Qatar Univ. Sci. J. (1994), 14 (C) : 32-38

THE EFFECT OF GOSSYPOL, GOSSYPOLONE AND APOGOSSYPOL ON THE LIPIDS OF MALE RAT SERUM AND SEMINAL FLUID

By

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تأثير جوسيبول وجوسيبولون وأبوجوسيبول على الليبيدات في مصل الدم والسائل المنوي في ذكور الفئران

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تمت دراسة تأثيرات خلات الجوسيبول وأبوجوسيبول وجوسيبولون على الدهون المتعادلة والدهون القطبية في مصل الدم وسائل المني في ذكور الفئران . أظهرت مجموعة الفسفوليبيدات ومجموع الليبيدات المتعادلة في المصل علاقة تزايدية معنوية ويقابلها علاقة تناقصية معنوية على الترتيب مع زمن المعالجة بخلات الجوسيبول في نفس الوقت زاد معنوياً محتوى الأحماض الدهنية الحرة تحت تأثير الجوسيبول والأبوجوسيبول .

في السائل المنوي – أظهر مجموع الليبيدات الكلية تغيراً غير معنوي بالرغم من وجود علاقة معنوية بين محتواها من الجلسريدات الثلاثية وأسترات الأستيريل . في المجموعة القياسية – أمكن تحديد علاقة تزايدية معنوية بين كمية الجلسريدات الثلاثية وزمن الدراسة يقابلها علاقة تناقصية في كمية أسترات ستيريل لنفس المجموعة . فقط تحت تأثيرات أبوجوسيبول أظهر علامة تناقصية بين أسترات ستيريل وزمن المعالجة . هذه النتيجة يمكن ربطها بالتأثير السلبي لأبوجوسيبول على الخصوية .

بدراسة تأثير خلات الجوسيبول وأبوجوسيبول وجوسيبولون على مكونات الفسفوليبيدات الكلية في مصل دم وسائل المني لذكور الفئران وجد أن فوسفاتيديل الإنيزيتول بالمصل انخفضت كميتها بدرجة معنوية تحت تأثير خلات جوسيبول والجوسيبولون وكانت العلاقة تناقصية معنوية بدرجة كبيرة بين كمية فوسفاتيديل الإنيزيتول في السائل المنوي وزمن المعالجة بخلات الجوسيبول والجوسيبولون .

تشير النتائج إلى أن الجرعات المسببة للعقم من جوسيبول وجوسيبولون تعطي بعض التأثيرات على الأيض من رفع لمعدل تحلل الدهون وبالتالي خفض في معدل بناء فوسفاتيديل أنيزوتول .

Key Words: Gossypol, Apogossypol, Gossypolone, Serum and Seminal fluid lipids.

ABSTRACT

The neutral and polar lipids of serum and seminal fluid of male rats were investigated under the effect of gossypol acetic acid, apogossypol and gossypolone. The total phospholipids and the neutral serum lipids showed positive and negative significant correlation with the duration of gossypol acetic acid treatment, respectively. At the same time, free fatty acids content significantly increased with apogossypol and gossypol acetic acid. In seminal fluids, the total lipid fraction showed insignificant variation in spite of the correlation between triglycerides and sterylester content. In control animals, triglycerides and sterylester contents showed positive and negative correlation, respectively. Only sterylester showed a negative significant correlation with the duration of apogossypol treatment. This result may be related to the negative effect of apogossypol on fertility. The effect of gossypol acetic acid, apogossypol and gossypolone on the total phospholipids constituents of male rat serum and seminal fluid were studied. Phosphatidyl inositol was significantly decreased under the effect of gossypol acetic acid and gossypolone in the serum, and negatively significant correlated with the duration of gossypol acetic acid and gossypolone treatment in the seminal fluid. The results indicate that infertility dose of gossypol and gossypolone exhibit some metabolic effect by elevation of lipolysis and perhaps depression of phospatidyl inositol biosynthesis.

