

SEXUAL AND AGGRESSIVE BEHAVIOR CHANGES IN GOSSYPOL TREATED MALE MICE

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ABSTRACT

Three groups of male mice, ten mice each, were treated by gossypol acetic acid for (38) day by oral rote successively: group one treated with normal saline served as control, group two treated with 20 mg/Kg. BW. and group three treated with 40 mg/Kg. BW. The treated males were direct faced individually with adult males for aggressive behavior tests, and virgin female diestrus for sexual behavior tests. The results were as follow: the group two and three did not mice show any copulation activities during the 3-hour of observation, group two as well as three mice had less initiative in attacking their opponents and tended to avoid any longer contacts in combating. Afterward group two and three mice got significant body weight changes compared with group one (control). Therefore, the suggested conclusion of the study was gossypol tended to reduce the aggressiveness and sexual activity, but moreover body weight of the male mice was decreased.

INTRODUCTION

Gossypol is one of the substances recommended for controlling fertility in men. In fertile men, gossypol was found to suppress effectively the testicular functions, especially in lowering sperm concentration associated with impairment steroidogenesis and spermatogenesis (1). The question then is whether gossypol is safe enough in short-term duration and has no side effect when aimed for contraception in men. This question is assumed to disrupt the hypothalamic-pituitary- Leydig cell axis, by direct or indirect mechanisms. First, the inhibition of

the Gonadotropin, Leutinizing hormone and testosterone production will cause further consequences in sexual male performance. Second, testosterone has an important role in controlling sexual as well as aggressive behavior. A positive correlation between testosterone concentration and the sexual as well as aggressive behavior was revealed in many species of animal including man. Third, sexual activities can be assessed by using many parameters, some of which are latency of copulation (Lc) (2) frequency of copulation (Fc), and duration of copulation (Dc) (3 and 4). A similar approach can also be applied to aggressive behavior. One of the parameters of the determination of aggressiveness are latency (La), frequency (Fa), duration (Da), and initiative of attack (5). This study was designed to find out the effects of gossypol on the some sexual and aggressive behavior using parameters mentioned above as well as the body weight changes in male mice.

MATERIALS AND METHODS

Experimental designs

Three groups of young-adult male mice, ten mice each, were used in current study. Mice in group one were treated by placebo as a control group. Group two mice were treated by gossypol (20 mg/Kg BW) per day individually, while mice in Group three were treated with gossypol (40 mg/Kg BW) per day. The treatments were given daily for (38) days (one spermatogenesis cycle). During the experiment each mouse were reared individually in a plastic cage Shoebox (29 x 15 x 12) cm, one side has glass window for monitoring and maintained under uniform environmental condition; temperature (25 –30) C°, (12) hours light and fed *ad libidum*. To obtain the body weight changes of the animals, each mouse was weighed before and after the experiment.

Gossypol preparation:

The gossypol acetic acid was prepared according to Al-Bayaty (6a) and Campbel (7).the different setting were prepared as following steps: cottonseed dehulitaton, delentation, defatting by petroleum ether, gossypol were extracted from cottonseed cake by ether then purified by thin layer chromatography and then HPLC.

The purity was 98%. It was suspended in saline suspension, which was given orally by stomach tube.

The sexual behavior test procedure:

The sexual-activity tests were carried out after the treatment periods. These tests were conducted by introducing a treated male into the cage occupied by an estrous virgin female. The sexual activities of the treated male were observed directly for three hours. The parameters recorded were the latency of copulation, the frequency of copulation, and the duration of copulation. The latency of copulation is the time span from the moment of introducing male into the females' cage until the first trial of copulation occurred. The number of copulation trials within a unit of time was determined as the frequency of copulation. Lastly, the total time spent by the animals to copulate is called the duration of copulation (8).

The aggressive test procedures

The aggressive behavior of the treated mice (Groups one, two and three) was assessed using four parameters as follow: latency, frequency, duration, and initiative of attacks. A mouse is assumed to attack when it bites, scratches, or slaps its opponent. It is faced with male in a neutral cage for ten minutes. The neutral cage is a cage strange to both treated and untreated mice. The time span, since the test animals converge at the cages until the first attack happens, was regarded as the latency of attacks. The number of attacks within an observation period was considered as the frequency of attacks. Next, the time spent by the mice for wrestling in an attack was called the duration of attacks. Subsequently, the percentage of the tested animals in a group initiating an attack was determined as initiative of attacks. Observation the activities of the tested animals were scanned with a video camera under dark condition and were saved on soft ware DVD. The picture was then replayed for detailed analysis (9).

Statistical analysis:

Results are expressed as mean \pm SEM. We used F test: one-way analysis of variance and LSD in this study. Differences between mean data were considered significant at $P < 0.05$ (10).

