Seminal plasma zinc level in users of gossypol

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Summary

Seminal plasma zinc levels were determined in 13 healthy male volunteers on gossypol, a triterpenoid aldehyde extracted to purity from cotton plant which has been found to be an orally active male fertility regulation agent that is simple, economical and reversible. Each subject was given 20 mg gossypol tablet daily until azoospermia occurred. Thereafter, a maintenance dose of 7.5 mg or 10 mg of gossypol was given orally to alternate subject. Each subject was followed up monthly for one year. Serial semen analysis and seminal plasma zinc levels were determined prior to and after administration of gossypol monthly for 52 weeks. The mean sperm count and motility decreased significantly until azoopermia occurred in all subjects at about the 20th week of follow up. However, there was no significant difference in the mean seminal plasma zinc levels throughout the period of observation irrespective of the dosage regimen of gossypol used by the subjects. It was concluded that gossypol has no effect on the seminal plasma zinc level in users of gossypol.

Keywords: *Zinc, Gossypol, Seminal Plasma, Sperm Parameters, Contraception*

Résumé

Les taux de zinc dans le plasma seminal ont été determinés chez 13 volontaires males en bonne sante sous traitement orale de la gossypol, un aldehyde triterpenoide extraite à purité de la plante de coton. Cette plante a été trouvé etre un agent actif regularit la fertilite masculine. Cet agent est simple, economique et reversible. Chaque sujet etait traite avec 20 mg de gossypol en comprime jusquà ce que l'azoospermie survienne. Les sujets etaient par la suite troite avec une dose de maintenance de 7.5 mg ou 10 mg de gossypol prise oralement aux sujets alternatifs. Chaque sujet etait suivive pendants une periode d'un mois à un an. L'analyse de semen et des taux de zinc dans le plasma seminal avaient été determiné mensuelement avant et apres l'administration de la gossypol pendant 52 semaines. La moyenne de conte compte de sperme et de la motilite avait reduit de mamiere significative j'usqua ce que l'azoospermie survienne. Chez tous les sujets au tour de la vingtieme semmaines de suivie. Cependant, il n'yavait pas eu de difference significative dans les taux moyen de zinc du plasma seminal pendant toute la periode d'observation sans rapport avec la dose de gossypol utilise par les sujets. Il avait été conclut que la gossypol n'a aucum effets sur le taux de zinc dans le plasma seminal chez les utilisateurs de gossypol.

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Introduction

Since gossypol was identified as a cause of male infertility in the rural communities of China about four decades ago, several attempts have been made to develop it as a male contraceptive. Consequently, anti-fertility properties of gossypol has been variously reported [1,2,3]. Daily oral administration of 20 mg of gossypol has been known to suppress spermategenesis without interfering with the Leydig cells [4]. Likewise, zinc plays an important role in sperm nucleic acid metabolism and its deficiency has been associated with oligospermia also, without effect on Leydig cells. However, information on zinc concentration in semen is conflicting [5,6,7,8].

Both low semen zinc level and gossypol ingestion have been shown to cause infertility in men [1,2,6,9,10]. It is most probable that zinc and gossypol affect spermatogenesis in different ways which are not interrelated and the two are not interdependent. We are unaware of any study that has been carried out to determine the relationship between zinc level in seminal plasma and gossypol administration. The aim of this study, therefore, was to determine the effect of gossypol administration on seminal plasma zinc levels.

Materials and methods

Thirteen healthy male Nigerians who had completed their family size were recruited into the study after counseling. Informed consent for participation was obtained from each of the men. They were also informed that they could withdraw from the study at any time. An approval for the study was also obtained from the Ethical Committee of the University College Hospital (UCH) Ibadan. The inclusion criteria were: normal semen analysis on two occasions that are two weeks apart; absence of biochemical abnormalities and absence of prostatitis on clinical examinations. Each subject was given 20 mg tablets of gossypol daily until azoopermia occurred. Thereafter, a maintenance dose of either 7.5 mg or 10 mg tablet of gossypol was given to alternate subjects daily for fifty-two weeks. They were followed-up on a monthly basis.