INTRODUCTION

It has been previously reported that gossypol, a yellow pigment isolated from cotton *Gossypium L. Malvaceae* plant seeds, has been used in the People's Republic of China as a male antifertility agent[1]. Gossypol is highly effective in reducing sperm count at the dosage of 5 mg/kg/day (i.p.)[2]. It was suggested that gossypol probably acts directly at the level of the seminiferous tubule to induce oligospermia[1] or at the activity level of some metabolic enzymes[3-5]. Ferguson et. al.[6] found that gossypol markedly reduced succinic dehydrogenase, cytochrome oxidase and xanthine oxidase activity in chicks, while malate dehydrogenase purified from mouse tissues was inactivated with low concentration of gossypol[3]. It was found that gossypol had no effect on succinic dehydrogenase in testis, renal and liver homogenate[7]. Gossypol has no effect on glucose-6-phosphate dehydrogenase activity in male rat, whereas the activity of glucose-6-phosphate dehydrogenase was slightly increased[8]. Others studied the inhibitory effect of gossypol on oxido-reductases. They found that gossypol was a strong inhibitor of alpha-hydroxy acid and malate dehydrogenase, NAD-linked enzyme, and of glutamate dehydrogenase, NADP-dependent enzymes[9]. No informations are available about such effects in human. According to the suggestion of Abou-Donia and Dieckert[10], gossypol is transferred *in vivo* to apogossypol and gossypolone (Fig. 1). We aimed to study the effect of gossypol and its metabolites on the lipid classes of sera and seminal fluid of treated male rats.

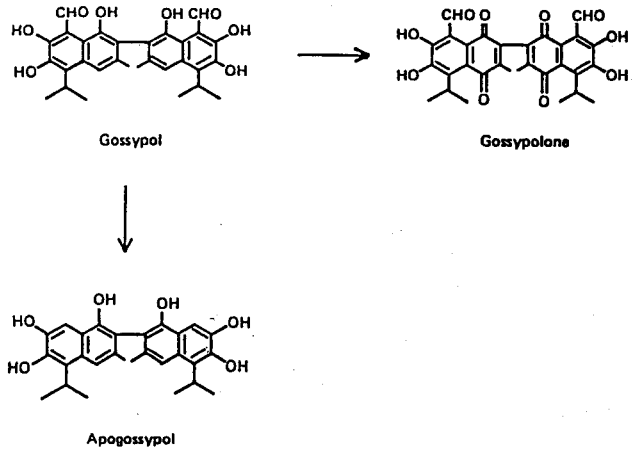

Fig. 1. Suggested gossypol metabolic pathways in the pig liver [10]

MATERIALS AND METHODS

Chemicals

Standard gossypol was obtained from Sigma Chem. Co., St. Louis, USA. Gossypol isolated from Egyptian cotton seeds according to a known method[11]. Apogossypol[12] and gossypolone[13] were prepared from gossypol acetic acid as described previously. Chemical purity was assessed by phloroglucinol reaction, infrared spectra and melting point. Assessments were found in agreement with those reported in the literature.

Animals and treatment

Adult, male Sprague-Dawley rats were used for the study. They were housed 8 per cage and maintained on a 12 hr light/dark cycle in a temperature controlled (25±5°C) room. Food and water were provided *ad Libitum*. Rats were randomly assigned to one of four groups. Group (DMSO), also designated as controls, were injected with dimethyl sulphoxide (1 ml/Kg body weight). Group (GA) were injected with gossypol acetic acid. Group (AG) were treated with apogossypol. Group (GN), gossypolone was applied. All drugs were prepared in DMSO and applied interapritoneally (i.p.) at a dose rate of 5 mg/ml DMSO/kg body weight. Injections were provided 5 days a week for 5 weeks. However, the animals of fourth group (GN) showed toxic features after the second week of injection. Four rats were sacrificed, 48 hours after the last injection, every week. Blood samples were collected by heart puncture. The coda and vas difference from each rat were taken, minced in Peterson and Freund medium[14], filtered through doubled 200 µm nylon mesh to remove large membrane fragments. The blood and seminal fluid centrifuged at 3000 RPM for 15 minutes. The total lipids of serum and seminal fluid were extracted according to the method of Folch et. al[15].

