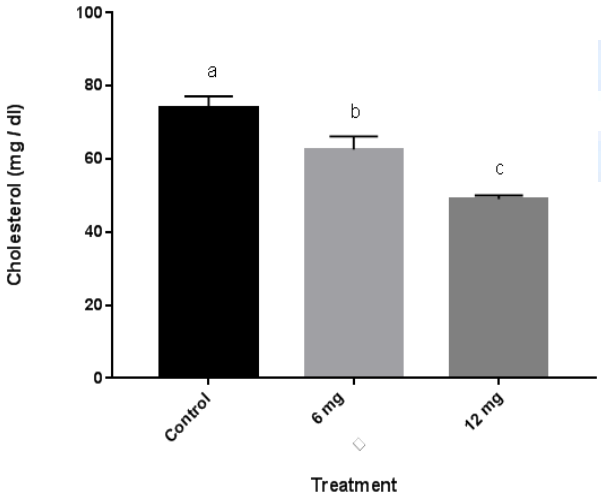

Figure 4: Total cholesterol concentration in the serum of control rabbits and rabbits treated with testosterone (6 mg/kg and 12 mg/kg) for 6 weeks. Results are mean ± SEM (n = 5). Different letters indicate significant differences between the means.

[18]demonstrated that Glut 4 expression and translocation was elevated following the addition of testosterone to cultured skeletal muscle cells of neonatal rats. Testosterone deprivation via castration of male rats led to decreased expression of Glut4 in liver, adipose, and muscle tissue[19]. This was accompanied by an elevation of blood glucose, reduced insulin levels and decreased glucose uptake in adipose and skeletal muscle tissue. Replacing testosterone in these animals restored Glut 4 expression and glucose uptake to wild-type levels.

In this study, testosterone caused an increase in the concentration of total serum protein. This increase could be due to the de novo synthesis of some plasma proteins such as fibrinogen, globulins, and sex hormones binding proteins. **[4]**reported that testosterone undecanoate at 6 mg/kg caused an increase in plasma fibrinogen in castrated rabbits. Similar increase in fibrinogen was reported by[20] in rabbits. However, [21]reported that testosterone at 10 mg had no effect on plasma fibrinogen or other coagulation proteins in rats but the plasma fibronectin was increased significantly by increasing the doses of testosterone. Furthermore, human studies [22]showed that testosterone had no adverse effect on fibrinogen levels.

In this study the serum albumin concentration was reduced slightly as a result of testosterone treatment, but this reduction was not statistically significant and still within the normal range. Albumin, which is produced only in the liver, is the major plasma protein that circulates in the blood stream. A low serum albumin indicates poor liver function. Albumin levels can be low in conditions other than liver disease, such as severe malnutrition and some kidney diseases that cause extensive protein wasting. Severe malnutrition should be excluded since the rabbits were well fed and increased in weight. Also, low serum albumin cannot be due to wasting or leakage through the kidney since we believe that testosterone reduced kidney filtration (unpublished data). Therefore, the reason for this low level of serum albumin could be due to poor liver function. This could be a possibility since liver enzymes were found to be high which indicates poor liver function (unpublished data). [23]also reported that testosterone caused a reduction in serum albumin.

The relationship between the doses of testosterone and total cholesterol in rabbits was also studied. The results showed a dose-dependent decrease in the level of total cholesterol. [24]reported that total cholesterol was significantly reduced in castrated rabbits injected with a physiological dose (6 mg/kg) of testosterone but the hypertestosteronemic dose (12 mg/kg) increased the total cholesterol. But**[3]** found that both doses of testosterone (6 and 12 mg/kg) significantly reduced total cholesterol in the serum of castrated rabbits. [25]found that testosterone caused a suppression of the levels of total cholesterol in healthy young men. Injection of a single dose (500 mg) of testosterone into obese middle-aged men had no significant effect on cholesterol level[26]. The concentration of total cholesterol was reduced after injection of 6 mg/kg testosterone into castrated rabbits after 6 weeks treatment period[4]. **[5]**also found that testosterone at 10 mg reduced cholesterol level in castrated rabbits. Furthermore, several clinical and epidemiological studies have reported that serum testosterone levels are inversely correlated with total cholesterol[27, 28].

