Research Journal of Animal Sciences 8 (1): 4-8, 2014
ISSN: 1993-5269

Effects of Camel Urine on Serum Testosterone Level in Male Rats of Different Testosterone Levels

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Abstract: Twenty four adult Wistar albino male rats, weighing 140-200 g were used to evaluate the effect of oral administration of she-camel urine in rats of different testosterone levels. The rats were divided into four groups; high serum testosterone group (HT) and low serum testosterone group (LT), prepared by subcutaneously injection of testosterone enanthate and intraperitoneally injection of lead acetate respectively for 21 days and other two groups (Normal Testosterone (NT) and Control (C)) were used without pre treatments. Then, all groups except the control group were treated daily by an oral dose of 2 mL/100 g body weight of she-camel urine for another 21 days. Rat's body weight was measured and blood samples were taken, before camel urine treatment and weekly after camel urine to determine the serum levels of testosterone. The results revealed that, camel urine regulated the high and low serum testosterone levels as they returned to their normal values. At the same time, no change on testosterone level was seen in the group of NT. After the 1st 2 weeks of the treatment HT group showed significant decrease in the serum testosterone level from 21.60-14.5 ng mL^{-1} while LT group appeared significant increase from 2.55-7.28 ng mL^{-1}. In the 3rd week, all treated groups showed significant ($p<0.01$) increase in testosterone level compared to the previous week. Camel urine treatment also improved the rate of weight growth in rats compared to the pretreated period in HT and LT groups. The study concluded that camel urine can regulate serum testosterone levels and rectify the resulted changes accompanied abnormal hormone levels.

Key words: LT, HT, NT, level, weight

INTRODUCTION

Camels milk and urine proved to have medicinal effects, so Islam encouraged and permitted the drinking of camel milk and camel urine is permitted in case of necessary medical treatment. Urine of one humped camel (*Camelus dromedaries*) is medically used for centuries in different parts of Arab countries. There are many well known health benefits drawn from camel urine as shown throughout the history of medical science till today as urine has profound medical uses such as effectiveness against allergies, psoriasis and all skin problems. Natalie (2002) reported beneficial effects of urine on fever, burns, tuberculosis and fertility.

According to Ohaj (1998) camel urine was reported to be used for the treatment of alopecia and to promote hair growth which indicated that camel urine can influence hormonal changes involved in hair loss. Testosterone conversion to dihydrotestosterone which is a more potent androgen results in miniaturization of hair follicle and change in cyclic phase of hair growth cycle (Pandit *et al*., 2008). Hormonal problems are one of the hair losses causes (Peter and Nicholas, 2008).

Testosterone is the major hormone that affects fertility in men which is needed to initiate spermatogenesis at puberty and to maintain this process in adults (Waters *et al*., 2003).

Several studies discussed the effects of natural products like herbs and medicinal plants on testosterone levels (Khaki *et al*., 2009). But research that concerned with the effects of camel urine on hormones are lacking, so this study is designed to examine the effects of camel urine on the different serum testosterone levels as well as body weight on Wistar albino male rats.

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MATERIALS AND METHODS

Experimental animals: Twenty four Wistar albino male rats, weighing 140-200 g were used in this study. They were kept under standard conditions of temperature (23°C) and relative humidity (65%) with a 12 h light and 12 h dark cycle and adequate ventilation in the Central Veterinary Research Laboratories premises at Soba, Khartoum, Sudan. Daily they received a balanced diet and water, *ad libitum*.

Experimental design: Rats were randomly selected and assigned into four groups each group represented in two replicates (3 rats in each).

Group 1: Untreated rats used as Control (C).

Group 2: Normal Testosterone level rats (NT).

Group 3: Low Testosterone level rats (LT) which were injected intraperitoneally with lead acetate at a dose of 8.0 mg (1.0 mL distilled water)/kg body weight/day according to Biswas and Ghosh (2003) for 3 weeks before the start of the experiment.

Group 4: High Testosterone level rats (HT) which were injected subcutaneously with 200 μL of sesame oil containing 1.5 mg of testosterone (Testoviron-Depot-50; Schering, Berlin, Germany) twice per week for 3 weeks before the start of the experiment. According to Brunner *et al.* (1992) , these doses were continued during the rest of the experimental period.

