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Effects of an aqueous extract of cotton seed (*Gossypium barbadense* Linn.) on adult male rats

K.D. THOMAS (1), A.E. CAXTON-MARTINS (2), A.A. ELUJOBA (3) AND O.O. OYELOLA (1)

(1) Department of Chemical Pathology, Faculty of Health Sciences,
(2) Department of Anatomy and Cell Biology, Faculty of Health Sciences,
(3) Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

Twenty adult male rats per group in 4 treatment groups were injected intraperitoneally at 08.00 hours with 0.1 ml of an aqueous cotton seed extract (*Gossypium barbadense* Linn.) (Malvaceae) in concentrations of (a) 105.25, (b) 21.21, (c) 4.65, (d) 2.325 mg ml^{-1} (kg body weight)$^{-1}$, respectively. A fifth group (control) was given 0.1 ml of pyrogen free distilled water per rat. Five rats per treatment group were sacrificed at 2, 8, 24 and 168 hours respectively after treatment. Plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) showed no change. Plasma testosterone was lower ($p<0.05$) than that of control at 2 and 8 hours, with recovery by 168 hours post treatment. Plasma creatinine was raised by 2 hours, with recovery by 8 hours. Plasma urea rose gradually but persistently to a maximum of 168 hours. Plasma aspartate (AST) and alanine (ALT) transaminases were significantly higher ($p<0.001$) than that of controls throughout the study. Testicular histology showed early germ cell disorganization followed by progressive fibrosis (sperm cytoskeleton) by 24 hours. There was evidence of recovery by 168 hours. It is concluded that aqueous extract of cotton seed meal contains substances that can rapidly cause damage to testicular, liver, kidney and muscular tissues.

Introduction

Cotton seed oil is used as cooking oil in some parts of Nigeria, especially among the Yorubas, where it is known as 'Ororo koro owu'. It is well known to be rich in the essential fatty acid, linoleic acid [1].

It is also known to contain a polyphenol bissesquiterpene called gossypol, which is used in China as a male contraceptive [2]. The cotton plant has been consistently used

by Mexicans [3] and Indians [4] as an abortifacient. Gossypol is, however, now under intensive investigation for its activity as a male contraceptive [5–7]. Most of the reports on the antifertility effects of either the cotton seed extract or gossypol are on the effect of daily oral treatment after at least 2 weeks. The antifertility effect has been ascribed to a direct effect on some membrane-bound mitochondrial enzymes, reduction of ATP production through uncoupling of oxidative phosphorylation [8], formation of oxygen radicals [6] and damaging round spermatids [9].

A preliminary investigation by the present authors suggests that treatment with cotton seed extract has very significant early effects. This prompted us to design the present study.

Materials and methods

One hundred adult male rats (Vom-Ife strain) between 6 and 8 months old were engaged in this study. Each rat was weighed at the beginning of the study. Rats were kept five to a cage (46 cm × 20 cm × 18 cm) in a well-aerated laboratory in 12 hours of alternate light and dark conditions and fed mouse cubes (12% protein) (Livestock Rations Nigeria Ltd) and given water *ad libitum*.

Twenty rats were assigned to each of the following treatment groups:

Group 1: Rats given intraperitoneal (i.p.) injection of 0.1 ml of undiluted cotton seed extract (106.30 mg ml^{-1} (kg body weight)$^{-1}$).

Group 2: Rats given i.p. injection of 0.1 ml of cotton seed extract diluted 1 in 5 (21.26 mg ml^{-1} (kg body weight)$^{-1}$).

Group 3: Rats given i.p. injection of 0.1 ml of cotton seed extract diluted 1 in 25 (4.65 mg ml^{-1} (kg body weight)$^{-1}$).

Group 4: Rats given i.p. injection of 0.1 ml of cotton seed extract diluted 1 in 50 (2.32 mg ml^{-1} (kg body weight)$^{-1}$)

Group 5: Controls, rats given i.p. injection of 0.1 ml pyrogen free distilled water.

