Original article

Effect of gossypol extracted from Cottonseed Cake on Body Weight, Fertility and Histopathology of Wistar Rats

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Abstract

The purpose of this study is to assess the effects of gossypol on body weight, female and male hormones (estrogen and testosterone) and morphological structures of ovary and testes of wistar rats. Thirty wistar rats were classified according to gender into two groups (each group contains fifteen rats). Each group was divided into three sub-groups, sub-group one represented none – treated rats (Control), sub-group two represented rats that received 0.3 g/kg extracted gossypol and sub- group three represented rats received 0.6 g/kg extracted gossypol (5 rats/sub-group). The results indicated a significant reduction in body weight for males and females group compared with the control group, decrease of estrogens level for low and high doses (P =0.04), decrease of testosterone level in low dose (P =0.035) and decrease in high dose (P = 0.384). The present results concluded that extracted gossypol had hazardous effects on body weight and hormones of for both male and female. Changes in structure of testis and ovarian follicles were evident on sexually mature males and females rats that received extracted gossypol for 21 days at dosage know to induce sterility in male and female rats (0.3 and 0.3 g/kg body weight, orally) were observed.

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Introduction

Gossypol is a chemical constituent of the lysigenous foliar (pigment) glands of cotton plants stems (Gadelha *et al.,* 2014) and is also found in glands in cottonseed. Its name is derived from the scientific name of cotton (*Gossypium* spp.) and it is phenol compound in its main chemical structure. The name of gossypol is derived from the plant genus scientific name (*Gossypium*) combined with the ending "ol" from name of the phenol. The concentration of gossypol increased linearly in plasma, liver, kidney, and muscle with dietary levels of free gossypol. Liver had the highest concentration of total gossypol (71.4 - 313.6 mg/kg dry matter) followed by kidney (9.2 to 36.3 mg/kg dry matter (, plasma (3.0-14.6 µg/ml (, and muscle) 2.1 - 9.8 mg/kg dry matter). The proportion of (-) gossypol was higher than that of (+) gossypol by 16-27 % in all tissues (Gamboa *et al.,* 2001). Gossypol is a terpenoid aldehyde, which is formed metabolically through acetate via the isoprenoid pathway (Burgos *et al*., 1997). The relatively long half-life of gossypol observed after oral administration to humans and animals may be explained by high plasma and tissue protein binding that prevents gossypol to be cleared from the blood stream as an unbound free molecule) Abou-Donia, 1976 and Jia *et al*., 2008). Gossypol is a male anti-fertility agent with anti-spermatogenic activity (Coutinho, 2002) and has been shown to contain anti-tumor, anti-viral, and anti-oxidant properties. Gossypol is a reversible inhibitor of protein phosphatase 2B, mitotic kinesin Eg5, and protein kinase C. Gossypol also potentially inhibits PAF-R, Bcl-2, 5-LO (5-lipoxygenase), 12-LO, and leukotriene-induced guinea pig parenchyma contractions. The acute toxicity of gossypol is low, but there are substantial chronic effects in non-ruminant animals. According to Randel *et al*., (1992), immature ruminants with an ill functionally developed rumen are highly susceptible to gossypol toxicity (Morgan, 1989, 2014). A dosage about 20 mg gossypol/month/day for 2-3 months leads to reduce sperm counts (less 4 million/ml). The dosage is reduced to maintain a range from 50 mg/week to 75-100 mg/twice a month (Polsky, 1989). The contraceptive effect gossypol in men is 99.5% and fertility usually returns to normal within three months of discontinuation. However, inhibition of spermatogenesis may persist up-to 20 % men two years after discontinuation (Polsky, 1989). This research was proposed to study the effect of gossypol on body weight, male and female hormones of Wistar rats that orally consumed extracted gossypol from cake of cotton seed. In addition, the morphology of testis and ovary follicles of the rats was studied.