RESULTS

Sexual behavior

Test on sexual behavior of the treated mice presented in Table 1. All parameters clearly pictured photograph that the reared mice were not attracted enough to do sexual intercourse. As long as observed for 3 hours, none of group two as well as group three male was engaged in mating. Whereas the control group was showed normal stand up and mating

Table (1):The effect of gossypol acetic acid treatment on mice sexual behavior parameters in response to different concentration.
Values are presented as mean \pm SEM (n = 10) mice.
The differences in letters mean significant differences at P < 0.05 from other group.

Parameters Groups	Latency of copulation (hour)	Frequency of copulation (per-hour)	Duration of copulation (second)
Group one Control (placebo)	20.18 \pm 1.58	29.91 \pm 3.54	30.27 \pm 1.00
Group two 20 mg/Kg per day	178.53 \pm 21.19 a	0.62 \pm 0.03 a	3.27 \pm 0.09 a
Group two 40 mg/Kg per day	180 a	0.00 a	0.00 a

Aggressive behavior

The effects of gossypol on the aggressive parameters of the treated mice are shown in the Table 2. Based on the data in Table 2, mice treated with gossypol tended to be less aggressive than that of control. This assumption was verified by latency of attacks of the treated mice was significantly (P<0.05) longer than that of control mice. The duration as well as the initiative of attacks was likewise. Both group one and group tow were significantly (P<0.05) shorter in duration of attacks

compared the control mice, and significantly ($P < 0.05$) lower in percentage of initiative of attacks.

Table (2): The effect of Gossypol acetic acid treatment on mice aggressive behavior parameters in response to different concentration.

Values are presented as mean \pm SEM (n = 10) mice.

The differences in letters mean significant differences at $P < 0.05$

Parameters	Latency of aggressiveness (second)	Frequency of aggressiveness (per-hour)	Duration of aggressiveness (second)	Initiative of attack (%)
Group one Control (placebo)	28.37 \pm 3.05	2.18 \pm 0.03	1.42 \pm 0.96	98.51 \pm 8.84
Group two 20 mg/Kg per day	76.08 \pm 11.49 a	1.35 \pm 0.07 a	0.87 \pm 0.09 a	53.15 \pm 6.10 a
Group two 40 mg/Kg per day	92.09 \pm 27.43 b	0.59 \pm 0.02 b	0.22 \pm 0.03 b	24.27 \pm 3.08 b

from other group.

Body weight changes

The body weight changes of the treated mice were compiled in Table 3. Based on those data, groups one and two of mice body weight changes was significantly ($P < 0.05$) greater compared with control group mice. Yet, there was no significant ($P > 0.05$) difference between groups one and two.

Parameters	Body weight (g)
Group one Control (placebo)	30.27± 0.58
Group two 20 mg/Kg per day	27.12 ± 1.46 _a
Group two 40 mg/Kg per day	24.82 ± 2.79 _b

Table (3): The effect of Gossypol acetic acid treatment on mice body weight in response to different concentration.

Values are presented as mean ± SEM (n=10) mice.

The differences in letters mean significant differences at P < 0.05 from other group.

Regarding the data in Table 1 and Table 2, they exhibited that male mice treated with Gossypol were less desirous of mating and were less aggressive. Sexual desire as well as aggressiveness in male animals has been widely known to be controlled by the androgen. The less sexual desire and aggressive behavior in male mice treated by Gossypol was assumed to be related to the decrease of testosterone production from Leydig cells (11 and 12). The great role of testosterone in controlling libido has been revealed for a long time. Sufficient testosterone in blood will promote sexual activity in many species of animals. In rats, testosterone triggers male motivation in mating (13). In an experiment on a group of men, testosterone injection resulted in increased frequency of masturbation. (14) On the contrary, low concentration of the androgen caused the animals to be depressive and less motivated in mate. Male rats treated with testosterone inhibitor distinctly showed less frequent mating (9). In these instances, testosterone was assumed to stimulate sexual-reflex sector of peripheral nerves responsible for controlling animal libido (12). Aggressive behavior is defined as a tendency to attack or to fight. In rodents, especially rats and mice, aggressive behavior is prominent in males particularly among the unfamiliar or isolated ones (15). The most important factor affecting the aggressive behavior is testosterone. Without this hormone aggressiveness will not appear. Some positive correlations between the androgen and aggressiveness were found in many species of animal, i.e. in finchs *Phophila guttata*, (16) in mice (17), in