All injections were given at 08.00 hours and each rat received only one injection in this study. Five rats from each group were sacrificed at 2, 8, 24 and 168 hours respectively after the injection.

Extraction procedure

One hundred grams of powdered cotton seed (*Gossypium barbadense* Linn.) (Malvaceae), purchased locally from the market in Ile-Ife as well as those obtained from a cultivated plant, specimens of which were compared with authentic herbarium

specimens at the Forestry Research Institute of Nigeria, Ibadan, Nigeria were extracted by reflux (45 minutes) in 70% ethanol. The ethanolic suspension was cooled, filtered and the filtrate concentrated to a small volume *in vacuo*. The resulting aqueous suspension was passed through a cationic exchange resin (Amberlite IR-120) in the H^+ form by soaking overnight in 1 N HCl, and rinsed with deionized water until neutral to litmus. The aqueous concentrate of the extract was passed through the column at 1.0 ml/min.

The effluent, which gave positive reaction to phenolics, was concentrated to dryness, and later made to 430 ml with pyrogen-free deionized water. From this stock solution, dilutions with pyrogen-free deionized water of (a) 1 in 5, (b) 1 in 25 and (c) 1 in 50 were made ready for animal dosing.

Testicular histology

The testes of each rat were removed after sacrifice by decapitation and fixed within 5 minutes in Bouin's fluid for histological analysis. Each testis was further chopped into small blocks with a clean razor in Bouin's fluid bath. They were then dehydrated in ascending grades of ethanol, cleared in benzene and blocked out in paraffin wax. The tissues were sectioned and then stained routinely in hematoxylin-eosin.

Plasma biochemistry

Blood was collected after decapitation of rats in each group at the different time interval outlined above into lithium heparinized tubes. The plasma was separated and used for the following estimations.

Plasma FSH and LH were estimated by the radioimmunoassay (RIA) method, using RIA assay kits (Radio Amersham, England) at the Department of Obstetrics and Gynecology, College of Medicine, Ibadan. The results are expressed in terms of NIAMDD-Rat-FSH RPI (2.1XNIH-FSH-SI) and NIAMDD-Rat-LH-RPI (0.03XNIH-LH-SI) for FSH and LH levels, respectively.

Plasma testosterone was determined by a modified RIA method described by Furayama *et al.* (1970) [10]. Plasma urea was measured by the diacetylmonoxime method [11]. Plasma creatinine was measured by the alkaline picrate method [12]. Plasma alanine and aspartate transaminase activities were determined by a spectrophotometric method [13].

Statistical analysis

The study was analyzed as a 5 × 4 factorial design in which the first factor (A) was treatment with cotton seed extract at 5 levels:

(i) undiluted extract
(ii) extract diluted 1 in 5

(iii) extract diluted 1 in 25
(iv) extract diluted 1 in 50
(v) controls (pyrogen free water)

The second factor (B) was the duration of treatment before sacrifice at 4 levels: (i) 2 hours, (ii) 8 hours, (iii) 24 hours, (iv) 168 hours.

The significance of the effect of treatment with the cotton seed extract on the different parameters was assessed using a two-way analysis of variance (ANOVA) as described by Kirk (1982) [14].

Results

The mean weight of the rats in the study was 202 ± 15.2 g. The mean values for the plasma urea and creatinine and for aspartate and alanine transaminase activities are shown in Table 1. In the analysis of variance for each of the four parameters, the values were significantly higher ($p<0.001$) than the control values at each of the treatment levels (concentration of extract) and the time of sacrifice after treatment.

The results for the plasma FSH, LH and testosterone levels for all stages of sacrifice in rats treated with 1 in 50 dilutions (2.325 mg ml^{-1} (kg body weight)$^{-1}$) as compared with control values are shown in Table 2. The plasma FSH amd LH values were not significantly different from the control values. However, plasma testosterone levels were significantly lower ($p<0.05$) than their respective controls at 2 and 8 hours after treatment, but the value returned to the control value by 168 hours.

