Qatar Univ. Sci. J. (1994), 14 (C) : 68-74

EFFECT OF GOSSYPOL, APOGOSSYPOL AND GOSSYPOLONE ON FATTY ACIDS PATTERN OF LIPID FRACTIONS IN RAT SERUM AND SEMINAL FLUID

By

A. S. EL-SHARAKY, S. M. EL-SEWEDY*, M. M. EL-SAYED** AND I. N. EL-SHAHAWY***
Chemistry Department, Faculty of Science, Qatar University,
*Applied Medicinal Biochemistry Department, Medical Research Institute
**Biochemistry Department, Faculty of Sciennce, Alexandria Univeristy and
***Salt and Soda Co., Kafr Al-Zayat, Egypt

تأثير جوسيبول وأبوجوسيبول وجوسيبولون على نمط الأحماض الدهنية لأقسام الليبيدات في مصل الدم والسائل المنوي للفأر

أحمد صبحي الشراكي و شحاته محمود السويدي
محمد محمود السيد و إبراهيم ناجي الشهاري

دُرست أنماط الأحماض الدهنية لأقسام الليبيدات الكلية في مصل الدم وسائل منوي من ذكر الفأر وذلك تحت تأثير جوسيبول وبعض مشتقات الأيض الخاصة به (أبوجوسيبول وجوسيبولون) . أظهرت النتائج أن الجرعة المستخدمة من جوسيبول وأبوجوسيبول وجوسيبولون تؤثر على أيض بعض مركبات مثل خفض الأحماض الدهنية المشبعة وزيادة معنوية للأحماض الدهنية عديدة الروابط الغير مشبعة .

في مصل الدم زاد معنوياً حمض (ك ٢٠ :٥) في قسم استرالاستيرولات وذلك تحت تأثير أبوجوسيبول . وبينما نقص معنوياً حمض الباليتيك من قسم الأحماض الدهنية الحرة وظهر في نفس الوقت زيادة معنوية للحمض الدهني خماسي الروابط الغير مشبعة (ك ٢٢:٥) بالمعالجة بأبوجوسيبول وجوسيبولون . وفي قسم الفسفوليبيدات الكلية انخفض معنوياً حمض إستياريك (ك ١٨: ٠) نتيجة للمعالجة بواسطة خلات الجوسيبول وجوسيبولون وأبوجوسيبول .

في السائل المنوي زاد أيضاً معنوياً محتوى استرات ستيرولات من حمض (ك ٢٠:٥) بالمعالجة بأبوجوسيبول . وفي أقسام الجلسريدات الثلاثية وقسم الأحماض الدهنية الحرة وأقسام الليبيدات الفسفاتية انخفض معنوياً محتواها من الأحماض الدهنية وحيدة الرابطة الغير مشبعة ، وذلك لمعالجتها بجوسيبول وجوسيبولون حيث ظهرت زيادة معنوية واضحة لحمض اللينوليك في القسم الرئيسي للدهون (جلسريدات ثلاثية) . ويعطي هذا احتمالية أن مسببات العقم من جوسيبول وجوسيبولون لها تأثير معنوي على ن اد وإنزيمات تعتمد على ن اد الذي لايسبب العقم ليس له تأثير على إنتاجية الحيوانات المنوية ولابيئتها من الأحماض الدهنية (أحادية الروابط الغير مشبعة) .

Key Words: Gossypol, Gossypolone and apogossypol, Fatty acids, Serum and seminal fluid lipids.