Camel urine treatment: The three groups NT, LT and HT were treated orally by 2 mL/100 g body weight of young she-camel urine daily for 3 weeks (Khougli *et al*., 2009). Camel urine was collected at early morning (at dawn) either by frequent urination or by Tashweel Method (Ohaj, 1998). All rats were weighed weekly throughout the treatment period by sensitive balance to an accuracy 0.1 g according to Axell *et al.* (2006).

Blood samples: Blood samples were taken before administration of camel urine and weekly afterwards. All samples were collected in the morning in order to minimize the diurnal variation of hormone levels. The samples were collected in a plain vial and allowed to stand for 3 h and thereafter, centrifuged at 2000 rpm for 10 min to separate serum from the blood clots. Serum obtained from the animals was kept frozen at -20°C until tested for the quantification of serum testosterone.

Hormonal assay: Serum testosterone was measured by Enzyme Linked Immunosorbent Assay (ELISA) Microwell Method described by Rajkowski *et al.* (1977).

Principle of the assay: Testosterone (antigen) in the sample competes with horseradish peroxidase testosterone (enzyme-labelled antigen) for binding onto the limited number of anti-testosterone (antibody) sites on the microplates (solid phase). After incubation, the bound/free separation was performed by a simple solid-phase washing.

The enzyme substrate (H_2O_2) and the TMB-Substrate (Tetra Methyl Benzidine) were added. After an appropriate time was elapsed for maximum colour development, the enzyme reaction was stopped and the absorbencies were determined. Testosterone concentration in the sample was calculated based on a series of calibrators. The colour intensity is inversely proportional to the testosterone concentration in the sample.

Statistical analysis: The data obtained from this experiment were subjected to Statistical Analysis of Variance (ANOVA) for CRD using computer Software Package Statistix (Version 8) and comparing the significant difference between them (Day and Quinn, 1989).

RESULTS AND DISCUSSION

The effect of she-camel urine on serum testosterone level: The effect of camel urine on three different serum testosterone levels in rats is presented in Table 1. Treatment with camel urine in wistar albino male rats resulted in transient lowering of the testosterone level in HT group, increased the level for two fold in the NT group and increased the testosterone level four fold in the LT group. At day 0 before the camel urine was application, the testosterone level in the HT group was higher significantly ($p<0.01$) by approximately two fold than the level in the C and NT groups. In the LT group the level was reduced to about 25% of the C group. But the NT group showed serum testosterone level at the same range observed in the C group.

The 7 days after camel urine administration, a highly significant ($p<0.01$) reduction in the treatment of high testosterone level was observed which was at the same range of the control without significant difference. Also, numerical increase in the low testosterone level was reported compared to day 0 but still the level was significantly ($p<0.01$) lower than the control group.

After the 2nd week of camel urine treatment there was no significant change in all treated groups compared to the 1st week but a little change was observed in the

LT group serum testosterone, resulted in a level of the same range of the control with no significant difference. At day 21 there was an increase in the serum testosterone level in all groups treated by camel urine compared with the previous week. The level was significantly ($p<0.05$) higher than day14 in both HT and NT groups. All groups' serum testosterone levels were numerically higher than the level in the C group, except in the HT group which showed a significantly ($p<0.01$) higher level than the C group and it reached the same range observed at day 0.

The effect of she-camel urine on rat's body weight: The effect of camel urine on the body weight of rats with different testosterone levels is presented in Table 2. The manipulation of testosterone level in Wistar albino rats reduced significantly the growth rate of both high and low T levels but the effect was more significant and pronounced in the LT group. Then, treatment with camel urine improved growth rate especially in the LT group. The NT group was not affected by camel urine treatment when compared to the C group as they maintained similar growth rates. The initial body weight was at the same range with no significant difference between the different groups. At day 0 the animals weights showed significant increase in all groups compared to the initial weight except LT group which it showed significantly ($p<0.01$) lower weight compared to its initial weight. These findings highlighted that low serum testosterone level reduced the rat's body weight significantly and the high levels reduced the growth rate of the rats but to lesser extent when both groups compared to the NT group and C group. Treatment of all groups with camel urine for 7 days resulted in similar rate of weight growth about 20 g in each group except the group of low testosterone level which increased by about 40 g. After 14 days the increase of body weight in all groups was not significant with a just higher level in the HT group compared to the previous week. Similar effects were observed at day 21 as all animals maintained similar weights compared to day14.