Thin layer chromatography scanner was used for quantitative determination of the lipid classes. Thin-layer chromatoplates were prepared and washed[16]. Aliquots of the total lipids (50-100 µg lipids) were applied as spots on chromoplates. Authentic solutions were applied as single spots on the side of the chromatoplates. The plates were developed with petroleum ether (b. p. 60-80°C)-diethyl ether- acetic acid (80:20:1, v/v/v). After development, air-dried plate was sprayed with 50% aqueous sulfuric acid, heated at 180°C for 30 minutes to complete charring, intensity of the spots were measured with thin-layer scanner (GS-910, Shimadzu) and the fractions content were determined according to the density of the authentic samples. Phosphorus content of different phospholipid fractions

(minor components not included) was determined[17] after TLC separation[18].

Statistical analysis

Results are expressed as average value for each group of rats and statistically correlated with the duration of drug treatment with corelation coefficient. The significant variation for total lipid fractions and the phospholipid components have been calculated by two-way analysis of variance (F-test), compared to control.

RESULTS

Sterylesters, triglycerides, free fatty acids, sterols and total phospholipids were determined quantitatively in the serum of the four studied groups. Table 1 illustrates the results, as well as the correlation coefficient with the duration of treatment. The results showed a significant positive correlation between total phospholipids and the duration of treatment with gossypol acetic acid. At the same time, the sum of neutral lipids showed significant negative correlation with the duration of treatment with gossypol. Statistical analysis of the results, showed insignificant variation between different related groups except free fatty acids class where apogossypol and gossypol acetic acid showed significant increase compared to DMSO group (Table 2). The total lipid content and seminal fluid neutral lipid classes were determined. The results and their correlation coefficients were presented in Table 3. While triglycerides were positively significant correlated in DMSO group ($p<0.05$), they showed insignificant correlation in other groups (GA, AG and GN).

Furthermore, the results showed high significant negative correlation ($P<0.01$) between sterylester and time of apogossypol treatment. The effect of different treatments (GA, AG, GN) on the phospholipid fractions was analyzed statistically, with two-way ANOVA's (F-test). The results showed insignificant variation (Table 4).

The main phospholipid components of serum are phosphatidyl serine, phosphatidyl inositol, sphingomyeline, phosphatidyl choline and phosphatidyl ethanolamine. Phosphatidyl choline represents the major phopholipid component. The total phospholipids were different from the sum of phospholipid components as the minor components were below the detection limits of the method[17]. The results and their correlation coefficients were determined and presented in Table 5. There are insignificant correlation with duration of drugs treatment. Data presented in Table 6 showed that phosphatidyl inositol was significantly decreased in gossypol acetic acid and gossypolone group compared to DMSO-group. Furthermore phosphatidyl ethanolamine showed significant decrease with gossypolone treatment compared to controls.

On the other hand, phosphatidyl inositol, phosphatidyl serine, sphingomyeline, phosphatidyl choline and phosphatidyl ethanolamine were the main phospholipid components of the seminal fluid. The results and their correlation coefficients with the duration of treatment were presented in Table 7. The statistical analysis showed a significantly negative correlation ($P<0.01$) in the gossypol acetic acid group (G) and gossypolone group (GN) between phosphatidyl inositol values and the duration of treatment. Two-way ANOVA'S analysis (F-test) showed insignificant variation between different groups (GA, AG and GN) compared to DMSO group (Table 8).

DISCUSSION

Sprague-Dawely male rats treated with gossypol acetic acid at a dose rate of 5 mg/Kg body weight, showed a positively significant correlation between the total phospholipids and the duration of treatment. At the same time total neutral lipids showed negative significant correlation with duration of treatment. At the same time, total neutral lipids showed negative significant correlation with duration of gossypol treatment. These results were attributed to the negative correlation of the sterylester under the effect of gossypol acetic acid. There is a possibility that gossypol may affect the activity of Lecithin-Cholesterol acyl transferase (LCAT) that catalyzes the transfer of fatty acid residue from second position of lecithin to cholesterol forming cholestrylester.

