Proteinaceous diet inhibits gossypol-induced spermatotoxicity

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The present study was designed to investigate the effect of a proteinaceous dietary supplement, fishmeal, on gossypol-induced spermatotoxicity. Twenty-five adult male Wistar rats, averaging 205 g b.w., were randomly sorted into four experimental groups (I-IV) of 5 animals each, and a control group. Crude cottonseed oil was administered orally to each animal in groups I-IV at a rate that provided 14 mg/kg/d free gossypol; in addition, 3 g/d, 7 g/d, and 10 g/d of fishmeal was provided as meal supplement to each animal in groups I, II and III respectively. The control group received rat pellets and water freely. At the end of the 53-day treatment period, all animals were placed under chloroform anaesthesia; the caudal epididymides were removed, minced and placed in Ham's F10 solution for the evaluation of sperm count and motility. The testes were also processed for histological studies using the eosin and haematoxylin (H & E) method. Our findings revealed a dose-dependent inhibition of gossypol-induced spermatotoxicity by the supplemented fishmeal; this suggests that proteinaceous diets are protective against gossypol-induced male infertility.

Key words: Gossypol, fishmeal, spermatogenesis.

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Cottonseed oil has become a household ingredient packaged in different brands for human consumption. This oil contains a yellow polyphenolic binaphthalene pigment, gossypol, which is reputed to induce infertility in man (Gu *et al.,* 2000; Coutinho, 2002). Gossypol also increases erythrocyte osmotic fragility (Risco *et al.,* 2002) and causes hypokalaemia (Yu and Chang, 1998), among other side effects. The reported mechanisms of action of gossypol as a male contraceptive include inhibition of acrosin and aryl sulphase (Yuan and Shi, 2000), impairment of ATP production in spermatozoa and the arrest of spermatogenesis (Coutinho, 2002).

However, it is interesting to note that despite the commercial production of cottonseed oil for domestic uses, most males do not usually present with infertility. Although the packaged oil is refined, the refining process does not entirely eliminate gossypol; even relatively low doses of the latter are known to be a potent testicular toxin (Coutinho, 2002). Thus, certain mechanism must exist *in vivo* that palliates gossypol-induced spermatoxicity. In the gut, gossypol had been reported to demonstrate affinity for soluble proteins (Reiser and Fu, 1962). Similarly, Wang *et al.,* (1992) observed that the biological activity of gossypol resulted from its stereospecific binding to extracellular and intracellular proteins *in vivo;* furthermore, amino acids such as lysine, histidine, cystine and glycine have also been reported to possess the potential of binding to gossypol (Javed and Khan, 1999; Henry *et al.,* 2001). Thus, the objective of this study was to test the efficacy of a proteinaceous dietary supplement (fishmeal) in inhibiting the spermatotoxic effect of gossypol in an animal model.

Materials and Methods

Crude cottonseed oil (CCO) was prepared using the solvent (hexane) extraction method as described by Zhang *et al.* (2002). The composition of the her-

Table 1. Composition of Herring-type fishmeal.

Composition	*Quantity (g/kg)*
Crude Protein	730
Calcium	20
Phosphorus	15

Table 2. Amino acid composition of Herring-type fishmeal.

Amino Acid	*Quantity (g/kg)*
Arginine	40.5
Cystine	6.7
Glycine	50.6
Histidine	14.1
Isoleucine	26.1
Leucine	44.6
Lysine	48.2
Methionine	15.2
Phenylalanine	28.2
Serine	28.3
Threonine	24.9
Tryptophan	6.9
Tyrosine	21.4
Valine	30.7

ring-type fishmeal (Shepherd Touch Mill, Ilorin, Nigeria) used as a supplement in this work is shown in Tables 1 and 2 (McDonald *et al.,* 1995).

Animals and diets

Twenty-five sexually mature (10 weeks old) male Wistar rats, averaging 205g b.w., were used. All animals were housed in well-ventilated cages in the Laboratory for Reproductive Biology, Department of Anatomy, University of Ilorin. They were exposed to the conventional light/dark cycle and fed rat pellets (Shepherd Touch Mill) and water freely. Animals were randomly distributed into four experimental groups (I-IV) and control (n=5). Each animal in experimental groups I-IV was administered CCO orally to provide 14 mg/kg/d free gossypol; in addition, a daily supplement of 3 g, 7g and 10 g of fishmeal was assigned to each animal in groups I, II and III respectively. CCO administration and treatment with fishmeal supplement was for a period of 53 days. Group V served as control and was given rat pellets and water only. At the end of the 53-day treatment period, animals were placed under chloroform anaesthesia; their caudal epididymides were excised, minced and placed in Ham's F10 solution for the purpose of evaluating sperm count and motility. The testes were also harvested for histological analysis (*see below*).