Table 1: Effect of she-camel urine on serum testosterone levels (ng/mL)

Treatments	Day 0	Day 7	Day 14	Day 21
HT	21.600 ± 3.2^{Aa}	13.65 ± 2.1^{Ba}	14.50 ± 2.0^{Ba}	22.950 ± 3.2^{Aa}
NT	8.250 ± 0.6^{Bb}	10.20 ± 1.1^{Bb}	9.05 ± 1.7^{Bab}	15.750 ± 6.3^{Aab}
LT	2.550 ± 3.7^{Bc}	6.05 ± 3.6^{Bc}	7.28 ± 2.2^{ABb}	12.130 ± 4.9^{Ab}
C	9.175 ± 0.9^{Ab}	11.25 ± 0.6^{Aab}	10.05 ± 7.4^{Aab}	9.075 ± 5.6^{Ab}

Means±SD within the same row followed by different capital letters are significantly different. Means within the same column followed by different small letters are significantly different

The effect of she-camel urine on serum testosterone level: In the present research, high serum testosterone in male rats showed highly significant ($p<0.01$) lower level after she-camel urine consumption compared to pre-treatment. But this effect was transient, that on day 21 the level of the hormone was raised to nearly the same level showed before camel urine treatment. This may be due to the influence of camel urine components which contain many of saturated and unsaturated fatty acids (Khougli, 2005). The presence of nonesterified fatty acids in the urine can inhibit testosterone synthesis by affecting cholesterol utilization or endogenous concentration as stated previously by Meikle *et al*. (1989). Similar to this finding was obtained by Meikle *et al*. (1996) who found that high testosterone concentration in isolated mouse Leydig cells that induced by luteinizing hormone was reduced significantly by oleic acid treatment which inhibit the testosterone synthesis by inhibiting cholesterol esterase activity. Also, Liang and Liao (1992) found that some unsaturated fatty acids like linoleic acid, palmitoleic acid, oleic acid and myristoleic acid are potent inhibitors of 5α-reductase enzyme that convert the testosterone into 5α-dihydrotestosterone. This can explain in part the elevation of testosterone that occurred after the reduction in response to camel urine consumption which suggests the body benefited from some free fatty acids in reducing the high testosterone level to a normal level but other fatty acids may affect 5α-reductase enzyme and cause retained serum testosterone concentration by preventing the conversion of it to dihydrotestosterone.

In NT group, oral she-camel urine administration did not affect serum testosterone till day 21 which showed significantly higher serum testosterone level than the previous weeks. This increase might be due to consumption of camel urine for long period and absorption of urinary testosterone founds in the camel urine which is the endogenous testosterone excreted in the camel urine (Abdel Hadi *et al*., 1998). Long standing

Table 2: Effect of she-camel urine on rat's body weight (g)

Treatments	Initial body weight	Day 0	Day 7	Day 14	Day 21
HT	177.50 ± 2.890^{Ca}	181.00 ± 22.08^{Bca}	202.50 ± 23.45^{ABb}	212.00 ± 9.090^{Ab}	219.25 ± 8.690^{Aa}
NT	168.75 ± 7.500^{Ca}	208.75 ± 13.77^{Ba}	228.50 ± 8.060^{Aa}	229.25 ± 10.05^{Aab}	226.00 ± 12.81^{Aa}
LT	176.25 ± 12.50^{Aa}	137.00 ± 6.270^{Bc}	178.25 ± 17.74^{Ac}	179.50 ± 19.05^{Ac}	184.75 ± 21.33^{Ab}
C	172.50 ± 20.21^{Ca}	206.25 ± 7.500^{Ba}	224.50 ± 10.21^{Aab}	231.75 ± 2.060^{Aa}	232.00 ± 2.160^{Aa}

Means±SD within the same row followed by different capital letters are significantly different. Means within the same column followed by different small letters are significantly different

to urine may affect the physiological capacity and the level of absorption by endothelial cells (Rang *et al*., 1995) and this leads to absorption of large quantity of chemically active substances of urine which probably leads to accumulative effects (Khougli *et al*., 2009).