The testicular histology of the rats after treatment with the various concentrations of the cotton seed extract is shown in Table 3. The histology photographs of rats treated with 1 in 50 dilution (2.325 mg ml^{-1} (kg body weight)$^{-1}$) and that of the controls are shown in Figures 1 and 2.

Discussion

The present study shows considerable but reversible histological damage to testicular germinal epithelium only a few hours after treatment with a very dilute cotton seed extract (2.325 mg ml^{-1} (kg body weight)$^{-1}$). Our histological findings support the report of Xue *et al.* [15], that the damage to germinal epithelium by gossypol and its related compounds is dose dependent. It contradicts a report by Xue [16], however, that it requires daily administration of gossypol for 2 to 4 weeks for demonstrable histological damage to seminiferous epithelium. This may be due to the route of administration. In the oral route, the slow rate of gut absorption [17] and metabolism may be protective.

Plasma creatinine levels of 5 times the control after only 2 hours of the administration of the undiluted cotton seed extract (Table 1) indicate not only an early renal glomerular damage but probable muscular (cardiac and skeletal) degeneration as well. However, there was biochemical recovery by 8 hours after the treatment.

Table 1 Mean plasma urea and creatinine concentrations, aspartate (AST) and alanine (ALT) transaminase activities after treatment with cotton seed extract

Period after treatment	*Treatment*	*Urea* (mg/100 ml)	*Creatinine* (mg/100 ml)	*AST* (IU/litre)	*ALT* (IU/litre)
B1	A1	48.56 ± 2.4	5.82 ± 1.23	89.6 ± 5.4	153.2 ± 7.2
	A2	31.18 ± 2.7	4.26 ± 0.94	103.6 ± 7.1	122.8 ± 6.8
2 hours	A3	26.22 ± 1.6	3.70 ± 0.74	292.0 ± 8.2	101.6 ± 5.7
	A4	21.22 ± 1.9	3.72 ± 0.78	315.2 ± 9.5	69.2 ± 4.2
	A5 control	19.70 ± 1.7	1.10 ± 0.47	20.0 ± 2.4	16.6 ± 2.3
B2	A1	74.14 ± 3.7	2.00 ± 0.8	197.6 ± 7.8	199.0 ± 7.8
	A2	58.14 ± 3.3	1.38 ± 0.42	269.6 ± 9.8	142.6 ± 6.3
8 hours	A3	41.82 ± 3.2	1.48 ± 0.47	837.4 ± 12.6	118.6 ± 5.9
	A4	46.68 ± 2.8	1.54 ± 0.45	874.0 ± 13.7	86.4 ± 4.1
	A5 control	17.44 ± 1.2	1.14 ± 0.32	16.6 ± 2.3	13.0 ± 2.7
B3	A1	59.56 ± 2.8	1.6 ± 0.48	102.4 ± 7.7	232.0 ± 10.2
	A2	43.94 ± 2.2	1.56 ± 0.52	110.0 ± 6.2	173.2 ± 8.5
24 hours	A3	60.78 ± 3.7	1.52 ± 0.47	398.2 ± 10.4	133.0 ± 7.4
	A4	39.06 ± 2.9	1.46 ± 0.42	445.0 ± 11.7	107.0 ± 6.9
	A5 control	16.84 ± 1.7	1.34 ± 0.28	13.78 ± 2.9	12.1 ± 2.1
B4	A1	97.76 ± 4.2	1.58 ± 0.42	84.8 ± 6.3	225.0 ± 12.7
	A2	80.04 ± 3.6	1.44 ± 0.31	102.2 ± 8.3	166.4 ± 9.4
168 hours	A3	99.14 ± 4.5	1.44 ± 0.33	376.2 ± 11.2	140.2 ± 8.1
	A4	100.70 ± 4.7	1.36 ± 0.24	398.0 ± 9.7	99.6 ± 5.4
	A5 control	17.92 ± 1.3	1.14 ± 0.27	14.6 ± 2.4	13.4 ± 2.7

A1 A2 A3 and A4 are treatments with the extract in concentrations of 106.3, 21.21, 4.65 and 2.325 mg ml^{-1} (kg body weight)$^{-1}$) respectively
A5 (control) is treatment with pyrogen free distilled water.
Each result is the mean of five observations.