On the otherhand there was a significant increase ($P<0.05$) in free fatty acids fraction noticed in apogossypol and gossypol acetic acid groups compared to DMSO group. The high level of free fatty acid groups compared to DMSO group may be attributed to one or more of the following factors:

(i) The increase of lipolysis rate over the esterification rate;
(ii) Increasing rate of fatty acids biosynthesis;
(iii) Some hormonal effect on lipolysis;

As the enzymes controlling the lipolysis and reesterification are mobilizing lipase and thiokinase, apogossypol and gossypol may activate mobilizing lipase or inhibit thiokinase enzyme. Thiokinase catalyzes the reesterification accompanied by expenditure on one high energy phosphate bond (ATP). Dou and Fu[19] found that gossypol acetic acid inhibits mitochondrial ATPase. The activity of pyruvate dehydrogenase, acetyl-CoA carboxylase and glycerolphosphate acyl transferase may be decreased as the synthesis of glycerolipids was inhibited by gossypol acetic acid[20]. Gerez de Burgos et al.[9] found that, gossypol was a powerful inhibitor to NADP-dependent enzymes. Accordingly, lipogensis may be inhibited with apogossypol and gossypol acetic acid.

The total lipid of the seminal fluid showed insignificant variation under the effect of the drugs. Yet, the control group (DMSO) showed positively significant correlation ($P<0.05$) between the content of triglycerides and the duration treatment. Also, sterylester of the control showed negative correlation. These results indicate the normal relationship between triglycerides and sterylester content. Only, apogossypol had significantly decreased sterylester content during duration of the treatment. This result, in comparison with controls, explains the fertility effect of apogossypol.

Table 1

The effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on the serum lipid classes of male rats*

Duration of experiment (no. of rats)	Total phospholipids				Sterols				Free fatty acids				Triglycerides				Sterylesters				Total neutral lipid			
	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN
1st week (4)	13.6	15.0	17.4	20.7	4.5	6.7	10.3	8.5	2.4	2.6	11.7	6.0	44.8	37.1	20.6	16.2	33.8	38.4	40.1	48.7	85.5	84.8	82.7	79.4
2nd week (4)	24.2	19.5	22.1	16.8	11.7	9.8	10.2	12.1	8.9	12.0	9.2	7.1	22.4	12.0	25.2	20.6	32.7	46.8	33.5	43.3	75.4	80.6	78.1	83.1
3rd week (4)	49.5	17.8	29.3	25.3	16.1	17.0	17.9	26.9	2.5	19.6	22.6	14.8	4.6	23.5	8.9	8.9	27.3	22.0	21.3	24.2	50.0	82.1	70.7	74.8
4th week (4)	34.2	28.4	28.0	21.3	14.7	16.7	13.5	10.2	9.5	19.0	21.1	21.6	20.8	10.8	14.6	30.1	20.8	25.2	22.8	16.7	65.3	71.7	72.0	78.0
5th week (4)	34.2	30.3	29.1	23.7	14.1	14.5	17.7	20.5	8.8	8.9	13.1	7.2	17.0	22.9	18.6	16.8	25.9	23.3	21.5	31.8	65.8	69.6	70.9	76.3
6th week (3)	23.9	38.9	19.8	35.5	22.4	22.7	12.2	19.7	3.4	2.1	0.9	5.0	24.2	22.5	34.7	5.8	26.1	13.8	32.8	34.0	76.1	61.1	80.1	64.5
7th week (3)	30.6	31.7	22.8	23.8	9.7	15.3	8.3	8.3	4.5	8.3	5.6	6.0	13.2	18.6	11.0	8.8	42.0	26.1	52.3	53.0	69.4	68.3	77.2	76.1
Correlation	+0.24		+0.18		+0.49		-0.05		+0.09		-0.44		-0.49		-0.001		+0.11		+0.23		-0.22		-0.2	
Coefficient		+0.9**		+0.8		+0.73		+0.09		+0.15		-0.15		-0.31		-0.4		-0.71		+0.01		-0.9**		-0.6

* The values are expessed as average percent for each group.
** The values are statistically highly significant (P<0.01).