Materials and Methods

Cotton seeds were collected from Gezira Board, Wad Madni from growing season 2015. Samples were prepared according to AOAC (1990). Dust, sand and any foreign matters were removed from the sample. Then seeds were soaked in water overnight at room temperature. The outer layer of the seeds was removed from by hand (Dehulling seeds). Dehulled seeds were dried overnight at lab temperature and are grounded into fine particles. Then oils of fine particle of cottonseeds were removed by hexane solvent in ration 3:1 (ml/g) by using the cold extraction method at room temperature. Then cake of Dehulled seeds were collected, dried at room temperature and kept in a dark bottle for later use.

Solvent was 400 ml (made from 368 ml Butanol plus 32 ml HCl, in ratio 1:20 (g/ml) for extraction uses. The mixture was thoroughly mixed by magnetic stirring for 15 minutes at room temperature. Then the mixture was filtrated by filter paper (Whatman No.1). The filtration solution was left at room temperature to dry. The dried - extracted gossypol was collected and kept in glass bottle until start the experiment.

The current experiment was conducted at the Animal laboratory and the biochemical analysis was done at the laboratory of Biochemistry and Molecular Biology Department, Faculty of Science and Technology, Al-Neelain University in Khartoum. Thirty Wistar rats were classified into males and females, fifteen males were divided into three groups (5 Rats per group) namely: G1 represented non treated male rats (control), G2 received a low dose of extracted gossypol (0.3 g\kg body weight, male), G3 received the high dose of extracted gossypol (0.6 g/kg body weight, male), While other fifteen female Wister rats were divided into three groups (5 Rats per group) namely: G4 represented the non treated female rats (control), G5 was received low dose of extracted gossypol (0.3 g/kg body weight, Female) G6 received high dose of extracted gossypol (0.6 g/kg body weight, Female). Before the start of the experiment, all the rats were fed the basal diet without gossypol for two weeks for adaptation as shown in Table (1). The water was distributed to the rats by pipe with nipple drinkers to each cage. Experiment period was extended to four weeks.

Table (1). Basal diet fed to experimental rats for adaptation before gossypol addition

Ingredients	Sorghum	Carrot	Meat	Wheat bran	Total
Kg/ton	600	165	90	95	1000

Determination of testosterone

STA-ALA-PACK testosterone is a competitive enzyme immunoassay which iszperformed entirely in the STA-ALA-PACK testosterone test cups. Testosterone which available in test sample competes with enzyme labeled testosterone for a limit number of binding for binding sites of testosterone specific monoclonal anti-body immobilized on a magnetic solid phase. The magnetic beads are washed to remove unbound enzyme labeled testosterone and are then incubated with flourogenic substrate, 4- methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled testosterone that binds the beads is inversely proportional to testosterone concentration in the test sample. Then a standard curve was constructed and unknown test sample are calculated by using standard curve.

Determination of estrogen

STA-ALA-PACK estrogen is a competitive enzyme immunoassay which is performed entirely in the STA-ALA-PACK estrogen test cups. Estrogen which available in test sample competes with enzyme labeled estrogen for a limit number of binding for binding sites on anti - estrogen specific monoclonal anti-body immobilized on a magnetic solid phase. The magnetic beads are washed to remove unbound enzyme labeled estrogen and are then incubated with flourogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme labeled estrogen that binds the beads is inversely proportional to steroid concentration in the test sample. Then standard curve was constructed and unknown test sample are calculated by using standard curve.

The morphological analysis of ovary and testis was carried out according to method described by Ivan *et al*., (2014).

Statistical analysis

Statistical analysis was performed using SPSS versionl6 (Statistical Package for the Social Sciences). The differences between the groups were tested for significance by student's T-test, One-way ANOVA test and Chi-Square Test. Data were expressed as the mean ± SD. *P* values < 0.05 are considered statistically significant

Results and Discussion

Effect of extracted gossypol on Body weight of experimental rats

Figure (1) and (2) indicated that body weight of male and female treated rats was reduced with increasing dose (0.3 and 0.6 g/kg) of extracted gossypol, respectively, compared with control group during four weeks. It was clearly observed that there was a significant reduction in body weight of the treated males and females with extracted gossypol compared with control groups. These results are agreed with those findings reported by Robert and Jim (2008). It is also confirm the results obtained by Oko and Hrudka (1984).