ABSTRACT

The fatty acids pattern of the total lipid fractions of male rat serum and seminal fluids were studied under the effect of gossypol and some of its metabolites (Apogossypol and gossypolone). The applied dose of gossypol and its derivatives exhibits some metabolic effects such as significant decrease of the saturated fatty acids and a significant increase of polyunsaturated fatty acids. In the serum, eicosapentaenoic acid (C20:5) in sterylester fraction was significantly increased under the effect of apogossypol. While in the free fatty acids fraction, palmitic acid (C16:0) content was significantly decreased, meanwhile significant increase of polyunsaturated fatty acid (C22:5) with apogossypol and gossypolone treatment. Stearic acid (C18:0) was significantly decreased in the total phospholipids fraction due to the treatment with gossypol acetic acid, apogossypol and gossypolone. In the seminal fluid, also eicosapentaenoic acid (C20:5), in sterylester fraction, was significantly increased with apogossypol treatment. In triglycerides, free fatty acids and phospholipids fractions, mono-unsaturated fatty acids were significantly decreased after gossypol and gossypolone treatment. Also, linoleic acid was significantly increased in the main fractions (triglycerides) under the effect of gossypol and gossypolone. Gossypol and gossypolone which induced sterility, may have significant effect on NAD and NADP-dependent enzymes, ATPase and/or have local effect on the germinal cells. Therefore, apogossypol which did not cause sterility, has no effect on the process of spermatogenesis as well as the content of seminal monounsaturated fatty acids.

INTRODUCTION

The reversible antifertility effect of gossypol in males was reported in man and rat[7]. Since then much interest was paid to this yellow phenolic compound extracted from cotton seeds. Several mechanism have been proposed to explain its action, particularly its capability to inhibit a number of enzyme activities; the (Mg^{++}/Ca^{++})-ATPase in sperm[2], the lactate dehydrogenase isoenzymes-X[3], adenylate cyclase[4], the phospholipid-and Ca^{+2} dependent protein kinase from testis[5] and 5-lipoxygenase from rat basophilic leukemia cells and human neutrophils. In addition, the 12-lipoxygenase and prostaglandines synthethase from platelets. The effect of gossypol acetic acid on luteinizing hormone (LH), and testosterone were studied. Gossypol also inhibits luteinizing hormone, 8-bromo-adenosine-3', 5'-monophate-included testosterone formation, and the conversion of pregnenolone to testosterone in isolated rat interestitial cells[6]. On the otherhand, gossypol had a dose-dependent inhibitory action on releasing of testosterone form dispersed mouse Leydig cells[7]. Also, gossypol acetate (implanted in one testis) lead to significantly decreased blood testosterone and LH[8].

The germinal epithelial cells showed vaculisation, pycnosis, disconnections of junctions, cytolysis and excavation of germ cells from the epithelium[9,10]. Also. gossypol cause mild proximal tubule vaculisation similar to that observed in animals on a K^+-free diet and small brownish pigment deposit in a few tubular cells[11]. The local and biochemical changes may be attributed to the effect of gossypol and/or its metabolites. It was suggested that gossypol may be biotransformed to apogossypol and gossypolone[12].

Accordingly, the present study deals with the possible relatioships between the effect of gossypol, apogossypol and gossypolone on the fattya acids pattern of the total lipid fractions of male rat serum and seminal fluids.

MATERIALS AND METHODS

Preparation of gossypol, apogossypol and gossypolone

Gossypol acetic acid was isolated from Egyptian cotton seeds according to the mehtod described early[13]. Apogossypol[14] and gossypolone[15] were prepared from gossypol acetic acid according to the method of Carruth[13] and Lyman et al[14]. The chemical purity was assessed by infrared spectra[14,15], phloroglucinol reaction, thin-layer chromatography and melting points.

Animals and Treatments

Adult male Sprague-Dawley male rats were used for this study. They were housed 8 per cage and maintained on 12 hr light/dark cycle in a temperature controlled room (25 ± 5 °C) and food and water were provided *ad libitum*. Rats were randomly assigned to one of four groups. Group designated as controls, were injected with dimethyl sulphoxide (DMSO) in a dose amount to 1 ml/Kg body weight. Group (GA) were injected with gossypol acetic acid dissolved in DMSO in dose amount to 5 mg/Kg body weight. Group (AG) and group (GN) were treated with apogossypol and gossypolone respectively using the same dose rate as group (GA). Injections were applied intraperitoneally 5 days a week for 5 weeks. Animals of fourth group (GN) showed high toxic features after the second week of injection.