Table 2 Mean plasma FSH, LH and testosterone levels of adult male rats after treatment with 1 in 50 dilution of cotton seed extract (2.325 mg ml^{-1} (kg body weight)$^{-1}$) and controls

Hours after treatment	*FSH (ng/ml)*		*LH (ng/ml)*		*Testosterone (ng/ml)*	
	1 in 50 dilution	*Control*	*1 in 50 dilution*	*Control*	*1 in 50 dilution*	*Control*
2 h	1008 ± 46.2	908 ± 47.4	15.6 ± 6.8	12.4 ± 5.7	3.1* ± 0.9	8.4 ± 2.4
8 h	802 ± 32.2	803 ± 51.7	10.9 ± 4.2	13.7 ± 8.4	2.8* ± 1.2	6.8 ± 1.9
24 h	808 ± 38.7	972 ± 48.7	9.7 ± 3.4	11.9 ± 7.9	3.4 ± 1.7	7.2 ± 2.6
168 h	913 ± 41.7	953 ± 58.2	13.6 ± 4.9	14.6 ± 6.9	5.7 ± 1.9	5.2 ± 1.7

* $p < 0.05$

Table 3A Histology of the testis of adult male rats at 2, 8, 24 and 168 hours, respectively, after treatment with cotton seed extract

Period after treatment	*Undiluted extract (106.30 mg ml^{-1} (kg body weight)$^{-1}$)*	*1 in 5 dilution (21.26 mg ml^{-1} (kg body weight)$^{-1}$)*	*Control*
2 hours	Severe distruption of peritubular connective tissue; evidence of fibrosis; probably cytoskeleton of damaged sperm cells, spermatogenic cells beginning to slough off.	Obliteration of morphological details of tubules, peritubular connective tissue further disrupted.	Normal
8 hours	Extensive fibrosis (cytoskeleton); peritubular connective tissue not visible.	Extensive degree of fibrosis; obliteration of germ cells; peritubular connective tissue not visible.	Normal
24 hours	Extensive fibrosis (cytoskeleton); tubular connective tissue lacking.	Germ cells are beginning to reappear but their morphological integrity is not quite certain.	Normal
168 hours	Germ cells are beginning to reappear in a few of the seminiferous tubules.	Fibrosis appears to be receding, germ cells show orderly arrangement while peritubular connective tissue appears to be reconstituted.	Normal

Table 3B Histology of the testis of adult male rats at 2, 8, 24 and 168 hours, respectively, after treatment with cotton seed extract

Period after treatment	*1 in 25 dilution (4.65 mg ml^{-1} (kg body weight)$^{-1}$)*	*1 in 50 dilution (2.325 mg ml^{-1} (kg body weight)$^{-1}$)*	*Control*
2 hours	Fibrosis still prominent in lumen of tubules, germ cells obliterated and arrangement disorganized; peritubular connective tissue appears thickened.	Germ cells appear slightly distorted in morphology with concomitant disorganization in arrangement.	Normal
8 hours	Degree of fibrosis (cytoskeleton) greater; sloughing off of germ cells; peritubular connective tissue bloated where visible.	Spermatogenic cells appear distorted; fibrosis in the lumen of the tubules.	Normal
24 hours	Many tubules appear obliterated, luminar fibrosis still strongly visible while peritubular connective tissue appears to have broken down.	Order is returning to germ cell arrangement, peritubular connective tissue shows some interruptions.	Normal
168 hours	Spermatogenic cells appear shrunken in size and stained intensively; organization of germ cell appears to be returning to normal, peritubular connective tissue appears slightly thicker but intact.	Minimal fibrosis, germ cell progression now orderly with complete reconstruction of peritubular connective tissue.	Normal