Table 2.

The two way analysis (F-test) for serum lipid classes of male rats.

Lipid Class	F-value	Percent of total lipids (mean value)**			
		DMSO	GA	AG	GN
Total phospholipids	1.15	30.03	25.94	24.07	23.87
Total neutral lipids	1.26	69.78	74.03	23.87	76.11
Cholesterol	0.66	13.31	14.67	12.87	15.17
Free fatty acids	3.16*	5.7	10.36*	12.03*	9.67
Triglycerides	0.64	21.0	21.06	19.09	15.31
Sterylesters	1.74	29.8	27.94	31.97	35.96

*These values are statistically significant compared to DMSO group ($P < 0.05$).
** The mean value is across all treatment times.

Table 3

The effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on the seminal fluid lipid classes of male rats*

Duration of experiment (no. of rats)	Total phospholipids				Sterols				Free fatty acids				Triglycerides				Sterylesters				Total neutral lipid			
	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN
1st week (4)	12.1	12.4	10.6	27.7	7.2	6.0	3.2	1.9	9.7	3.3	0.4	1.2	31.8	41.0	41.2	21.8	39.1	37.2	44.6	47.3	87.8	87.5	89.4	72.2
2nd week (4)	15.8	22.6	25.6	32.8	10.3	9.1	12.1	9.5	5.5	6.5	9.1	5.0	55.2	51.4	5.0	22.9	13.2	10.3	48.2	29.2	84.2	77.3	74.4	67.3
3rd week (4)	15.3	10.6	10.6	21.2	15.8	7.5	12.5	16.2	10.9	3.7	2.6	18.1	42.3	40.7	44.9	26.9	15.7	38.0	29.3	17.6	84.7	89.9	89.3	78.8
4th week (4)	16.4	33.3	19.4	33.5	13.4	8.6	7.7	9.8	13.4	10.5	11.1	8.8	45.6	32.1	50.3	29.8	11.2	15.2	11.5	18.1	83.6	66.4	80.6	66.5
5th week (4)	22.3	35.9	16.5	18.0	13.6	15.9	16.2	10.5	0.4	0.4	2.5	5.4	49.3	29.0	52.0	52.0	14.5	18.5	12.7	14.0	77.8	63.8	83.6	81.9
6th week (3)	12.8	22.1	53.8	26.7	8.8	10.0	12.1	15.6	1.2	3.7	3.4	9.4	61.6	36.2	6.7	15.3	15.6	28.0	24.1	33.1	87.2	77.9	46.3	73.4
7th week (3)	20.7	18.4	21.3	17.9	5.0	7.2	9.1	7.5	0.6	1.9	5.3	3.5	69.0	39.3	59.0	49.0	4.7	33.5	5.3	22.1	79.3	81.7	78.7	82.4
Correlation	+0.55		+0.49		-0.23		+0.4		-0.66		+0.06		+0.81*		+0.22		-0.71		-0.87**		-0.53		-0.49	
Coefficient		+0.33		-0.53		+0.33		+0.37		-0.3		+0.04		-0.50		+0.5		+0.03		-0.48		-0.33		+0.53

* The values are expessed as average percent for each group.
* The values are statistically significant (P<0.05).
** The value is statistically highly significant (P<0.01).

Table 4

The two way analysis (F-test) for the seminal fluid lipid classes of male rats.