Four rats were sacrificed every week, 48 hours after the last injection. Blood samples were collected by heart puncture. The coda and vas difference from each rat were taken minced in 1 ml Peterson and Freund[16] salt medium filtered through doubled 200 um mesh nylon cloth to remove large membrane fragments. The blood and the seminal fluid were centrifuged at 3000 RPM for 15 minutes. The total lipid of the serum and the seminal fluid were extracted according to the method of Folch et al[17].

Fatty acids analysis was performed by coupling of thin layer chromatography with gas chromatography and the fatty acids were analyzed in the form of methylester[18]. The methylesters were purified by chromatography on silica gel G chromatoplates using petroleum ether (b.p. 60-80 °C) - diethyl ether, 90:10, v/v), using authentic sample of fatty acids methylesters as reference[19].

Gas chromatographic analysis was done using a Perkin-Elmer instrument (3-SIGMA) using glass cloumn (6 ft x 5 mm diameter) packed with DC-200. The detector and injector temperatures were 250 °C and oven temperature was 190 °C. Nitrogen flow rate was 40 ml/minute. Quality of the fatty acids were determined by triangulation[20].

Statistical Analysis

Results are expressed as average value for each group per week and statistically correlated with the duration of experiment with correlation coefficient. The significant variations for the fatty acids of total lipid fraction compared to the control have been calculated with two way analysis (F-test).

RESULTS

Fatty acids identified in the serum total lipid fraction were myristic (C14:0), pentadecanoic (C15:0), dodecylecrylate (15:1), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), linoleic (C18:2), arachidonic (C20:4), eicosapentaenoic acid (C20:5) and docosapentaenoic acid (C22:5). In sterylester fraction, the percent value of the fatty acids as well as their statistical analysis (F-values) were presented in Table 1. Only, eicosapentaenoic acid (C20:5) was significantly increased ($P < 0.05$) under the effect of apogossypol. On the other hand, mono-unsaturated fatty acids decrease and polyunsaturated fatty acids increased under the effect of the compounds (GA, AG and GN) compared to control (DMSO).

Table 1

The two ways analysis (F-test) of fatty acids pattern of the sterylester class for serum of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	0.19	29.47	29.90	29.95	25.90
C14:0	0.66	1.36	1.47	1.90	1.36
C15:0	2.13	6.11	2.07	0.50	2.90
C16:0	0.33	19.10	18.30	19.70	16.38
C18:0	2.46	2.93	8.04	7.83	5.09
Mono-unsaturated fatty acids	1.52	35.50	22.30	27.40	24.40
C15:1	0.95	0.20	0.46	0.47	0.20
C16:1	1.09	3.42	2.11	3.80	2.90
C18:1	1.67	31.89	19.89	22.77	21.01
Poly-unsaturated fatty acids	1.08	35.01	47.74	42.61	49.66
C18:2	1.76	3.40	5.19	5.70	6.40
C20:4	0.11	16.29	13.27	14.73	15.39
C20:5	3.68*	0.00	2.10	4.4*	0.00
C22:5	1.02	15.33	27.18	17.74	27.80

* These values are statistically significant compared to DMSO group ($p<0.05$).

In triglycerides, the drugs have no significant effect on the fatty acids pattern (Table 2). While in the free fraction, palmitic acids (C16:0) was significantly decreased and docospentaenoic acid (C22:5) was significantly increased under the effect of apogossypol and gossypolone (Table 3). Stearic acid (C18:0) in phospholipid classes was significantly decreased under the effect of the compounds (GA, AG and GN) compared to the control (Table 4).