Semen samples were collected from the subjects at each visit by masturbation into a sterile, wide-mouth, acid-washed, screw cap plastic container after three to six days of sexual abstinence. Each sample was analysed as previously described [11], and the semen centrifuged at 2500 g thereafter. The seminal plasma was collected and stored at –20 ^{0}C until analyzed for zinc.

Seminal zinc levels were estimated after thawing each frozen sample and diluting with deiodinised distilled water to make a 1:10 solution. The zinc concentration was estimated in the solution using Perking Elmer atomic absorption spectrophotometer, model 403 (Norwalk, USA) with a hollow cathode lamp at an optical wave length of 213.8 mm [12,13,14]. The mean of triplicate readings of each sample was used. Inter-assay and intra-assay coefficient of variations were 4.8% and 4.3%, respectively. Zinc does not decompose

in semen on storage as earlier reported [5], so the value obtained at analysis represented the real value at collection. The results were expressed in the mean ± standard deviation. Unpaired t test was used to determine the significance of difference between two means. The significant level was fixed at $P<0.05$.

Results

The characteristics of the subjects are shown in Table 1.

Table 1: Characteristics of the subjects on gossypol.

Characteristics	Mean value ± SD (n = 13)
Age	40 ± 2.61 years
Weight at recruitment	68.48 ± 8.3 kg
Height at recruitment	167.15 ± 4.9 cm
Mean systolic blood pressure at recruitment	116.15 ± 6.50 mmHg
Mean diastolic blood pressure at recruitment	74.62 ± 5.19 mmHg.

Their ages ranged from 35 to 42 years with the mean age at 40 ±.2.61 years. A small reduction in the sperm count was noted in the first month of treatment. Subsequently, the mean sperm count and motility continued to fall with the duration of use of gossypol until azoopermia or marked oligospermia occurred in all subjects at about the 20th week. The earliest changes noticed were in the mean sperm motility. These were followed by changes in the sperm count, but no significant change in the mean semen volume was noted throughout the study period (Table 2).

Table 2: Mean sperm motility, count and volume at different weeks of gossypol treatment.

Week of study	Mean sperm motility ± SD (%) (n = 13)	Mean sperm count ± SD (mil/ml) (n = 13)	Mean semen volume ± SD (ml) (n = 13)
0	82 ± 12	114 ± 11	2.35 ± 1.09
2	74 ± 19	103 ± 16	2.85 ± 1.46
4	61 ± 17	94 ± 28	3.5 ± 1.74
8	34 ± 16	66 ± 36	2.92 ± 1.37
12	16 ± 26	44 ± 32	2.83 ± 1.29
16	13 ± 15	36 ± 23	2.62 ± 1.02
20	0	0	3.0 ± 1.41
26	0	0	3.15 ± 1.38
52	0	0	2.77 ± 1.35

The mean seminal plasma zinc levels were similar throughout the period of observation.

Table 3: Mean seminal plasma zinc levels in subjects on oral gossypol.

Duration of use of gossypol (weeks)	Mean zinc level (Zn mmol/1 ± SD)
0	1.25 ± 0.07
2	1.15 ± 0.93
4	1.03 ± 0.07
8	0.99 ± 0.70
12	1.04 ± 0.35
16	0.95 ± 0.41
20	1.03 ± 0.35

In addition, there were no changes in the seminal plasma zinc levels during the maintenance period irrespective of the dose of gossypol used by the subjects *(P>0.05)*. The mean seminal plasma zinc levels at 6th and 12th month of continuous use of gossypol were 1.04 ± 0.66 mmol/l and 1.10 ± 0.67 mmol/1, respectively, for those on 10 mg maintenance dose while they were 1.09 ± 0.7 mmol/l and 1.13 + 0.66 mmol/1, respectively, for those on 7.5 mg maintenance dose (Table 4).

Table 4: Mean seminal plasma zinc levels in subjects on maintenance dose of gossypol tablets.