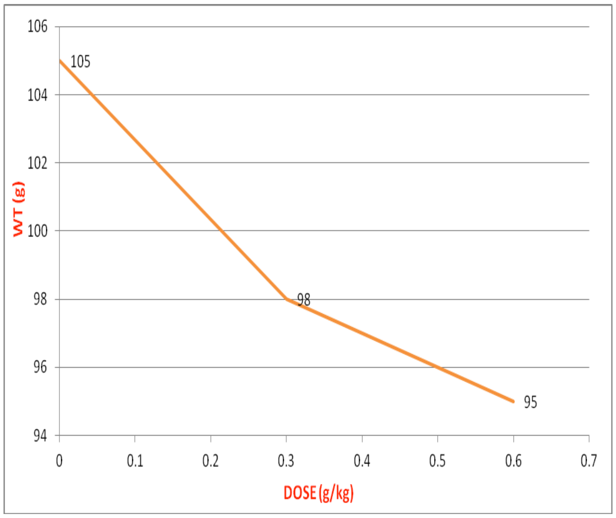

Figure (1): The effect of extracted gossypol on body weight of male rats. During four weeks (Each value is represent average of three reading)

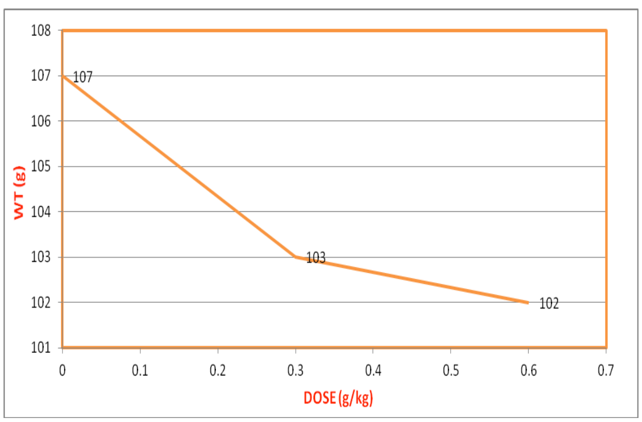

Figure (2): The effect of extracted gossypol on body weight of female rats during four weeks (Each value is represent average of three reading)

Effect of extracted gossypol on Estrogen hormone (Table 2) indicated that mean value of estrogen for 0.0, 0.3 and 0.6 g of extracted gossypol was 184, 18 and 18, respectively. The results are illustrated that there is reduction in estrogen in low and high gossypol level compared with control group during the four weeks of experiment. In addition there no significant difference in reduction between low and high dose of gossypol level at (P≥0.05)

Table (2) The effects of low (0.3g) and high dose (0.6 g) of extracted gossypol on Estrogen and Testerone of experimental Wister rats (Each value is represent average of three reading)

Hormone (Dose)	0.0 (g\kg)	0.3 0 (g\kg)	0.60 (g\kg)
Estrogen (g/ml)	184	18.0	18.0
Testerone(g/ml)	460	15.0	270

Effect of high and low of extracted gossypol on Testerone hormone (Table 3) indicated that mean value of Testerone for 0, 0.3 and 0.6 g gossypol was 460, 15.0 and 270, respectively. The results are illustrated that there is reduction in Testerone in low and high dose compared control group during the four weeks of experiment. In addition changes in serum level of testosterone at low dose is higher than the high dose of treated gossypol. These results are agreed with Chang, *et al*. (1982) who said that serum testosterone in male rats was significantly reduced at dose of 30 mg/kg during six weeks, but there was no significant changes in serum level of testosterone in animal received 7.5 and 15 mg\kg during 12 weeks. These findings are in line with those values reported by Oko and Hrudka (1984).