Table 2

The two ways analysis (F-test) of fatty acids pattern of the triglycerides class for the serum of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	0.77	38.54	36.26	43.07	38.30
C14:0	0.72	3.57	2.00	1.66	2.06
C15:0	0.83	3.73	1.44	1.10	2.93
C16:0	0.61	26.67	23.09	28.73	25.64
C18:0	1.37	4.50	9.73	11.60	6.71
Mono-unsaturated fatty acids					
C15:1	0.014	33.44	33.50	34.49	33.93
C16:1	0.97	3.01	0.51	0.74	0.57
C18:1	2.164	2.20	2.47	4.70	2.40
	0.09	28.27	30.50	28.99	31.07
Poly-unsaturated fatty acids	0.45	28.01	30.26	22.40	27.64
C18:2	2.00	6.21	4.24	3.33	7.41
C20:4	1.05	2.40	1.40	1.60	4.10
C20:5	2.38	---	----	3.60	0.66
C22:5	0.67	19.37	23.00	13.87	15.43

Table 3

The two ways analysis (F-test) of fatty acids pattern of the free fatty acids class for the serum of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	3.204*	47.80	46.63	37.47*	41.86
C14:0	0.250	1.77	2.13	1.16	2.06
C15:0	0.990	2.07	2.01	1.01	3.33
C16:0	4.97**	32.34	24.1*	24.1*	25.57*
C18:0	0.45	11.61	9.80	9.80	10.57
Mono-unsaturated fatty acids	0.93	30.90	34.87	31.84	25.97
C15:0	1.35	0.14	0.43	0.71	0.16
C16:1	0.79	2.73	2.49	3.77	2.57
C18:1	1.18	28.10	31.93	27.29	23.17
Poly-unsaturated fatty acids	2.07	21.29	18.49	30.69	32.17
C18:2	0.94	8.66	5.74	5.06	6.14
C22:5	4.75*	10.10	11.25	24.77*	25.66*

*These values are statistically significant comapred to DMSO group ($P < 0.05$).

Table 4

The two ways analysis (F-test) of fatty acids pattern of the phospholipids class for the serum of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	2.64	57.21	48.07	43.31	49.70
C14:0	1.90	1.09	0.57	0.81	1.27
C15:0	0.81	0.71	0.50	0.29	1.61
C16:0	0.27	26.76	25.26	24.30	27.07
C18:0	8.72**	28.65	21.74*	17.9**	19.23*
C20:0	3.93	0.0	0.0	0.0	5.56*
Mono-unsaturated fatty acids	0.94	23.91	22.91	24.23	31.90
C15:1	0.39	0.91	0.87	0.56	0.96
C16:1	1.78	0.80	0.67	0.93	1.97
C18:1	0.84	21.80	21.01	22.89	29.11
Poly-unsaturated fatty acids	2.57	19.26	29.77	32.39	18.43
C18:2	0.80	3.10	3.11	3.91	2.83
C20:4	0.46	8.30	7.13	8.64	6.23
C22:5	1.90	7.84	18.52	19.83	9.37

* These values are statistically significant compared to DMSO group ($P < 0.05$).
** These values are statistically highly significant compared to DMSO group ($P < 0.01$)

The fatty acids pattern for the seminal lipid fractions were presented in Table 5. Myristic acid (C14:0) pentadeconic acid (C15:0), dodecylacrylate (C15:1) palmitic acid (C16:0), palmitomoleic acid (C16:1), stearic acid (C18:0) oleic acid (C18:1), linoleic acid (C18:2), arachidonic acid (C20:4), eicosapentaenic acid (C20:5), docosapentaenoic acid (C22:5) were the major fatty acids.

Table 5

The two ways analysis (F-test) of fatty acids pattern of the sterylester class for seminal fluid of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	0.35	37.60	35.97	34.16	39.20
C14:0	0.27	2.27	2.71	2.40	2.37
C15:0	0.85	1.71	1.47	3.30	3.76
C16:0	0.49	23.00	18.10	20.50	19.80
C18:0	0.67	9.90	13.80	7.50	13.30
Mono-unsaturated fatty acids	1.25	24.81	18.85	17.59	22.14
C15:1	0.37	0.56	0.76	0.33	0.53
C16:1	0.79	2.89	2.60	3.50	3.80
C18:1	1.73	21.39	15.54	13.76	17.80
Poly-unsaturated fatty acids	0.84	37.99	45.17	48.14	38.64
C18:2	0.95	12.60	17.20	7.50	8.90
C20:4	3.35*	0.00	1.46	6.13*	0.00
C22:5	0.40	25.20	26.50	34.04	29.20

* These values are statistically significant compared to DMSO group ($P < 0.05$).