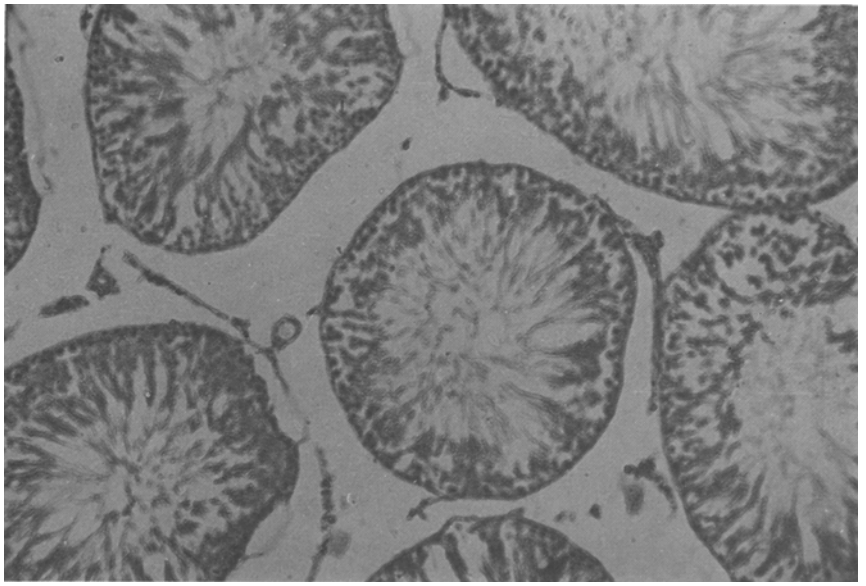

Figure 1A Testicular histology of rats given i.p. injection of 0.1 ml of 1 in 50 dilution (2.325 mg ml^{-1} (kg body weight)$^{-1}$) cotton seed extract at 8 hours after treatment

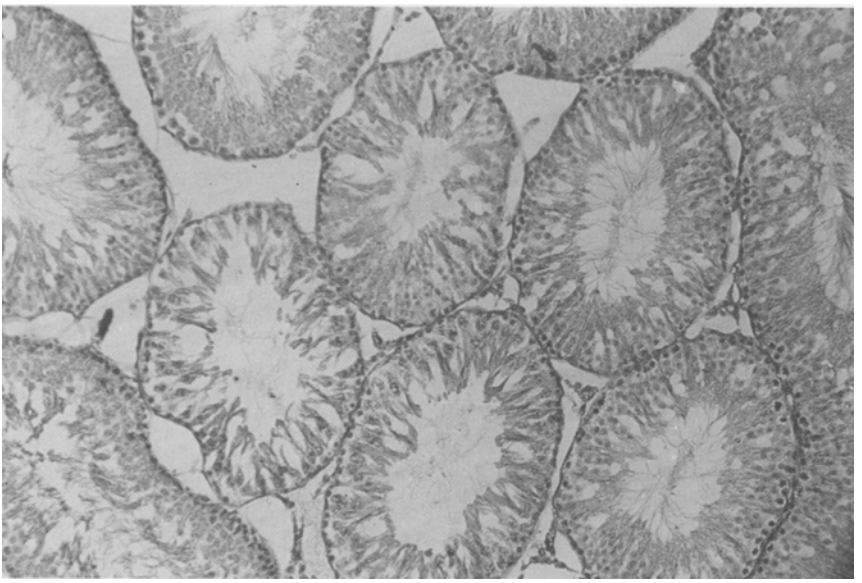

Figure 1B Testicular histology of rats given i.p. injection of 0.1 ml of 1 in 50 dilution (2.325 mg ml^{-1} (kg body weight)$^{-1}$) cotton seed extract at 24 hours after treatment