Effect of extracted gossypol on the morphology of testes and ovaries of rats

(a) Rats' testes

Figure (3) indicates testis with primary or early secondary stages of spermatogensis for rats none treated with gossypol for 3 weeks

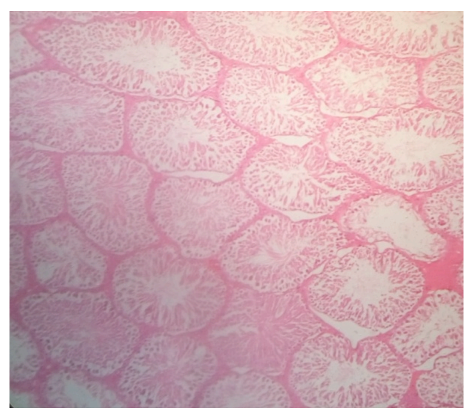

Figure (3): Testis with primary or early secondary stages of spermatogensis for male rats none treated with gossypol for 3 weeks.

Figure (4) indicates normal testis for rats none treated with gossypol for 3 weeks. However, it was obsreved that the testis of rats received 0.3 and 0.6 g extracted gossypol no primary or early secondary stages of spermatogensis for three weeks (Fig.5 and 6).

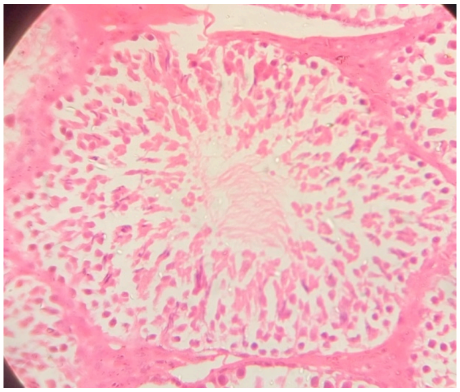

Figure (5): An inactive (dormant) testis of male rats treated with 0.3 g of extracted gossypol.

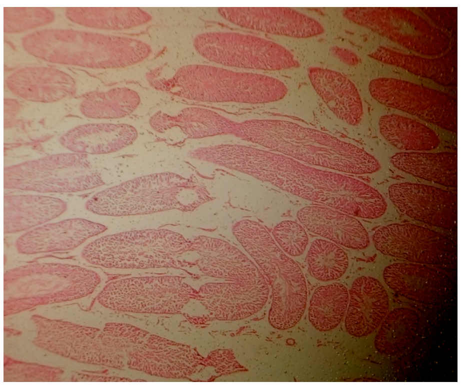

Figure (6): An inactive (dormant) testis of male rats treated with 0.6 g of extracted gossypol for 3 weeks

(b) Ovary folicles

Figure (7) indicates that there was one layer of flattened granulosa cells; this stage in ovary development is termed primordial follicles. In figure (8), one or two layers of cuboidal granulosa cells were observed The one layer cuboidal granulosa cells represented primary follicles while two layers cuboidal granulosa cells represented secondary follicles. Figure (9) in which antral cavity appeared, this stage represented antral follicles. While irregular antral follicles were observed for rats treated with extracted gossypol. All Ovaries from non-treated rats showed normal primordial, primary, secondary and antral follicles. These results are in line with those finding reported by Ivan *et al*., (2014) and Holly *et al.,* (1988).