In sterylester fraction, the per cent of fatty acids as well as the statistical analysis (F-value) are presented in Table 5. Only, eicosopentaenoic acid was increased significantly after apogossypol treatment ($P > 0.05$). The fatty acids of the

triglycerides fraction, Table 6, showed highly significant decrease for mono-unsaturated fatty acid during the treatment with gossypol acetic acid and gossypolone compared to DMSO-group. Palmitoleic acid (C16:1) and oleic acid (C18:1) were significantly decreased during both gossypol acetic acid and gossypolone treatment. Only, oleic acid (C18:1) was significantly decreased under the effect of gossypol acetic acid and gossypolone in the free fatty acids fraction Table 7. Also, mono-unsaturated fatty acids, (C15:1) and (C18:1), were significantly decreased in phospholipids fraction under the effect of gossypol acetic acid treatment Table 8.

Table 6

The two ways analysis (F-test) of fatty acids pattern of the triglycerides class for seminal fluid of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	0.34	32.87	36.17	35.40	36.80
C14:0	2.02	2.49	1.77	2.17	2.63
C15:0	0.70	0.57	0.99	1.03	1.87
C16:0	0.11	26.09	26.41	26.87	24.94
C18:0	2.17	3.73	7.00	5.36	7.44
Mono-unsaturated fatty acids	5.48**	51.59	32.22**	46.81	37.03**
C15:1	1.39	0.21	0.44	0.54	0.30
C16:1	6.09**	11.47	4.43**	7.96	7.11**
C18:1	4.05*	39.87	27.34*	38.28	29.60*
Poly-unsaturated fatty acids	2.52	15.54	31.60	17.79	26.20
C18:2	4.08*	2.91	6.14*	2.26	4.76*
C20:4	0.99	0.00	4.51	0.44	0.80
C20:5	0.99	0.66	0.00	2.96	5.05
C22:5	0.99	11.49	19.68	11.67	15.50

* These values are statistically significant compared to DMSO group ($P < 0.05$).
** These values are statistically highly significant compared to DMSI group ($P < 0.01$).

Table 7

The two ways analysis (F-test) of fatty acids pattern of the free fatty acids class for seminal fluid of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	1.48	43.10	35.16*	34.76	45.69
C14:0	3.73*	3.37	2.20*	2.24*	2.37
C15:0	0.41	2.43	2.33	1.73	3.76
C16:0	0.41	27.34	24.13	22.80	19.80
C18:0	1.38	7.84	7.00	7.90	13.30
Mono-unsaturated fatty acids	3.07	25.37	15.43	27.70	22.59
C15:1	0.13	1.41	0.84	1.01	1.44
C16:1	2.17	2.76	1.94	4.86	3.27
C18:1	3.90*	21.20	12.58	21.20	17.90*
Poly-unsaturated fatty acids	2.75	31.50	48.40	37.53	38.64
C18:2	0.85	5.63	6.99	6.53	4.74
C20:4	0.00	1.30	4.97	1.03	3.97
C22:5	1.32	24.60	34.47	29.99	23.00

*These values are statistically significant compared to DMSO group ($P < 0.05$).