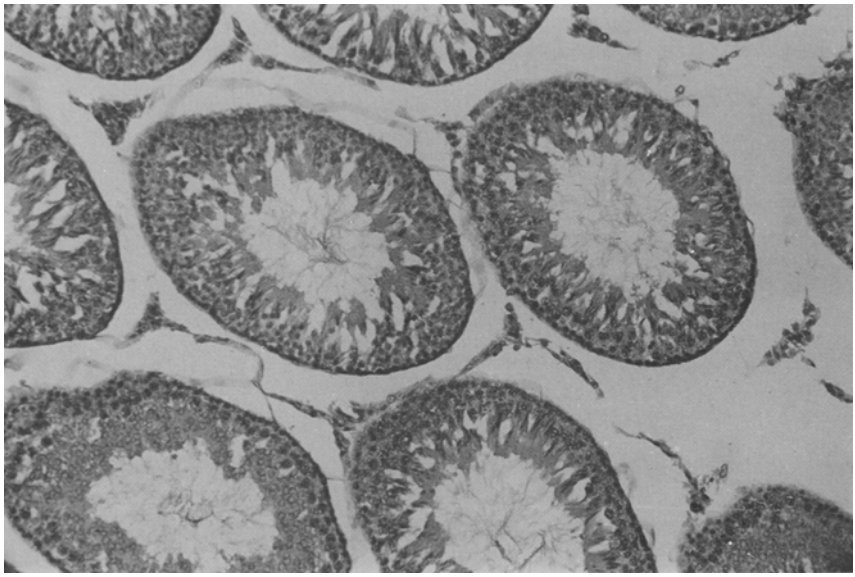

Figure 1C Testicular histology of rats given i.p. injection of 0.1 ml of 1 in 50 dilution (2.325 mg ml^{-1} (kg body weight)$^{-1}$) cotton seed extract at 168 hours after treatment

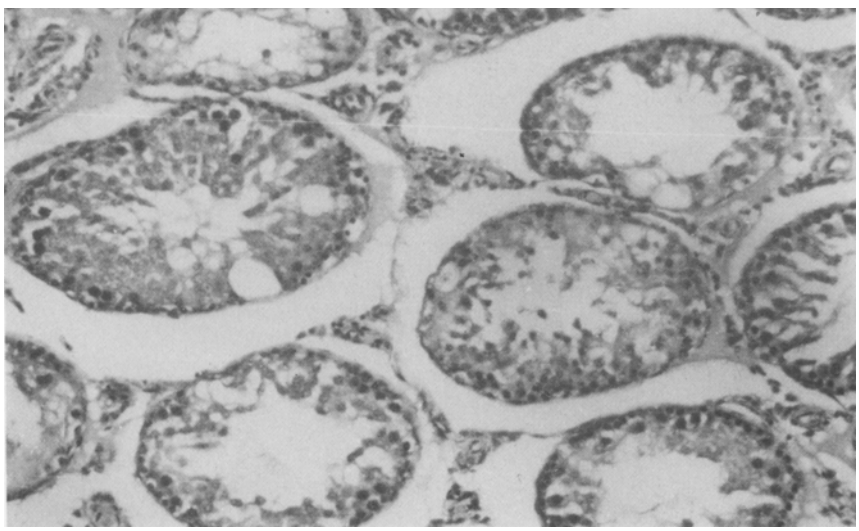

Figure 2 Testicular histology of rats given i.p. injection of 0.1 ml pyrogen-free distilled water (controls)

The persistently raised plasma urea levels at 8 to 168 hours after treatment in the presence of glomerular recovery may not only be coming from renal tubular damage but from prerenal causes such as hepatic catabolism of protein from damaged and necrotizing tissues. The early and persistent elevation of plasma aspartate (AST) and alanine (ALT) transaminases in this study gives supporting evidence to the presence of early and necrotizing liver cell damage. A higher value for AST than ALT up to 168 hours most probably indicates damage to, and necrosis of, cardiac muscle [18].

The observed elevated mean plasma testosterone levels at 2 and 8 hours with recovery at 168 hours supports an earlier report of an early reduction in Leydig cell junction in gossypol acetic acid treated rats [19].