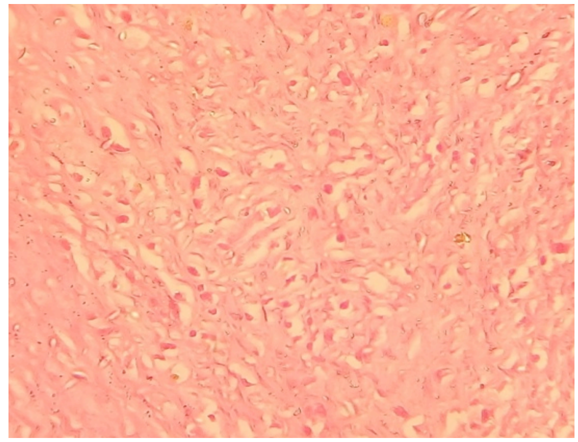

Figure (7): One layer of flattened granulose cells (Primordial folicles) for treated female rats with gossypol

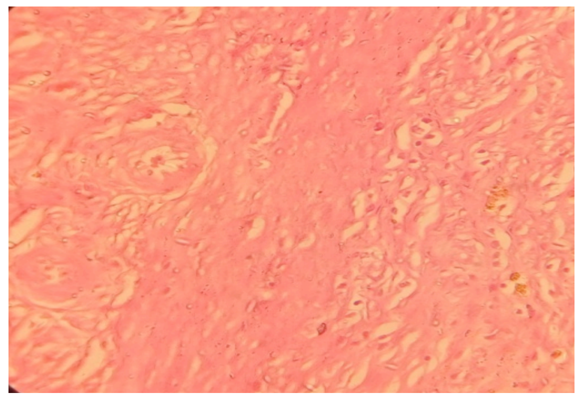

Figure (8): One or two layers of cuboidal granulosa cells (primary and secondary follicles) for non-treated female rats with gossypol.

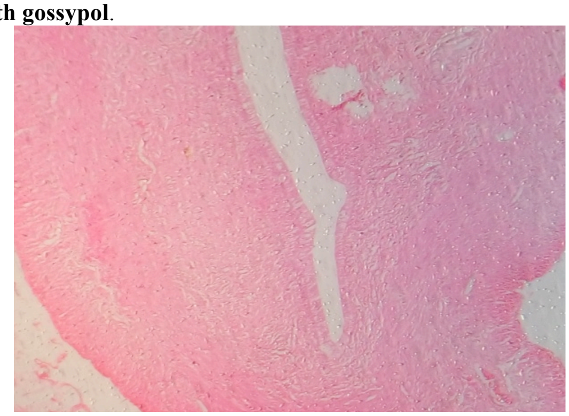

Figure (9): An antral cavity (antral follicles) for non-treated female rats with gossypol

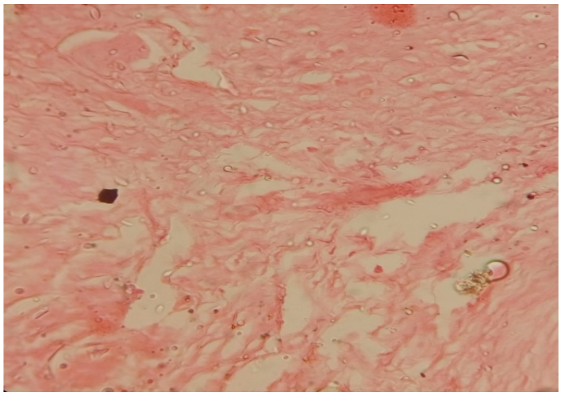

Figure (10): Inactive (dormant) ovary follicles for female rats treated with 0.3 g of extracted gossypol for 3 weeks

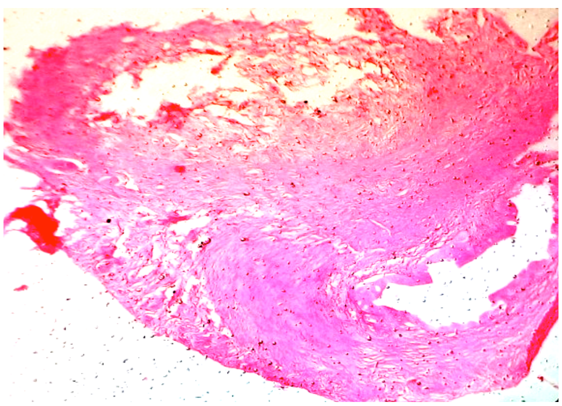

Figure (11): Inactive ovary follicles for female rats treated with 0.6 g of extracted gossypol for 3 weeks