Duration of continuous use of gossypol	Mean seminal plasma zinc levels during maintenance	
	On 10 mg gossypol	On 7.5 mg gossypol (n = 6)
26 weeks	1.04 ± 0.06	1.09 ± 0.7
52 weeks	1.10 ± 0.67	1.09 ± 0.66

No significant difference detected (P>0.05)

The zinc concentrations in all the samples analysed were within normal limits by World Health Organisation (WHO) standards [15,16]. There was no pregnancy reported by any of the volunteers throughout the period of observation.

Discussion

The anti-fertility properties of gossypol has been confirmed in this study. Continued ingestion of the triterpenoid aldehyde by each subject caused progressive asthenozoospermia earlier than oligospermioa. This observation suggests that gossypol damages sperm motor anoxeme responsible for motor activities faster than it affects sperm production. Studies have indicated that this occurs through the inhibition of dynien ATpase activity in spermatozoa by gossypol [2,17]. In doses as little as 100 pg/100 ul gossypol has been reported to produce a 90% reduction in sperm motility [18]. Gossypol also inhibits the development of immature spermatozoa through an uncertain mechanism. While some workers [19] have postulated that inhibition of testosterone production by gossypol was responsible, others (20) found no change in testosterone level in men taking gossypol for three months despite an occurrence of progressive oligospermia. Therefore, it will seem that gossypol acts directly on matured and immature spermatozoa without interfering with the Leydig cells, pituitary-gonadal axis or testicular hormone production. Consequently, the users' sex drive and hormone regulatory systems are not disturbed.

Zinc plays an important role in nucleic acid metabolism and its effect on DNA synthesis has been emphasised in some experimental studies [21,22,23,24]. Deficiency of zinc has been associated with hypogonadism in animals and man [25,26]. This seems to be through a direct effect on the pituitary-gonadal axis and prolactin secretion [27,28,29]. It has been reported that progressive decrease in activity of human semen was associated with decreasing zinc concentrations in the semen without a deficiency in zinc in the whole body [7]. Evidence in the literature indicates that semen with greater fertility potential in terms of sperm density contains higher zinc content than semen from oligospermic infertile males [5]. Moreover, the first prostatic ejaculate which contains most of the

spermatozoa also contains far more zinc that the vesicular fraction with less spermatozoa [30]. Therefore, a positive correlation between sperm density and zinc level in semen exists. This suggests that zinc has a local effect on sperm survival in the semen.

However, it will appear that the mechanism by which both zinc and gossypol affect spermatogenesis are unrelated. Since prostatic fluid contributes the highest concentration of zinc in semen, it can be confidently inferred that gossypol does not have any effect on prostatic zinc accumulation and possibly has no adverse effect on prostatic function judging by our results. As the controversy over the antifertility effects of gossypol deepens, it is possible that its racemose may be developed in future with a more promising effect. Therefore, our finding that gossypol has no effect on zinc level in seminal plasma is reassuring.