Finally, although the intraperitoneal administration probably results in a rapid and higher plasma level of the toxic agent in the cotton seed extract which readily transcends the blood–testis barrier, it is also possible that these effects may be more pronounced in rat than human tissues and at the very least may even be species dependent. Yet we still feel these observations should be reported because unrefined cotton seed oil is now extensively sold and eaten in some parts of Nigeria and possibly other third world countries.

In conclusion, this study has shown that a single intraperitoneal injection of an aqueous extract of cotton seed meal to the rat has demonstrable early damaging effects on testicular, hepatic, renal and muscular tissues.

References

1. Mayes, P.A. (1988). Lipids of physiologic significance. In: *Harpers Biochemistry*, R.K. Murray, D.K. Granner, P.A. Mayes and V.W. Rodwell, eds, 21st edition. Appleton and Lange, California, pp. 130–141
2. National Coordinating Group on Male Antifertility Agents (1978). Gossypol: A new antifertility agent for males. *Chin. Med. J.*, **4**, 417–428
3. Conway, G.A. and Slocumb, J.C. (1979). Plants used as abortifacients and emmenagogues by Spanish New Mexicans. *J. Ethnopharmacol.*, **1**, 241
4. Casey, R.C.D. (1960). Alleged antifertility plants of India. *Ind. J. Med. Sci.*, **14**, 590–600
5. Prasad, M.R.N. and Diczfalusy, E. (1982). Physiology and medical aspects of andrology. 'Gossypol'. *Int. J. Androl.* (Suppl.), **5**, 53–70
6. De Peyster, A., Quintanilha, A., Packer, L. and Smith, M.T. (1984). Oxygen radical formation induced by gossypol in rat liver microsomes and human sperm. *Biochem. Biophys. Res. Commun.*, **118**, 573–579
7. Edwards, J.G. (1986). Gossypol and rat germinal cells. *Res. Reprod.*, **18**, 4
8. Reyes, J., Aallen, J., Tanphaichitr, N., Bellve, A.R. and Benos, D.J. (1984). Molecular mechanisms of gossypol action on lipid membranes. *J. Biol. Chem.*, **259**, 9607–9615
9. Baccetti, B., Bigliardi, E., Burrini, A.G., Renieri, T. and Selmi, G. (1986). The action of gossypol on rat germinal cells. *Gamete Res.*, **13**, 1–7
10. Furayama, S., Mayes, D.M. and Nugent, C.A. (1970). A radioimmunoassay for plasma testosterone. *Steroids*, **16**, 415–428
11. Varley, H. (1967). *Practical Clinical Chemistry*. Interscience Publishers, Inc., New York, pp. 158–167
12. Giorgio, J.D. (1974). Manual determination of creatinine and creatine. In: *Clinical Chemistry Principles and Techniques*. R.J. Henry, D.C. Cannon and J.W. Winkelman, eds, 2nd edition. Harper and Row, New York, pp. 543–552
13. Henry, R.J., Chiamori, N., Golub, O.J. and Berkman, S. (1960). Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. *Am. J. Clin. Pathol.*, **34**, 381–398
14. Kirk, R.E. (1982). *Experimental Design: Procedures for the Behavioral Sciences*, 2nd Edition. Brooks/Cole Publishing Company, USA, p. 911

15. Xue, S.P., Zong, S.D., Su, S.Y., Wu, Y., Lih Zhou, Z.H. and Ma, X.X. (1980). Antifertility effect of gossypol on the germinal epithelium of the rat testis: a cytological autoradiographical and ultrastructural observation. *Sci. Sin.* (English edition), **23**, 642–657
16. Xue, S.P. (1981). Studies on the antifertility effect of gossypol, a new contraceptive for males. In: *Recent Advances in Fertility Regulation. Proceedings of a Symposium. Beijin, China.* C.F. Chang, D. Griffin and A. Wollman, eds, S.A. Atar, Geneva, pp. 122–146
17. Tang, X.C., Shu, M. and Shi, Q.X. (1980). Comparative studies on the absorption, distribution and excretion of ^{14}C-gossypol in four species of animals. *Acta Pharmacol. Sing.*, **15**, 212–217
18. Baron, D.N. (1985). The kidneys. In: *A Short Textbook of Chemical Pathology*, 4th edition. ELBS Edward Arnold, Great Britain, pp. 207–228
19. Hadley, M.A., Liu, C.Y. and Dym, M. (1981). Effects of gossypol on the reproductive system of male rats. *Int. J. Androl.*, **2**, 190–194