Gossypol is known to affect the heart, liver, reproductive tract (Coutinho, 2002), abomasums and kidney animals (Morgan, 2014). The effect manifest in many symptoms especially in young animals: sudden death, chronic labored breathing which resemble pneumonia, nasal discharge, red urine. In general, adult cattle can tolerate large amounts of free gossypol, but toxicity has been reported with 800 ppm fed for a long period. The problem with gossypol is the toxic effect seems to be cumulative, the longer they are on a ration that contains much gossypol; the more likely they are to have problems (Morgan, 2014 and 1989).

There are many studies e.g. (Randel *et al*., 1992, Hassan *et al.* 2004; Qian and Wung, 1984; Ueno *et al.* 1988 and Teng, 1997). That indicated the negative effects of gossypol on male and female gametogenisis; inhibition of spermatogenisis; promotion of embryo lesions; reduction of sperm motility; mitochondrial injury and damaging germinal epithelium. However, all these effects are reversible when gossypol is no longer ingested (Gadelha *et al*. 2011).

Conclusions

- Extracted gossypol had adverse effects on body weight for both males and females wister rats groups.
- Both males' and female' hormones were negatively affected.
- Changes in structure of testis and ovarian follicles were monitored for sexually mature males and females rats that were orally fed extracted gossypol for 21 days fed with a dose known to induce sterility in males and females rats (0.3 and 0.3 g/kg body weight).

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References

Abou–Donia, M. B. (1976). Physiological effects and metabolism of Gossypol. In Gunther F.A. (ed). *Residue Review* 61,126- 152.

Burgos, M., Ito, S., Segal, J. S.A. and Tran, T. P. (1997). Effect of Gossypol on Ultra-structure of Spisula Sperm. *Biol. Bull.* 193: 228–229.

Chang, C. C., Gu, Z. and Tsong, Y. Y. (19820. Studies on gossypol. Toxicity, anti-fertility, and endocrine analyses in male rats. *Int J Fertil.,* 27(4):213-8.

Coutinho, E. M. (2002). Gossypol: a contraceptive for men. *Contraception* 65(4), 259-263.

Fajana, O .O. (2013). Characterization of Alkaline Phosphatase (E.C 3.1.3.1).

Gadelha, I. C. N., Fonseca, N. B. R., Oloris, S. C. S. Melo, M. M. and Blanco, B. S. (2014). Gossypol toxicity from cotton products. *Scientific World Journal*, Vo. 2014, Article ID 231635, 11 pages.

Gadelha, I. C. N. , A. H. do Nascimento Rangel, A. R. Silva, and B. Soto-Blanco, "Efeitos do gossipol na reprodução animal," *Acta Veterinaria Brasilica*, vol. 5, no. 2, pp. 129–135, 2011. View at Google Scholar

Gamboa, D.A., Calhoun, M.C., Kuhlmann, S.W., Hag, A.U. and Bailey, C.A, (2001). Tissue distribution of gossypol enantiomers in broilers fed various cottonseed meals. *J. Poultry Sci*. 80, 920-925.

Hahn, D.W., Rusticus, C., Probst, A., Homm, R. and Johnson, A.N. (1981). Anti-fertility and endocrine activities of gossypol in rodents. *Contraception* 24(1), 97-105.

Hassan, M. E , G. W. Smith, and R. S. Ott, "Reversibility of the reproductive toxicity of gossypol in peripubertal bulls," *Theriogenology,* vol. 61, no. 6, pp. 1171–1179, 2004. View at Publisher · View at Google Scholar · View at Scopus

Herve, J.C., Pluciennik, F., Bastide, B., Cronier, L., Verrecchia, F., Malassine, A., Joffre, M. and Deleze, J. (1996). Contraceptive gossypol blocks cell-to-cell communication in human and rat cells. *Eur. J. Pharmacol*. 313, 243-255.