Moreover, animal studies have also demonstrated markedly increased serum cholesterol levels in testosterone deficient male mice[29, 30] and castrated pigs[31], and administration of testosterone to these testosterone-deficient animals reduced the level of

cholesterol. These findings suggest that testosterone serves an important role in regulating serum cholesterol metabolism. However, the potential molecular mechanisms whereby testosterone deficiency affects cholesterol metabolism are unclear. [31]suggested that the increased serum cholesterol levels observed in castrated pigs may be attributed in part to impaired cholesterol clearance and that testosterone repairs this defect.

REFERENCES

1- **Wilson, J. D. (1988).** Androgen abuse by athletes. *Endocr. Rev.,* 9: 181-199.

2- **Bhasin, S. and Bremner, W. J. (1997).** Clinical review 85: emerging issues in androgen replacement therapy. *J. Clin. Endocrinol. Metab.,* 82: 3-8.

3- **Gui, Q. F., Xu, Z. R., Lou, Y. M. and Yang, Y. M. (2008).** Effect of testosterone on neointimal proliferation and blood lipids after balloon-induced aorta injury in rabbits. *Zhejiang Da XueXue Boa Yi Xue Ban.,* 37 (4): 393-398.

4- **Zhao, C., Moon, D. G. and Park, J. K. (2013).** Effect of testosterone undecanoate on hematological profiles, blood lipid and viscosity and plasma testosterone level in castrated rabbits. *Can. Urol. Assoc. J.,* 7 (3-4): E221-E225.

5- **Aydilek, N. and Aksakal, M. (2005).** Effects of testosterone on lipid peroxidation, lipid profiles and some coagulation parameters in rabbits. *J. Vet. Med. A. Physiol. Patho. Clin. Med.,* 52 (9):436-439.

6- **Trinder, P. (1969).** Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.,* 6: 24-27.

7- **Young, D. S. (1990).** Effects of drugs on clinical laboratory tests. 3rd ed. *Washington: AACC press.*

8- **Flack, C. and Wollen, J. W. (1984).** Prevention of interference by dextran with biuret-type assay of serum proteins. *Clin. Chem.,* 30: 559-561.

9- **Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971).** Albumin standards and the measurement of serum albumin with bromcresol green. *Clin. Chem. Acta.,* 31 (1): 87-96.

10- **Roeschlau, P., Bernt, E. and Gruber, W. J. (1974).** Enzymatic determination of total cholesterol in serum. *Clin. Chem. Clin. Biochem.,* 12 (5): 226-230.

11- **Cooke, S. W. (2000).** Clinical Chemistry and Hematology. In: Manual of rabbit medicine and surgery. Paul Flecknell (Ed.). Pages 25-32. *British Small Animal Association. UK.*

12- **Wang, C., Jackson, G., Jones, T. H., Matsumoto, A. M., Nehra, A., Perelman, M. A., Swerdloff, R. S., Traish, A., Zitzmann, M. and Cunningham, G. (2011).** Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. *Diabetes Care.,* 34: 1669-1675.

13- **Marin, P., Krotkiewski, M. and Bjorntorp, P. (1992).** Androgen treatment of middle-aged, obese men: effects on metabolism, muscle and adipose tissue. *Eur. J. Med.*, 1: 329-336.

14- **Kapoor, D., Goodwin, E., Channer, K. S. and Jones, T. H. (2006).** Testosterone replacement therapy improves insulin resistance, glycemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur. J. Endocrinol.*, 154: 899-906.

15- **Bergamini, E. (1975).** Different mechanisms in testosterone action on glycogen metabolism in rat perineal and skeletal muscles. *Endocrinol.*, 96: 77-84.