Table 3. Sperm count and percentage sperm motility of control and experimental rats.

Group	*Sperm count (x10^6/mL)*	*Sperm motility (%)*
I	*16.1±2.8	*45.2±1.1
II	48.4±4.4	60.4±1.8
III	50.3±5.3	64.3±2.1
IV	*7.9±0.2	*10.2±1.1
V	54.8±3.5	68.5±3.2

*Values are expressed as Mean±SEM; *(p<0.05).*

Sperm count and motility

0.5 mL of the sperm suspension in Ham's F10 was appropriately diluted with 1% phenol, 4% $NaHCO_3$ (5 g), 35% formalin (1 mL) in distilled water. Sperm count was done by a light microscope with the aid of Neubauer counting chamber of a haemocytometer at a final magnification of x100. The percentages of motile and non-motile sperm were recorded by microscope scoring of freshly collected sperm suspension in Ham's F10 medium.

Statistical analysis

Data collected were analysed by the ANOVA statistical test.

Histological analysis

The testes from control and treated animals were fixed in 10% formalin and routinely processed and sectioned for microscopic studies; 5 μm-thick sections were finally stained with haematoxylin and eosin (H&E).

Results

Table 3 shows the sperm count and percentage sperm motility of control and experimental animals.

In animal groups I and IV (which had been administered CCO plus 3 g/d of fishmeal or CCO alone, respectively), sperm count and motility were significantly lower ($p<0.05$) than in controls (group V) (Table 3). No statistically significant difference in sperm count or sperm motility was observed between the control and groups II and III (which received CCO plus 7 or 10 g/d of fishmeal, respectively: Table 3). At light microscopy, no apparent changes were ever observed in the morphology of spermatozoa from any of the animal groups.

In the controls, the cytoarchitecture of the seminiferous tubules was well defined, with germ cells occupying the germinal epithelium while numerous spermatozoa filled the lumen; adjacent tubules were