Table 8

The two ways analysis (F-test) of fatty acids pattern of the phospholipids class for seminal fluid of male rats

Fatty acids	F-value	Mean value			
		DMSO	GA	AG	GN
Saturated fatty acids	0.89	39.60	35.97	34.16	39.20
C14:0	1.32	1.33	2.71	2.40	2.37
C15:0	0.82	2.41	1.47	3.30	3.76
C16:0	0.37	22.43	18.10	20.50	19.80
C18:0	0.77	13.49	13.80	7.50	13.30
Mono-unsaturated fatty acids	5.97**	17.03	18.85	17.59	22.14
C15:1	4.29*	0.67	0.76	0.33	0.53
C16:1	1.44	1.59	2.60	3.50	3.80
C18:1	5.20**	14.74	15.54	13.76	17.80
Poly-unsaturated fatty acids	2.60	43.37	55.70	42.19	49.57
C18:2	1.85	8.13	11.64	9.61	7.79
C20:4	2.30	1.80	0.00	2.07	0.40
C22:5	0.22	4.40	9.27	4.70	6.04
C22:5	0.96	29.04	34.80	25.37	35.30

* These value are statistically significant compared to DMSO group ($P < 0.05$).
** These values are statistically highly significant compared to DMSI group ($P < 0.01$).

DISCUSSION

The intraperitoneal injection of gossypol acetic acid, apogossypol and gossypolone (5 mg/Kg body weight) into male rats decreased significantly saturated fatty acids, (C16:0) and (C18:0) and a significant increase of the polyunsaturated fatty acid (C22:5) in the serum of male rat. An inverse relationship between the content of palmitic acid and the content of docosapentaenoic acid was statistically identified by apogossypol and gossypolone treatment. Also, mono-unsaturated fatty acids showed marked decrease during the treatment with the drugs. At the same time, stearic acid in serum phospholipids was significantly decreased. The possibility that these agents may decrease the *de novo* synthesis of palmitic acid. This system is found in the soluble (cytosol) fraction of many tissues and its factor requirements include NADPH, ATP, Mn^{++} and HCO_3^-. Accordingly, the treatment may affect the production of NADPH and the activity of acetyl Co-A carboxylase. Therefore, the diffusion of citrate from mitochondria to cytosol leading to elongation of polyenoic fatty acids (C18:2) or (C20:4) forming polyunsaturated fatty acids (C22:5). Gerez de Burgoe et al [21] found that gossypol was powerful inhibitor to α-hydroxylase and malate dehydrogenase (NAD-linked enzymes) and of glutamate, maleic acid and glucose-6-phosphate dehydrogenases (NADP-dependent enzymes). Also, Dou and Fu[22] concluded that gossypol may form an active complex with the enzyme-ATP.

Prostaglandins are hormone-like compounds derived from C20-polyene fatty acid (C20:4) by the activation of fatty acid cyclooxygenase. Linoleic acid (18:2) is chiefly converted into arachidonic acid in the organism. Prostaglandins biosynthesis involves consumption of two molecules of oxygen and requires two molecules of reduced glutathione. The significant increase of polyunsaturated fatty acids in the serum may be due to inhibition of prostaglandins biosynthesis caused by gossypol, aposgossypol and gossypolone treatment. This conclusion agree with the results of others, where they have showed that gossypol inhibits prostaglandins biosynthesis[23,25]. Also, El-Habet el al. [24] found that gossypol acetic acid (5 mg/Kg body weight) decreased liver glutathione content (34%).

In the seminal fluid, the fatty acid pattern for lipid classes showed some changes under the effect of gossypol, apogossypol and gossypolone treatment. In triglycerides, free acids and phospholipids fractions; mono-unsaturated fatty acids (C16:1 and C18:1) were significantly decreased under the effect of gossypol acetic acid and gossypolone (which induced sterility for male rats). The possibility that gossypol and gossypolone may inhibit palmitate biosynthesis in the testis as well as the significant decrease of the saturated fatty acids (C16:0 and C18:0) in blood. This conclusion agree with the results of others[2, 21, 22, 25] concerning the effect of gossypol on ATPase of the testis, NAD-linked enzymes, NADP-dependent enzymes and on enzymes-ATP complex. Linoleic acid (C18:2) was significantly increased in the triglycerides fraction for the seminal fluid lipid under the effect of gossypol acetic acid and gossypolone. The results means that linoleic acid may be acyl-transferred to glycerophosphate instead of being biotransformed to arachidonic acid (C20:4) which inturn is responsible for prostaglandins biosynthesis[23].