Holly, S.B., Geoffrey, K.S. and Hara, P. M.(1988). A histopathological study of the effect of gossypol on the female rat. *International Reproduction Health Journal of Contraception*, 38(5), 589 -592.

Ivan, C.N.G, Michelly, F. de M., Silvia, C.S..O, Marilia, M.M. and Benito, S.B. (2014). Gossypol promotes degeneration of ovarian follicles in Rats. *Scientific World Journal*,:986184. doi: 10.1155/2014/986184

Jia, L., Coward, L.C., Kerstner -Wood C.D, Cork, R.L., Gorman, G.S, Noker, P.E, Kitad, S., Pellecchia, M. and Reed, J.C. (2008).

Lena, S., M.D. (2013). Normal liver anatomy. *Clinical Liver Disease*. 2 (S1), 3.

Morgan, S. E. (2014). Gossypol toxicity in livestock. Oklahoma Cooperative Extension Service. VTMD-9116. Oklahoma State University.

Morgan, S. E. (1989). Gossypol as a toxicant in livestock. In Burrows G. E. (ed.): The Veterinary Clinics in North America: Food Animal Practice. Philadelphia, W. B. Saunders, pp 251-263.

Oko, R. and Hrudka, F. (1984). Comparison of the effect of gossypol, estradiol 17 beta and testosterone compensation on male rat reproduction organs, *Biol. Reprod*. 30 (5), 198 -207.

Polsky, B., Segal, S.J., Baron, P.A., Gold, J.W., Ueno, H., Armstrong, D. (1989). Inactivation of human immunodeficiency virus in vitro by gossypol. *Contraception* 39 (6): 579–608.

Qian, S. Z. , and Z. G. Wang (1984) "Gossypol: a potential antifertility agent for males," *Annual Review of Pharmacology and Toxicology*. 24, 329–360. View at Google Scholar · View at Scopus

Randel, R.D., Chase, C.C. and Wyse, S.J. (1992). Effects of Gossypol and Cottonseed Products on Reproduction of Mammalian. *Journal of Animal Science*, 7(6), 1028 -1038.

R. D. Randel, C. C. Chase Jr., and S. J. Wyse, "Effects of gossypol and cottonseed products on reproduction of mammals," *Journal of Animal Science*, vol. 70, no. 5, pp. 1628–1638, 1992. View at Google Scholar · *View at Scopus*

Robert, K.L. and Jim, N.T. (2008). The effect of Gossypol Acetic Acid on female reproduction. *Journal of Drug and Chemical Toxicology*, 8(6), 469 – 482.

Sahar, M., Srour Haleem, H. H. and Mahmoud, A., R, (2009), Hazard of Gossypol on Male Fertility with Trials to Overcome The Deleterious Effects; Egypt. *J. Comp path. & Clinic. Path*. 22(3), 4-5

Teng, C. S. (1997) "Reversible changes in the content of cellular and microtubular tubulin in spermatogenic cells after gossypol treatment," *Contraception*. 55 (1), 41–46,. View at Publisher · View at Google Scholar · View at Scopus

Ueno, H. , M. K. Sahni, S. J. Segal, and S. S. Koide, (1988) "Interaction of gossypol with sperm macromolecules and enzymes," *Contraception*. 37 (3) 333–341. View at Google Scholar · View at Scopus

Xing-Jiu, H., Yang, K. C., Hyung-Soon M., Oktay, Y., Euisik Y., and Hak-Sung, K. (2006). Aspartate Aminotransferase (AST/GOT) and Al Aminotransferase (ALT/GPT) Detection Techniques. *Sensors* , 6, 756-782.

Zhou, D.R., Zhou, Y.C., Cui, G.H., Guo, X., Qin, J., Gui, Y.T. and Cai, Z.M. (2008). Gossypol Repressed Gap functional Intercellular Communication between Sertoli Cells by Decreasing the Expression of Connexin43. *Toxicol. In Vitro* 22, 1719-1725.