16- **Ramamani, A., Aruldhas, M. M. and Govindarajulu, P. (1999).** Differential response of rat skeletal muscle glycogen metabolism to testosterone and estradiol. *Can. J. Physiol. Pharmacol.*, 77: 300-304.

17- **Chen, X., Li, X., Huang, H. Y., and Lin, J. F. (2006).** Effects of testosterone on insulin receptor substrate-1 and glucose transporter 4 expression in cells sensitive to insulin. *Zhonghua Yi Xue Za Zhi.*, 86: 1474-1477.

18- **Sato, K., Lemitsu, M., Aizawa, K., and Ajiska, R. (2008).** Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.*, 294: E961-E968.

19- **Muthusamy, T., Murugesa, P. and Balasubramanian, K. (2009).** Sex steroids deficiency impairs glucose transporter 4 expression and its translocation through defective Akt phosphorylation in target tissue of adult male rat. *Metabol.*, 58: 1581-1592.

20- **Zhao, C. and Park, J. K. (2012).** Role of testosterone on whole blood viscosity in rabbits. Trans. *Androl. Urol.*, 1: 3-7.

21- **Owens, M. R., Cimino, C. D. and Donnelly, J. (1987).** Testosterone effect on biosynthesis of coagulation proteins. *Thromb. Haemost.*, 57 (3): 259-262.

22- **Smith, A. M., English, K. M., Malkin, C. J., Jones, R. D., Jones, T. H. and Channer, K. S. (2005).** Testosterone does not adversely affect fibrinogen or tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) levels in 46 men with chronic stable angina. *Eur. J. Endocrinol.*, 152: 285-291.

23- **Young, N. R., Baker, H. W., Liu, G. and Seeman, E. (1993).** Body composition and muscle strength in healthy men receiving testosterone enanthate for contraception. *J. Clin. Endocrinol. Metab.*, 77 (4): 1028-1032.

24- **Wu, Q. and Li, X. Y. (2003).** Effects of serum testosterone level on plasma lipid and apolipoprotein spectrum in orchiectomized rabbits. *Zhongguo Ying Yong Sheng Li Xue Za Zhi.*, 19 (3): 295-297.

25- **Uyanik, B. S., Ari, Z., Gumus, B. and Arslan, T. (1997).** Beneficial effects of testosterone undecanate on the lipoprotein profiles in healthy elderly men. *Jpn. Heart J.*, 38 (1): 73-82.

26- **Rebuffe, M., Marin, P. and Biomtorp, P. (1991).** Effect of testosterone on abdominal adipose tissue in men. *Inter. J. Obesity.*, 15 (11): 791-795.

27- **Isidori, M. M., Giannetta, E., Grelo, E. A., Gianfrilli, D. and Boniacio, V. (2005).** Effect of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clin. Endocrinol.*, 63 (3): 280-293.

28- **Zhang, N., Zhang, H., Zhang, X., Zhang, B., Wang, F. and Wang, C. (2014).** The relationship between endogenous testosterone and lipid profile in middle-aged and elderly Chinese men. *Eur. J. Endocrinol.*, 170 (4): 487-494.

29- **Lee, C. E., Kang, J. S. and Kim, K. I. (2008).** Effects of gender, gonadectomy, and sex hormones on growth and plasma cholesterol level in rats. *Ann. Nutr. Metab.*, 53 (1): 1-5.

30- **Hatch, N. W., Srodulski, S. J., Chan, H. W., Zhang, X., Tannock, L. R. and King, V. L. (2012).** Endogenous androgen deficiency enhance diet-induced hypercholesterolemia and atherosclerosis in low-density lipoprotein receptor-deficient mice. *Gend. Med.*, 9 (5): 319-328.

31- **Zhaowei, C., Haito, X., Yongmeng, P., Xiaoling, J., Liang, C., Yuegin, C., Keyan, Z., Cheng, C., Xiaoping, X. and Minli, C. (2015).** Effect of testosterone deficiency on cholesterol metabolism in pigs fed a high-fat and high-cholesterol diet. *Lipids Health Dis.*, 1: 14-22.