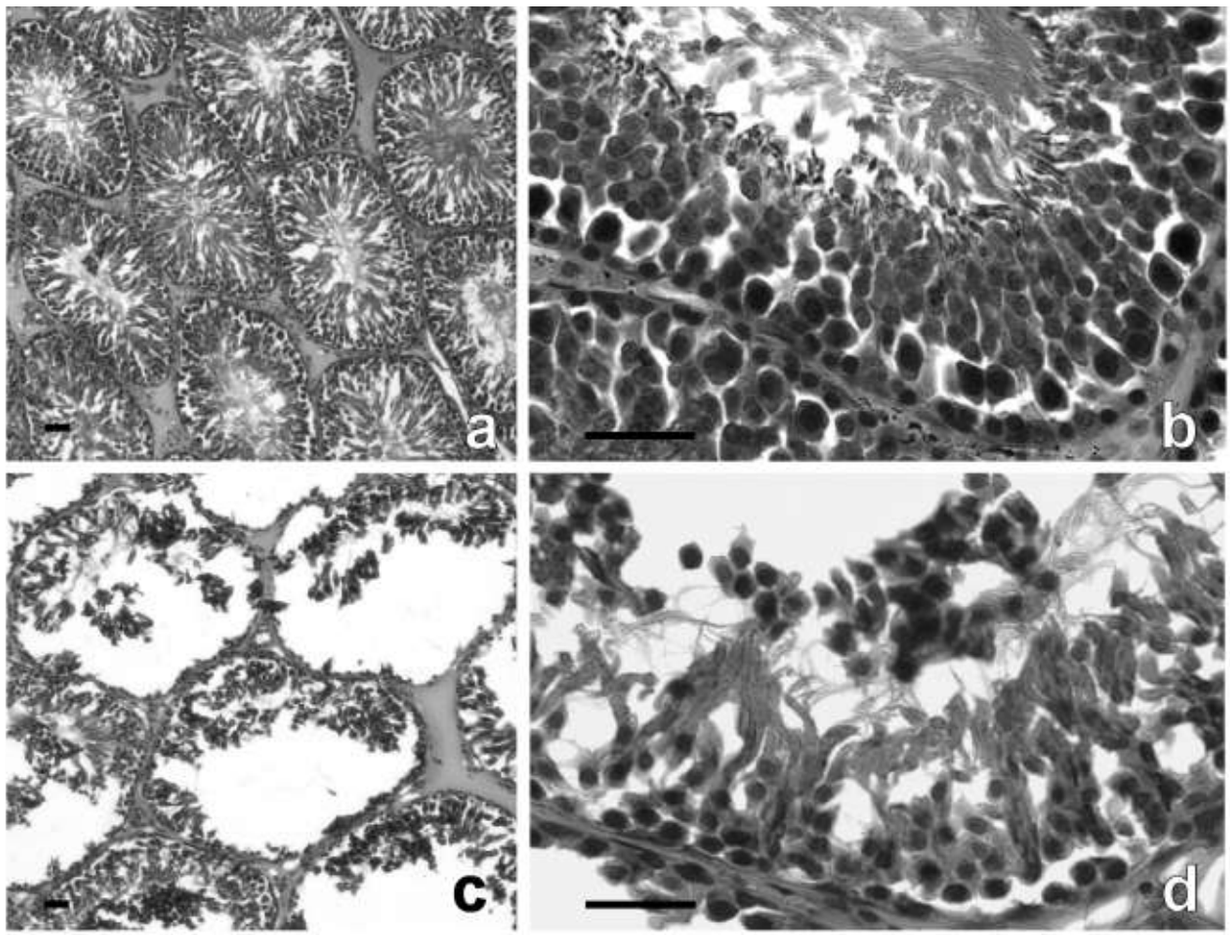

Figure 1. Cross sections of seminiferous tubules of control (a,b) and of group IV rats (c,d). Magnification 10x (a,c) and 40x (b,d); bars are for 20 microns.

separated by a thin layer of interstitial tissue (Figure 1 a,b). Testicular sections from animal groups II and III were similar to the controls. On the contrary, in groups I and IV, testicular sections revealed pronounced reduction in thickness of the germinal epithelium and the lumens were largely devoid of spermatozoa (Figure 1 c,d).

Discussion

The results of the present study suggest that proteinaceous diets have the potential of reversing the testicular damage induced by gossypol; this effect was observed to be dose-dependent.

Gossypol is a proven testicular toxin and male contraceptive (Yu and Chan, 1998; Yuan and Shi, 2000); its reported mechanism of action include inhibition of acrosomal enzymes (Yuan and Shi, 2000), affinity for extracelluar and intracellular proteins (Wang *et al.,* 1992), and inhibition of spermatogenesis and sperm motility (Kalla and Vasudev, 1980; Ridley and Blasco, 1981; Coutinho, 2002).

In animals exposed to CCO alone (group IV), sperm count and motility were significantly reduced ($p<0.05$). This confirms the reports of Kalla and Vasudev (1980) and Shi *et al.,* (1981) on the inhibitory effects of gossypol on these male fertility indices; the deleterious effect of this substance on sperm motility was reported to be due to its ability to deplete ATP in spermatozoa. Shi *et al.,* (1981) concluded that gossypol was capable of inducing spermatogonial damage, thereby arresting spermatogenesis. This could explain the reduction in sperm count observed in this work.

However, supplementing fishmeal to the diet of experimental animals resulted in a dose-dependant reversal of the adverse effects of gossypol on sperm count and motility. In previous studies, certain amino acid and proteins had been demonstrated to possess affinity for gossypol, to which they bind *in vitro* and *in vivo* (Wang *et al.*, 1992; Fombad and

Bryant, 2004). Of special importance in this respect are lysine, histidine, cystine, glycine, threonine and methionine which are all contained in the supplemented fishmeal used in the present investigation (McDonald *et al.,* 1995). Lysine reportedly has a high affinity for gossypol, to which it binds readily (Henry *et al.,* 2001); it had recently been demonstrated to be of importance in reducing the adverse effect of gossypol (Fombad and Bryrant, 2004). Furthermore, ruminants are capable of tolerating gossypol probably owing to the affinity of this substance for soluble proteins in the gut (Reiser and Fu, 1962); it is, however, not clear whether the inhibitory effect of supplemented fishmeal on gossypol-induced spermatotoxicity (observed in this study) was due to the binding of amino acids (in fishmeal) to gossypol in the gut or in the plasma, or both.

The marked reduction in the thickness of seminiferous tubules observed in the animals exposed to gossypol-containing CCO only (group IV) indicates a reduction in the density of the developing germ cells in the tubules; a similar finding had previously been observed by Oko and Hrudka (1984) who reported graded atrophy of the testes, degeneration of spermatogenic cells and impairment of Sertoli cells following exposure to gossypol. The evidence that group I animals (which received CCO plus 3 g/d of fishmeal) had similarly damaged testicular epithelium, whereas in the animals of groups II and III, the testicular micro-anatomy was more similar to the controls, suggests that the supplemented fishmeal, at high doses, was able to inhibit gossypol-induced testicular damage. This protective effect could likely result from the binding of the fishmeal amino acids to gossypol.

The potential of proteinaceous diets to inhibit the spermatotoxic effects of gossypol could likely explain why some habitual consumers of cottonseed oil do not usually present with infertility.

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