Prostaglandins stimulate the secretion of luteinizing hormone (LH) and Luteinizing hormone/follicle stimulating hormone-releasing hormone[26]. On the otherhand, LH is responsible for adenylate cyclase, cyclic-AMP and testosterone formation[27,28]. Therefore, the possibility that; gossypol acetic acid and gossypolone may affect the secretion of LH in the testis leading to decreased adenylate cyclase and testosterone. This conclusion agrees with that of others[4,6]. It may also concluded that the anti-spermatogenic effect of gossypol and gossypolone is mediated within the germinal epithelium rather elsewhere. This condition is in agreement with that reported by others[8-10, 29-31]. The antispermatogenic effect of gossypol acetic acid and gossypolone may be attributed to their significant effect on the enzymes and steroidogenesis via the local effect on the testis. However, apogossypol has metabolic effect on the total lipid of male rat serum, but has no antispermatogenic effect. Therefore, further studies should be done in the future on apogossypol to investigate its possible antispermatogenic effect.

REFERENCES

[1] **Nolional coordinating group on male antifertility agents, 1978**. Gossypol - a new antifertility agent for males. Chinese Med. J., 6: 417.

[2] **Kalla, N. R.** and **M. Vasudev, 1981**. Studies on the male antifertility agent-gossypol acetic acid. II. Effect of gossypol acetic acid on the motility and ATPase activity of human spermatozoa. Andrologia, 13: 95.

[3] **Olgiati, K. L.** and **W. A. Toscans, 1983**. Kinetics of gossypol inhibition of vine lactase. Biochem. Biophys. Res. Commun. 115: 130.

[4] **Olgiati, K. L., A. P. Hoffer** and **W. A. Jr. Toscano, 1984**. Gossypol modulation of nucleotide metabolizing enzyme in the reproductive tract of male rats. Biol. Reprod., 31: 759.

[5] **Kimura, K., K. Sakurada** and **N. Katoh, 1989**. Inhibition by gossypol of phospholipid-sensitive Ca^{+2} dependent protein kinase from pig testis. Biochem. Biophys. Acta. 839: 276.

[6] **Tulin, M. D., P. M. Eisuke, O. Jurnj, R. N. Howard** and **B. C. Patricia, 1981**. Gossypol inhibits testicular steroidogenesis. Fertil. Steril., 35: 563.

[7] **Donaldson, A., S. B. Sufi** and **S. L. Jeffcoate, 1985**. Inhibition by gossypol of testosterone production by mouse Leydig cells *in vitro*. Contraception, 31: 165.

[8] **Balwant, A., H. Frank** and **A. Winston, 1986**. Morphologic and endocrine changes in the

reproductive organs in rats implanted with gossypol acetate pellet in the testis. J. Andrology, 7: 254.

[9] **Xue, S. P., D. C. Liong, P. R. Fei, X. M. Chen, S. J. Ye, Y. Liu, Y. W. Wu, M. M. You** and **X. Y. Guo, 1983**. Subcellular site of antispermatogenic effect of gossypol and its possible molecular mechanism of action. Sci. Sin (B), 26: 612.

[10] **Yu, M. Y. Hu, Z. Liu, C. Zhang, X. Wu, X. Luo, H. Zhou** and **Y. Liu, 1987**. Ultrastructural observation of the effect of gossypol acetic acid on rat testis. Tongji Yike Daxue Xuebao 16: 196.

[11] **Van Ypersele de Strihou, C., J. P. Dieu** and **E. Marbaix, 1988**. Effect of gossypol on the kidney with special emphasis on potassium excretion. Contraception, 31: 173.

[12] **Abou-Donia, M. B.** and **J. W. Dieckert, 1975**. Metabolic fate of gossypol: The metabolism of ^{14}C-gossypol in swine. Toxicol. Applied Pharmacol., 31: 32.