She-camel urine administration to adult male rats with low serum testosterone level caused gradual increase in the serum testosterone until it reached the same range of the C group. This could be referred to the use of the camel urine which can reduce the oxidative stress, induced by lead acetate, due to its content of uric acid and creatinine which are known as potent antioxidants (Ames *et al*., 1981; Glazer, 1988). This finding agree also with the suggestion of Khougli *et al*. (2009) that the camel urine may have an active component which could play an important role as an endogenous antioxidant, also may act as cyto-protective agent against tissue damage mediated by toxic substances. Beside that camel urine has some healing power (Imam, 2006) which might repaired the damages on the cellular components of testis tissues. There are no available published data concerning the effect of camel urine on the serum testosterone level but Rubio *et al*. (2006) studied the role of *Lepidium meyenii* (Maca) in reversing the lead acetate induced damage on reproductive function in male rats and found that testosterone level was higher in the group of rats treated with Lead acetate plus Maca than that treated with Lead acetate alone. Also, *Tribulus alatus* extracts in male rats showed significant increase in the free serum testosterone when compared to control (El-Tantawy *et al*., 2007). Serum testosterone level also was increased by one of the medical herbals called ginger which is a strong anti-oxidant substance and may prevent generation of free radicals (Khaki *et al*., 2009).

The effect of she-camel urine on rats' body weight: In the present research un normal growth rate were seen in comparison to C group when high serum testosterone level was achieved in rats. This agrees with Bing *et al*. (1998) who found that body weight of rats treated by high doses of testosterone for 8 weeks was significantly lower than that of control. Also, Gray *et al*. (1979) reported that in male rats given high doses of testosterone, body weight gain was reduced by direct effects on adipose tissue metabolism. After camel urine treatment in the present research the body weight began to increase normally by the same growth rate observed in the control group, this may be due to the positive effect of camel urine on the rats feed intake and nutrient absorption in addition to the decrease in the testosterone level in this group.

Body weight of rats with normal testosterone level was insignificantly different from the C group before and after camel urine administration. The growth rate was increased significantly during the experiment period as normal increasing due to the normal growth as compared to the C group.

Rat's body weight in the group of low serum testosterone level showed significantly lower value when compared to the C group. Also the decrease from the initial body weight was great from 176.25-137.00 g. Similar observations were found by El-Nekeety *et al*. (2009) who stated that lead acetate significantly decreased the feed intake and consequently the body weight gain with significant decrease in the serum testosterone. Also, Kang *et al*. (2004) reported that testosterone level and the mean body weight of the animals treated with lead acetate was significantly lower than that of the other groups. After camel urine administration, in the present study, the decreasing of the body weight in LT group was stopped and replaced by significant increasing after only 7 days of camel urine treatment, this was accompanied by enhanced feed intake which indicated a restored, good, food absorption and metabolism by feeding she-camel urine which was interrupted by lead acetate application. Marchlewicz *et al*. (2007) concluded that reduction of body weight might be due to the interruption in absorption and metabolism of feed nutrients essential for health.

CONCLUSION

Daily oral administration of she-camel urine for 3 weeks resulted in transient lowering of the high testosterone level, gradual increase in the low testosterone level while the group of the normal level was not affected by camel urine administration.

REFERENCES

Abdel Hadi, A.A., I.A. Wasfi, M.A. Osman and N.S. Boni, 1998. Urinary testosterone equivalent levels in mature male and female racing camels. Proceedings of the 3rd Annual Meeting for Animal Production Under Arid Conditions, Volume 1, May, 1998, United Arab Emirates University, pp: 197-203.

Ames, B.N., R. Cathcart, E. Schwiers and P. Hochstein, 1981. Uric acid provides an antioxidant defense in humans against oxidant and radical causing aging and cancer: A hypothesis. Proc. Natl. Acad. Sci. USA., 78: 6858-6862.

Axell, A.M., H.E. MacLean, D.R. Plant, D.R. Harcourt and L.J. Davis *et al*., 2006. Continuous testosterone administration prevents skeletal muscle atrophy and enhances resistance to fatigue in orchidectomized male mice. Am. J. Physiol. Endocrinol. Metab., 291: E506-E516.

Bing, O., M. Heilig, P. Kakoulidis, C. Sundblad, L. Wiklund and E. Eriksson, 1998. High doses of testosterone increase anticonflict behaviour in rat. Eur. Neuropsychopharmacol., 8: 321-323.

Biswas, N.M. and P. Ghosh, 2003. Effect of lead on male gonadal activity in albino rats. Kathmandu Univ. Med. J., 2: 43-46.

Brunner, M., E.M. Schraner and P. Wild, 1992. Cellular changes in rat parathyroids provoked by progesterone and testosterone. Cell Tissue Res., 268: 283-286.

Day, R.W. and G.P. Quinn, 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr., 59: 433-463.