Lipid Class	F-value	Mean value*			
		DMSO	GA	AG	GN
Total phospholipids	1.134	16.69	22.13	22.54	25.40
Total neutral lipids	1.130	83.51	77.79	77.74	74.60
Cholesterol	0.36	10.59	9.19	10.41	10.14
Free fatty acids	0.87	5.96	4.29	4.91	7.34
Triglycerides	2.30	50.69	38.53	37.01	31.10
Sterylesters	1.69	16.29	25.79	25.10	26.01

*The mean value is across all treatment times.

Table 5

The effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on the serum lipid classes of male rats*

Duration of experiment (no. of rats)	Total phospholipids				Phosphatidylserine				Phosphatidylinositol				Sphingomyeline				Phosphatidylcholine				Phosphatidylethanolamine			
	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN
1st week (4)	36.7	36.9	37.0	34.4	2.1	2.4	3.1	2.8	7.0	6.9	6.9	6.3	5.0	5.1	6.0	5.7	12.8	11.1	11.8	13.2	3.7	3.1	3.7	2.6
2nd week (4)	35.6	35.2	35.7	35.2	0.8	3.0	4.2	2.6	6.0	4.6	4.9	4.0	6.3	4.3	5.2	5.3	13.0	13.8	12.9	13.3	5.8	3.5	3.4	4.0
3rd week (4)	44.3	38.8	37.8	35.8	2.9	1.3	2.7	1.8	8.1	5.3	4.0	5.7	7.6	6.2	5.5	6.7	13.9	16.4	13.7	11.5	5.8	4.0	4.9	4.3
4th week (4)	38.7	36.5	36.5	33.9	3.2	2.8	4.3	5.6	4.6	1.4	3.7	3.2	4.3	4.3	4.3	1.4	13.3	15.8	14.7	15.6	6.4	5.7	3.4	1.4
5th week (4)	34.7	38.2	37.1	29.6	2.7	3.9	2.3	1.9	1.8	1.9	5.0	4.5	2.2	6.6	2.9	5.4	14.7	17.3	16.0	9.8	6.8	3.0	4.9	2.9
6th week (3)	38.2	35.5	36.0	34.8	1.0	3.3	3.2	4.1	7.8	4.1	4.0	4.2	6.7	5.0	5.3	5.6	18.5	15.3	13.0	14.0	1.1	0.3	3.6	0.3
7th week (3)	36.5	40.3	34.0	39.4	4.2	3.6	5.1	4.4	7.5	4.5	5.6	4.2	4.6	6.1	4.1	7.2	11.2	16.5	10.2	13.2	3.0	3.3	3.3	1.6
Correlation Coefficient	-0.12	-0.57	+0.42	+0.21	+0.41	+0.28	+0.61	+0.43	-0.04	-0.32	-0.47	-0.52	-0.25	-0.59	-0.4	+0.16	+0.24	-0.09	+0.74	-0.01	-0.39	-0.09	-0.33	-0.63

* The values are expressed as average value (ug phosphorus/mg phospholipid).

Table 6

The two way analysis (F-test) for the serum main phospholipid components of male rats.

Lipid Class	F-value	Percent of total lipids (mean value)**			
		DMSO	GA	AG	GN
Total phospholipids	2.55	37.81	37.34	36.3	34.73
Phosphatidylserine	1.87	2.41	2.91	3.66	3.3
Phosphatidylinositol	3.48	6.11	4.1*	4.87	4.59*
Sphingomeylin	0.34	5.24	5.37	4.76	5.33
Phosphatidylcholine	1.79	13.91	15.17	13.19	12.94
Phosphatidylethanolamine	4.80	4.66	3.27	3.89	2.44*

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

** The mean value is across all treatment times.

Table 7

The effect of gossypol acetic acid (GA) apogossypol (AG) and gossypolone (GN) on the seminal fluid main phospholipids of male rats*