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References

1. Coutinho, EM, Melo JF and Barbosa I. (1985). Biphasic action of gossypol in men. In Segal, JS (ed). Gossypol, A potential contraceptive for men. Plenum Press. New York and London., pp. 25-31.
2. White IG., Vishwanath, Swam MA. and Woodman BPD. Studies on the mechanism of action of gossypol as a male anti-fertility agent. Contracept 1988; 37: 3269-77.
3. Srivastava A, Gupta G, and Setty, BS. Studies on the action of the anti-fertility action of gossypol in rat and hamster. Contracept 1989; 39: 337-55.
4. National Coordinating Group on Male Anti-fertility Agents Gossypol. A new antifertility agent for males. Chin Med J 1978; 4: 417
5. Marmar, JL, Kartz S, Praiss DE and Benedictis TL. Semen zinc levels in infertile and post vascetomy patients with prostatitis. Fertil, Steril 1975; 26: 1057-63.
6. Eliasson R. Standard for investigation of human semen. Andrologie 1971; 3: 49-51.
7. Stankovic, H. and Mikac-Davic D. Zinc and copper in human semen. Clin Chemica Acta 1976; 70: 123-125
8. Wood BJ, Lawrence DM, and McCarrigle HHG. Similar zinc levels in seminal fluid from normospermic, oligospermic and azoospermic men. Clin Chemica Acta 1982; 123: 329-32.
9. Adejumo CA, Ilesanmi AO, Ode EO and Akinlade KS. Biophysical and biochemical analysis of semen in infertile Nigerian males. Afr J Med Sci 1996; 25: 217-219.
10. Abyholm T, Kofstand J, Mone K and Stray-Pedresen S. Seminal plasma fructrose Zn+, Mg+ and acid phosphatase (ACP) in cases of male infertility. Int J Androl 1981; 4: 75-81.
11. Stewart, ID and Aitken, JR. Seminal fluid analysis and sperm function testing. Clin Andro Endocrinol Metab of North America 1994; 23: 752-758.
12. Canaie D, Bartelloni M, Negroni A, Menchini P, Izzo PL, Bianchi B and Menchini-Fabris GF. Zinc in human semen. Int J Androl 1986; 9: 477-480.
13. Smith JC, Bultimovitz GP and Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1979; 25: 1487–91.
14. Kelson J. Criticism of the proposed method. Direct measurement of zinc in plasma by atomic absorption spectroscopy (letter). Clin Chem 1980; 26: 349-350.
15. World Health Organisation (1992). Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interactions. 3rd Edition Cambridge University Press, pp 70-71.
16. Johnsen O and Eliasson R. Evaluation of commercially available kit for the calorimetric determination of zinc in seminal plasma. Int J Androl 1987; 10: 435-440.
17. Sato E, Segal, S and Koide S. Biochemical interaction of gossypol with spermatozoa. Adv. Contracept Del Sys 1986; II, 4: 379.
18. Ridley AJ and Blasco L. Testosterone and gossypol effects on human sperm motility. Fertil Steril 1981; 36 (5): 638-642.
19. Tu-lin, Murono EP, Osterman J, Monklin HR, and Coulson, PB. Gossypol inhibited testicular steroidogenesis. Fertil Steril 1981; 5: 563.
20. Frick, J. and Danner (1985). Effect of gossypol on human testicular function: Evaluation of seminal and hormonal parameters. In: Segal SJ (ed.). Gossypol: A potential contraceptive for men. Plenum Press. New York and London, pp 17-23.
21. Stanstead H and Rinaldi RA. Impairment of deoxyribonucleic acid synthesis of dietary zinc deficiency in the rat. J. Cell Physiol 1969; 73: 81-85.
22. Andez-Madrid FF, Prasad AS, and Oberleas D. Effect of zinc deficiency on nucleic acid, collagen and non-collagen protein of connective tissues. J Lab Clin Med 1973; 82: 951-961.
23. Chapil, M. Effect of zinc in cells and bimembranes. Med Clin North Am 1976; 60: 799-812.
24. Wang YC and Rao PN. Effect of gossypol on DNA synthesis and cell cycle progression of mammalian cell in vitro. Cancer Research 1984; 44: 35-38.
25. Diamond I and Hurley L.S. Testicular and eosophageal lesions in zinc deficient rats and their reversibility. J Nutri 1971; 101: 77-84.
26. Orgebin-Crist MC, Freeman M and Barney GH. Sperm formation in zinc deficient rats. Ann, Biol Ann Biochem Biophys 1971; 11: 547-548.

27. Lei KY, Abbasi AA and Prasad AS. Function of pituitary gonadal axis in zinc deficient rats. Am J Physiol 1976; 1730-2.

28. Mahajan SK, Abassi AA and Prasad AS. Effect of zinc therapy on gonadal function in haemodialysis patients. A double blind study. Ann Int Med 1982; 97: 357-361

29. Login IS, Thorner MO and Macleod RM. Zinc may have a physiological role in regulating pituitary prolactin secretion. Neuroendocrinology 1983; 37: 317-320.

30. Lindholmer, C and Elliason, R. The effect of albumin, magnesium and zinc on human sperm survival in different fractions of slit ejaculates. Fertil Steril 1974; 25: 424-31.