sheep, (18), as well as in humans (19) In addition, testosterone provided the animals' strength since the androgen promote the body muscles enlargement. Huang and Wang (20) reported the effects of gossypol on testosterone secretion, which was attributed to the contribution of adrenergic receptor inhibition in the hypothalamus by gossypol; this adrenergic receptor inhibition might decrease GnRH, which led to decrease of LH-Testosterone (8 and 21). Furthermore, Leydig cells secreted testosterone and estrogen, both testosterone and estrogen act on the hypothalamus and pituitary gland through regulating the negative feed back mechanism in of the quantity of GnRH release (22). Zhang (23) reported that the mechanism of testosterone and estrogen releasing was disrupted by gossypol, through inhibition of hypothalamic estrogenic receptor. Kalla and Sud (24) and Udho and Palil (25) suggested that this effect leads to increase of GnRH and testosterone, the increase of testosterone played an important role in decrease GnRH releasing through feed back mechanism and the result of GnRH reduction decreased testosterone secretion. Whereas Grey (26) reported that, the inhibition of testosterone release due to inhibition of LH receptor depend adenyl cyclase - c.AMP system of Leydig cell. Al-Bayaty (27) recorded decrease plasma testosterone level through reduction of Leydig cell number per Leydig cell cluster per seminiferous tubule due to direct effect of gossypol on previous Leydig cell. For that reason, a substance suppressing LH, such as sexual disrupting compound; gossypol can be used in controlling fertility (28). This is why male mice treated by gossypol in current study tend to be less aggressive (29)

Higher changes in body weight of the gossypol treated groups two and three (Table 3), is inseparable from the testosterone. Some experiments have successfully revealed that some obesity incidents are correlated to low concentration of blood testosterone. However, in this instance testosterone is responsible for controlling body musculature (30). The lower the testosterone concentration the retarded muscles cells development can be atrophy. As the consequences, lack of testosterone will decrease anabolic mechanism causing lost of body weight. This is why mice treated with gossypol (groups two and three) in this study showed significant body weight changes (19). Conclusion based overall results of the current studies it can be

suggested that the male mice treated with gossypol tend to be less aggressive and less sexual motivation, but tend to have higher body weight changes compared with the placebo ones.

دراسة السلوك الجنسي والعدواني في ذكور الفئران المختبرية المعاملة بحامض الكوسبيول
مهند عبد الستار البياتي
فرع الادوية والسموم، كلية الطب البيطري، جامعة البصرة، البصرة، العراق

الخلاصة

ثلاثة مجاميع من ذكور الفئران قسمت إلى ثلاثة مجاميع تحوي على عشرة فئران. الأولى مجموعة سيطرة عوملت بالمحلول الفسيولوجي، والمجموعة الثانية والثالثة عوملت بالكوسبيول اسبتك اسد (٢٠) و (٤٠) ملغم/كغم من وزن الجسم لمدة (٣٨) يوم على التوالي. الذكور المعاملة قبلت فرادى مع ذكور مجموعة السيطرة فتيه بالغه لفحوصات السلوك العدواني، وقوبل مع إناث عذراوات غير معاملة لغرض فحوصات السلوك الجنسي. أظهرت النتائج وكما يلي مجموعتا فئران المعاملة لم تبتدأ فعالية الجماع الجنسي خلال (٣) ساعات الملاحظه وأظهرتا فعالية عدوانية أقل متجنبه الاتصال لمدة طويلة ضمن مدة المنافسه وانخفاضاً معنوياً في معايير العدوانية كما وعرضت النتائج انخفاضاً معنوياً في وزن الجسم مقارنة بمجموعة السيطرة وبذلك قد يعزى انخفاض السلوك العدواني والجنسي إلى انخفاض في مستوى التستوستيرون ضمن محور الإفرازي تحت المهاد- الغدة النخامية – خلايا ليديك فضلاً عن ذلك إن النقص الحاصل في وزن الجسم قد يعزى إليه أيضاً أو يعزى إلى انخفاض كمية الغذاء المتناول.

REFERENCES

1. Chase, C.; Bastidas, P.; Ruttle, Jr.; Lony, C. and Randel, R. (1994) *Growth and Reproductive Development in Brahman Bulls Fed Diets Containing Gossypol*. J. Anim. Sci. 15:204(4):642-643.
2. Moelock, N., and Sutyarso, K. (1995) *progesteron dengan androgen untuk kontrasepsi hormonal pada pria*. Maj. Kesehatan Masyarakat Indonesia, 13(11):737-9.
3. Herbert, J. (2003) *Hormones and behaviors*. Proc R Soc. London, 199:425-43.
4. Stokes, W. (1999) *Animal behavior in laboratory and field*. San Francisco: WH Freeman Co.
5. Al-Maliki, S. (1994) *The behavioral and reproductive consequences in offspring (first generation) of mice continuously exposed to a strong magnetic field*. Behavioral Processes, 32:229-34.
6. Al - Bayaty, M. A. (1999) *The reproductive effect of cottonseed extract on male mice*. Ph.D. College of Veterinary Medicine, University of Baghdad, P 40.