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Resumé

On a injecté à 8 heures par voie intrapéritonéale à 20 rats mâles, divisés en 4 groupes de traitement, 0,1 ml d'une solution aqueuse d'extrait de graine de coton (*Gossypium barbedense Linn*) (Malvacée) en concentrations respectives de (a) 105,25, (b) 21,21, (c) 4,65, et (d) 2,325 mg/ml/kg de poids corporel. On a administré, par rat, à un cinquième groupe (témoins) 0,1 ml d'eau distillée exempte de pyrogène. Dans chaque groupe de traitement, 5 rats ont été sacrifiés respectivement à 2, 8, 24 et 168 heures après le traitement. Aucun changement n'a été constaté en ce qui concernait l'hormone folliculostimulante (FSH) et l'hormone lutéinisante (LH) plasmatiques. La testostérone plasmatique était moins élevée ($p<0,05$) que chez les témoins à 2 et 8 heures, mais était remontée 168 heures après le traitement. La créatinine plasmatique s'était accrue 2 heures après, mais avait retrouvé son niveau d'origine après 8 heures. L'urée plasmatique augmentait progressivement mais de façon persistante jusqu'à une maximum à 168 heures. Les transaminases plasmatiques d'aspartate (AST) et d'alanine (ALT) étaient significativement plus élevées ($p<0,001$) que chez les témoins pendant toute la durée de l'étude. L'histologie testiculaire a révélé une désorganisation précoce des cellules germinales, suivie d'une fibrose progressive (cytosquelette du sperme) après 24 heures. On a constaté des signes de retour à la normale après 168 heures. Cette étude a permis de conclure qu'une solution aqueuse d'extrait de graine de coton contient des substances qui peuvent rapidement entraîner des dégats dans les tissus testiculaires, hépatiques, rénaux et musculaires.

Resumen

Veinte ratas macho adultas por grupo, en 4 grupos de tratamiento, fueron inyectadas por vía intraperitoneal a las 8 horas con 0,1 ml de una solución acuosa de extracto de semillas de algodón (*Gossypium barbedense Linn*) (malvácea) en concentraciones de (a) 105,25, (b) 21,21, (c) 4,65 y (d) 2,325 mg/ml/kg de peso corporal respectivamente. Se administró, por rata, a un quinto grupo (testigos) 0,1 ml de agua destilada libre de pirógeno. En cada grupo de tratamiento, 5 ratas fueron sacrificadas respectivamente a las 2, 8, 24 y 168 horas de la iniciación del tratamiento. No se verificó ningún cambio en lo que respecta a la hormona foliculoestimulante (FSH) y a la hormona luteinizante (LH) plasmáticas. La testosterona plasmática fue menos elevada $p<0,05$ que en los testigos a las 2 y 8 horas, con recuperación a las 168 horas de iniciado el tratamiento. La creatinina plasmática se elevó a las 2 horas, pero recuperó su nivel original al cabo de 8 horas. La urea plasmática aumentó progresiva pero persistentemente a un nivel máximo a las 168 horas. Las transaminasas plasmáticas aspartate (AST) y alanina (ALT) fueron significativamente más altas ($p<0,001$) que en los testigos durante todo el curso del estudio. La histología testicular reveló una desorganización precoz de las células germinales, seguida de una fibrosis progresiva (cistoesqueleto del esperma) al cabo de 24 horas. Se observaron signos de retorno a lo normal al cabo de 168 horas. Se pudo llegar a la conclusión de que el extracto de semillas de algodón contiene sustancias que puedon dañar rápidamente los tejidos testiculares, hepáticos, renales y musculares.