Duration of experiment (no. of rats)	Total phospholipids				Phosphatidylserine				Phosphatidylinositol				Sphingomyeline				Phosphatidylcholine				Phosphatidylethanolamine			
	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN	DMSO	GA	AG	GN
1st week (4)	36.7	35.8	31.6	36.0	4.0	1.1	1.5	3.2	3.3	5.1	2.2	4.5	7.1	8.5	8.0	8.6	10.7	11.2	12.1	11.5	3.5	6.5	5.1	4.5
2nd week (4)	35.9	37.6	35.5	33.4	1.8	3.2	0.9	2.0	4.7	4.6	3.9	3.5	10.0	7.1	10.0	7.3	14.8	12.8	15.1	12.8	2.5	3.5	3.4	2.7
3rd week (4)	33.9	27.2	26.0	36.0	3.5	4.9	3.6	1.1	3.3	3.9	2.9	2.5	7.7	5.6	5.0	9.1	10.9	7.2	6.3	12.0	3.6	2.3	4.5	8.0
4th week (4)	33.0	37.0	35.3	37.0	6.4	9.1	3.0	6.0	0.1	1.6	0.6	0.0	2.4	4.9	4.3	1.2	13.5	10.7	7.6	11.5	3.0	2.6	13.6	12.3
5th week (4)	38.3	39.3	32.1	32.6	4.6	2.5	1.5	3.9	1.1	0.6	1.2	0.5	7.6	12.6	11.5	7.8	9.1	10.9	6.9	10.8	8.7	2.9	6.5	5.6
6th week (3)	34.7	39.7	29.7	34.9	2.3	5.6	3.9	3.0	6.7	0.0	3.7	0.9	3.3	9.1	5.1	7.0	11.5	16.6	11.15	13.1	6.7	0.6	2.3	3.5
7th week (3)	37.5	39.6	37.1	36.8	5.1	6.3	4.7	10.1	7.4	0.0	0.0	0.0	7.3	10.3	8.3	6.1	8.3	11.5	10.2	6.1	3.1	3.2	6.5	2.7
Correlation	+0.58		+0.22		+0.26		+0.72		+0.41		-0.44		-0.37		-0.07		-0.53		-0.30		+0.41		+0.08	
Coefficient		+0.49		+0.14		+0.52		+0.65		-0.97**		-0.88**		+0.47		-0.28		+0.34		-0.55		-0.66		-0.14

* The values are expressed as average values for each group.
**The values are statistically highly significant (P<0.01).

Table 8

The two way analysis (F-test) for the seminal fluid mainphospholipid components of male rats.

Lipid Class	F-value	Percent of total lipids (mean value)**			
		DMSO	GA	AG	GN
Total phospholipids	2.81	35.14	36.6	32.47	35.1
Phosphatidylserine	1.71	3.96	4.67	2.73	4.19
Phosphatidylinositol	1.8	3.8	2.26	2.07	1.7
Sphingomeylin	1.35	6.49	8.3	7.46	6.73
Phosphatidylcholine	0.76	11.26	11.56	9.96	11.11
Phosphatidylethanolamine	1.56	4.44	3.09	5.99	5.61

* The mean value is across all treatment times.

Like neutral lipids, phospholipids also might act as a source of oxidizable fatty acids for spermatozoa[21]. The statistical studies for phospholipid content of male rat serum and seminal fluid showed that gossypol acetic acid and gossypolone, that caused sterility, significantly decreased phosphatidyl inositol. Studies in cell-free extracts have shown that gossypol can inhibit a variety of mitochondrial and cytoplasmic enzymes including glutathione-S-transferase and lipooxygenase[3] Ca^{+2}-dependant protein kinase C[4] phospholipase[2] glycerophosphate acyltransferase[20]. Similar studies on apogossypol and gossypolone are not available in literature. There is a relationship between the sterility effect and the significant decrease of phophatidyl inositol. This possibility is suggested by the significant decrease of the body weight under the effect of gossypol and gossypolone. The deficiency of inositol in experimental animals leads to failure of lactation and growth.

From the forgoing results, it was found that gossypol increases lipolysis and inhibits lipogenesis. However, it participates with gossypolone to affect fertility and it causes a decrease in phosphatidyl inositol content and growth failure. However, biotransformation of gossypol to apogossypol and gossypolone is of a vital importance and needs further extensive investigation.

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