7. Campbell, K.; Morris, R.; and Adams, R. (1937) *The structure of gossypol*. *J. Am. Chem. Soc.* 59: 1723-1728.
8. Dennis, F. and Anthony, G. (1999) *Facilitation of sexual behavior and enhanced dopamine efflux in the nucleus accumbens of male rat after D-amphetamine-induced behavioral sensitization*. *J. Neuroscience*, 19(1):456-463.
9. Ewing, L.; Gorski, R. and Robaire, B. (1979) *Testosterone-estradiol filled polydimethylsiloxane subdermal implants: effect on fertility and masculine sexual and aggressive behavior of male rats*. *Biology of Reproduction*, 21:765-772.
10. Steel, R.G.D. and Torrie, J.H. (1980) *Principles and procedures of statistic. A biometrical lab roach* (2nd edition).
11. O'Neill, R.D.; Lowery, J.P. and Mas, M. (1998) *Monitoring brain chemistry in vivo: voltammetric techniques, sensors and behavioral applications*. *Crit Rev Neurobiology*, 12:69-127.
12. Carlson, N. (1977) *Physiology of behavior*. Boston: Allyn and Bacon Inc. 8.
13. Beyer, C.; Mollal, G. and Larson, K. (2000) *Steroid regulation of sexual behavior*. *Excerpta Medica*, 38(2):145.
14. Monti, P.; Brown, W. and Corriveau, D. (1977) *Testosterone and components of aggressive and sexual behavior in man*. *Excerpta Medica*, 38(8):642.
15. Gühl, A. and Schein, M. (1969) *A glossary of terms used in animal behavior*. In: *W. Stokes (ed). Animal behavior in laboratory and field*. San Francisco and London: W.H. Freeman Co.
16. Arnold, A. (1975) *The effects of castration and androgen replacement on song, courtship, and aggression in zebra finches*. *J Exp Zool*, 191:309-26.
17. Barkley, M. and Goldman, B. (1978) *A quantitative study of serum testosterone, sex accessory organ growth, and development of inter-male aggression in the mouse*. *Excerpta Medica*, 38(5):410.
18. Lincon, G. and Davidson, W. (1976) *The relationship between sexual and aggressive behavior and social dominance in man*. *Excerpta Medica*, 34(2):167.
19. Clarenburg, R. (1992) *Physiological chemistry of domestic animals*. St. Louis: Mosby Year Book Inc.
20. Huang, H. F. and Wang, M. (1994) *Effect of gossypol acetate, danazol progesterone and GnRH-A on estrogen and progesterone receptors of human*

endometrial cell. *Chung. Kuo. Chung. Hsi. I. Chieh. Ho. Tsa. Chih.* 14(6):352-353.

21. Kono, H.; Chhin, L.Y.; Gu, Y.; Yamaguchi, M.; Zuspan, F.P.; Furuhashi, N.; Takagama, K. and Yajima, A. (1991) Gossypol effects on monoamine oxidase (MAO) activity in several organ of term rat. *J. Exp. med.* 163 (3):1-6.
22. West, J. B. (1982) Best and Taylor's: *Physiological bases of medical practice* 11th edition, William and Wilkins Baltimore London.
23. Zhang, Y.W.; Han, M.L. and Wang, Y.F. (1994) *Estrogen and progesterone cytosol receptor concentrations in patients with endometriosis and their changes after gossypol.* *Chung. Hua. Fu. Chan. Ko. Tsa. Chih* 29, 4: 220-253.
24. Kalla, N.R. and Sud, S. (1990) *Distribution of gossypol.* *Acta Eur. Fertil.* 21 (2): 77-88.
25. Udho, P. and Palil, D.R. (1992) *Histopathological and biochemical effects of gossypol acetate on pituitary gonadal axis of male albino rats.* *Contraception* 46, 5:493-509.
26. Grey, M. I.; Greene, L.W. and Williams, G.L. *Effects of dietary gossypol consumption on metabolic homeostasis and reproductive homeostasis and reproductive endocrine function in beef heifers and cows.* *J. Anim. Sci.* 71 (11):3052-3059.
27. Al - Bayaty, M. A. (2005) *Quantitation of Leydig cell in testicular biopsies of gossypol treated rats.* *The Iraqi Journal of veterinary medicine* 29,1,179
28. Sutyarso, M. (2000) *The results of testes biopsy on male cynomolgus monkey (Macaca fascicularis) injected by androgen and progesterone combination.* *Maj Kedokl Indon,* 50(12):544-8.
29. Sealmanoff, M.; Abreu, E.; Goldman, B. and Ginsburg, B. (1978) *Manipulation of aggressive behavior in adult DBA/2/Bg and C57/BL10Bg male mice implanted with testosterone in silastic tubing.* *Excerpta Medica,* 38(9):730.
30. Bhasin, S.; Storer, T. and Berman, N. (1996) *The effects of supra-physiologic doses of testosterone on muscle size and strength in normal men.* *N Eng J Med.* 335:1-7.