El-Nekeety, A.A., A.A. El-Kady, M.S. Soliman, N.S. Hassan and M.A.A. Wahhab, 2009. Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats. Food Chem. Toxicol., 47: 2209-2215.

El-Tantawy, W.H., A. Temraz and O.D. El-Gindi, 2007. Free serum testosterone level in male rats treated with *Tribulus alatus* extracts. Int. Braz J. Urol, 33: 554-558.

Glazer, A.N., 1988. Fluorescence-based assay for reactive oxygen species: A protective role for creatinine. FASEB J., 2: 2487-2491.

Gray, J.M., A.A. Nunez, L.I. Siegel and G.N. Wade, 1979. Effects of testosterone on body weight and adipose tissue: Role of aromatization. Physiol. Behav., 23: 465-469.

Imam, Y.O., 2006. Healing in Islam. http://irfi.org/articles3/articles_4201_4300/healing%20in%20islam html.htm.

Kang, J.K., D. Sul, J.K. Kang, S.Y. Nam, H.J. Kim and E. Lee, 2004. Effects of lead exposure on the expression of phospholipid hydroperoxidase glutathione peroxide mrna in the rat brain. Toxicol. Sci., 82: 228-236.

Khaki, A., F. Fathiazad, M. Nouri, A.A. Khaki, C.C. Ozanci, M. Ghafari-Novin and M. Hamadeh, 2009. The effects of ginger on spermatogenesis and sperm parameters of rat. Iran. J. Reprod. Med., 7: 7-12.

Khougli, S.M., A.M. El-Hassan, O.Y. Mohamed and A.A. Majid, 2009. Hepatoprotective effect of camel urine against carbon tetrachloride induced hepatotoxicity in rats. J. Sci. Tech., 10: 130-137.

Khougli, S.M.E., 2005. Hepatoprotective and anti parasitic effect of dromedary female camel urine. Ph.D. Thesis, University of Kartoum, Sudan.

Liang, T. and S. Liao, 1992. Inhibition of steroid 5α-reductase by specific aliphatic unsaturated fatty acids. Biochem. J., 285: 557-562.

Marchlewicz, M., B. Wiszniewska, B. Gonet, I. Baranowska-Bosiacka and K. Safranow *et al*., 2007. Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of rats chronically exposed to lead. Biometals, 20: 13-19.

Meikle, A.W., J.C. Cardoso de Sousa, J. Hanzalova and D.K. Murray, 1996. Oleic acid inhibits cholesteryl esterase and cholesterol utilization for testosterone synthesis in mouse Leydig cells. Metabolism, 45: 293-299.

Meikle, A.W., S.J. Benson, X.H. Liu, W.D. Boam and J.D. Stringham, 1989. Nonesterified fatty acids modulate steroidogenesis in mouse Leydig cells. Am. J. Physiol. Endocrinol. Metab., 257: E937-E942.

Natalie, B., 2002. Urine therapy (drinking urine). J. Berkeley Medicine.

Ohaj, H.M., 1998. Clinical trials for treatment of ascitis with camel urine. M.Sc. Thesis, University of Gezira, Sudan.

Pandit, S., N.S. Chauhan and V.K. Dixit, 2008. Effect of *Cuscuta reflexa* Roxb on androgen-induced alopecia. J. Cosm. Dermat., 7: 199-204.

Peter, Y.L. and A. Nicholas, 2008. Male pattern hair losses. Monash Institute of Medical Research, monash Medical Centre, 246 Clayton Road, Clayton, Vic 3168.

Rajkowski, K.M., N. Cittanova, B. Desfosses and M.F. Jayle, 1977. The conjugation of testosterone with horseradish peroxidase and a sensitive enzyme assay for the conjugate. Steroids, 29: 701-713.

Rang, H.P., M.M. Dale and J.M Ritter, 1995. Pharmacology. 5th Edn., Charchill Livingstone, London.

Rubio, J., M.I. Riqueros, M. Gasco, S. Yucra, S. Miranda and G.F. Gonzales, 2006. *Lepidium meyenii* (Maca) reversed the lead acetate induced-damage on reproductive function in male rats. Food Chem. Toxicol., 44: 1114-1122.

Waters, D.L., C.L. Yau, G.D. Montoya and R.N. Baumgartner, 2003. Serum sex hormones, igf-1 and igfbp3 exert a sexually dimorphic effect on lean body mass in aging. J. Gerontol.: Med. Sci., 58: 648-652.