[13] **Carruth, F. E., 1918**. Contribution to the chemistry of gossypol the toxic principle of cottonseed. J. Amer. Chem. Soc., 40: 647.

[14] **Lyman, C. M., J. T. Cronin, M. M. Trant** and **G. V. Odell, 1969**. Metabolism of gossypol in the chick. J. Amer. Oil Chem. Soc. 46: 100.

[15] **Haas, R. H.** and **D. A. Shirly, 1965**. The oxidation of gossypol II. Formation of gossypolone with ferric chloride. J. Organ. Chem., 30: 441.

[16] **Peterson, R. N.** and **M. Freund, 1971**. Factors affecting fructose utilization and lactic acid formation by human semen. The role of glucose and pyruvic acid. Fertil. Steril., 22: 639.

[17] **Folch, J., M. Lee** and **G. H. Sloane-Stanly, 1957**. A simple method for the resolution and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497.

[18] **Radwan, S. S., 1978**. Coupling of two-dimensional thin-layer chromatography and gas chromatography for the quantitative analysis of lipid classes and their constituent fatty acids. J. Chromatogr. Sci., 16: 538.

[19] **Mangold, H. K.** and **D. C. Malins, 1960**. Fractionation of fats, oils, and waxes on thin-layers of silicic acid. J. Amer. Soc., 37: 383.

[20] **Bubzen, J., N. Guichard, J. Labbe, A. Prevot, Sperpinet** and **J. Tranchant, 1969**. In Tranchant J. (ed): Practical manual of gas-liquid chromatography. Elsevier Publishing Company, Amsterdam, London, New York.

[21] **Geies-de-Burgos, N. M., C. Burges, E. E. Moninal, L. E. Rovai** and **A. Blanco, 1984**. Inhibition by gossypol of oxidoreductase from Trypanosoma Cruzi. Biochem. Pharmacol., 33: 955.

[22] **Dou, S.** and **Y. Fu, 1988**. Effects of gossypol acetic acid on heart mitochondrial membranes ATPase. Zhongguo Yaoli Xuebao, 9: 233.

[23] **Hamasaki, Y.** and **H. H. Tai, 1985**. Gossypol a potent inhibitor of arachidonate 5-md 12-lipoxygenases. Biochem. Biophys. Acta, 834: 37.

[24] **El-Habet, A. E., A. S. El-Sharaky** and **S. M. El-Sewedy, 1986**. Effect of gossypol on liver metabolic enzymes in male rats. J. Med. Res. Inst., 7: 163.

[25] **Kalla, N. R., T. W. Foo** and **A. R. Sheth, 1982**. Studies on the male antifertility agent-gossypol acetic acid. V. Effect of gossypol acetic acid on the fertility of male rats. Andrologia, 14: 492.

[26] **Gerold, M. G., 1979**. Chemistry and functions of hormones. II. Pituitary and hypothalamus. In H. A., V. W. Radwell and P. A. Mayes (ed): Review of physiological Chemistry, 17th edition p. 564. Lange Medical Publication, Californai.

[27] **Burger, H. G., D. M. de Kretser** and **B. Hudson, 1976**. Spermatogenesis and its endocrine control. In E. S. E. Hafez (ed.): Human semen and fertility regulation in men. The C. V. Mosby Company, Saint Louis.

[28] **Fronchiment, P.** and **H. G. Burger, 1975**. Human growth hormone and gonadotropins in health and disease. North-Holland Publishing Co., Amesterdam.

[29] **Ravan, E., N. R. Kalla, J. Frick** and **H. Adam, 1984**. Effect of gossypol on bull spermatozoa *in vitro*. Urol. Res., 12: 187.

[30] **Nadakavukaren, M. J., R. H. Sorensen** and **J. N. Tone, 1979**. Effect of gossypol on the ultrastructure of rat spermatozoa cell. Tissue Res., 204: 293.

[31] **Hoffer, A. P., 1983**. Effects of gossypol on the seminiferous epithelium in the rat: A light and electron microscope study. Biol. Reprod., 28: 1007.