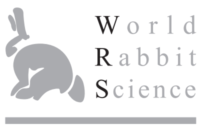

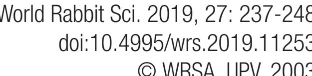

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EFFECTS OF SUBLETHAL DOSES OF GOSSYPOL ON HAEMATOLOGICAL PROPERTIES AND BIOCHEMICAL METABOLITES OF MALE RABBIT

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Abstract: The purpose of this study was to investigate the effects of two sublethal doses of gossypol (GOS) (4 and 20 mg/kg of body weight), administered every other day, on some haematological, biochemical, enzymatic and electrolytic properties and amino and fatty acids in male rabbit blood plasma. The experiment lasted for 16 wk and included two phases: 1) administration period; rabbits were given the experimental doses of GOS for 8 wk; and 2) recovery period; rabbits were allowed 8 wk for complete withdrawal of drugs from the plasma. Results showed that low levels of gossypol increased (P<0.01) haemoglobin, mean corpuscular haemoglobin and white blood cells compared to control. Plasma total protein was increased (P<0.01) by the low GOS dose in both experimental phases. Likewise, glucose concentration was increased (P<0.01) by the high GOS dose during the recovery period. Aspartate aminotransferase and alanine aminotransferase enzymes were increased (P<0.01) by the high dose of GOS treatment only. Low GOS dose increased (P<0.01) blood plasma Na^+ concentration in the recovery period only. Results revealed that total essential amino acids (EAA), and EAA/non-EAA ratio were not affected in a dose-dependent manner during the treatment phase expect for plasma proline, which was increased along with non-EAA (P<0.01) by high GOS dose. Additionally, GOS administration did not affect total unsaturated fatty acids (USFA), total saturated fatty acids (SFA) and SFA/USFA ratio in a dose-dependent manner. In conclusion, Gossypol treatment affected rabbit haematological parameters and biochemical properties of blood plasma in a dose-dependent manner.

Key Words: gossypol, rabbit, haematological parameters, biochemical parameters, amino, fatty acids.

INTRODUCTION

Gossypol (GOS) is a polyphenolic compound found in cottonseed oil (Gadelha *et al.,* 2014). Early studies on GOS focused mainly on how to reduce its toxicity, as it is abundantly present in cotton seeds used for feeding animals. Cottonseed meal is extensively used as a protein source in ruminant feeding, either as a supplement or in formation of the mixed rations in commercial beef and dairy cattle operations (Risco *et al.,* 1992), and in feeds for monogastric animals including pigs, chickens, horses, pets and catfish (Jones, 1991). Gossypol residues remain in cottonseed oil and cottonseed meal after industrial processing (Lin *et al.,* 1994). The estimated average amount of free GOS contained in whole cottonseed was 6000 ppm, in cottonseed meal 400-3000 ppm, in cottonseed hulls 409 ppm and in decorticated cottonseed 12400 ppm (Jones, 1991).

Subsequent studies revealed that GOS possesses many pharmaceutical properties, including antifungal, anti-inflammatory, anti-tumour and anti-fertility activities (Moon *et al.,* 2011). Because of the protein-binding property of GOS, the latter's cytotoxicity may be alleviated when bound by serum protein (Haspel *et al.,* 1984), but the increase in protein concentration in the blood plasma of GOS-treated animals can be considered as a biological response that occurs to counteract its deleterious effect. GOS is highly lipophilic and can interact directly with the phospholipid

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bilayer of biological membranes to alter their structure, electrostatic charge and transmembrane ion fluxes. A major side effect of GOS administration is the change in electrolyte status of serum; reduction in K levels (Liu *et al.,* 1988) that is accompanied by a marked increase in serum Na concentration (Lohiya *et al.,* 1990). Daily ingestion of GOS provokes infertility in various animal species, including man. The contraceptive effect of GOS in human beings was first discovered in China and continues to be tested as a favourable alternative for male contraception (Cui *et al.,* 2004). Signs of GOS toxicosis include decreased growth rate, anorexia, laboured breathing, and dyspnoea (Randel *et al.,* 1992).

Some preventive schemes to minimise or eliminate the toxic effects of GOS have been tested; these schemes include heat treatment of grains (Arieli, 1998), pelleting of the diet and dietary supplementation with ferric sulfate (Soto-Blanco, 2008) or sodium selenite (El-Mokadem *et al.,* 2012). Even though whole cottonseed and cottonseed meal are important sources of protein for ruminants, they contain toxic polyphenolic pigment GOS, but the toxicity could be reduced depending on the detoxification capacity of the rumen (Beradi and Goldblatt, 1980). However, ruminants may exhibit similar pathological syndromes to those in monogastrics if dietary levels of GOS exceed the detoxification capacity of the rumen. Increasing GOS dose resulted in significant reduction in haemoglobin concentration and packed cell volume in calves (Risco *et al.,* 1992; Velasquez-Pereira *et al.,* 1999) and increased erythrocyte fragility in goats (Solamin *et al.,* 2009).

Several reports indicated that gossypol administration resulted in reducing both lysine and histidine concentrations, which was explained by the involvement of lysine (Morris *et al.,* 1986) and histidine (Javed and Waqar, 1995) in the metabolism of GOS. Furthermore, GOS administration hindered the intestinal absorptive function of leucine and alanine (Chadha *et al.,* 1988). On the other hand, GOS displays various promising biological properties including contraceptive (Porat, 1990), anticancer (Shelley *et al.,* 2000; Moon *et al.,* 2008), antiviral (Radloff *et al.,* 1986), and antifungal properties (Przybylski *et al.,* 2009). The objectives of the present study were to evaluate the effects of two sublethal doses of GOS on haematological parameters, biochemical properties, enzymatic and electrolyte contents, and profiles of some free amino and fatty acids in blood plasma of male rabbits.

MATERIALS AND METHODS

Animals and management

This study was carried out at the Department of Animal Production, Faculty of Agriculture, Alexandria University. Experimentation was performed according to standard scientific procedures without compromising animal welfare rights and without any commercial profit purpose. Fifteen adult male New Zealand white rabbits aged 6 to 8 mo and weighing 2.76±0.39 kg at the beginning of the experiment were used during the reproductive season, which starts in September. The rabbits were individually housed in cages. Pelleted food was offered *ad libitum* and water was available excessively. The pellets consisted of (per kg) 330 g of berseem (*Trifolium alexandrinum*) hay, 170 g soybean meal, 165 g ground maize, 160 g barley, 120 g wheat bran, 38 g molasses, 10 g salt, 4 g dibasic calcium phosphate and 3 g vitamins. The chemical analysis according to the Association of Official Analytical Chemists (AOAC, 1995) indicated that the pellets contained (per kg) 175 g of crude protein, 140 g of crude fibre and 27 g of fat. All animals were allowed an adjustment period of days to the experimental environmental conditions before starting the treatment.

Experimental design

Gossypol was extracted from cottonseeds and purified according to Boatner (1948), as described by Taha *et al.* (2006). The rabbits were randomly allotted into three groups each of five animals. Groups were assigned at random to one of the following treatments: 1) control; the animals were given an equivalent GOS free dose of maize oil+acetone; 2) low dose; 1/100 of median lethal dose, 4 mg/kg live weight and 3) high dose; 1/20 of median lethal dose, 20 mg/kg live weight. Doses were orally applied directly into the nasopharyngeal region every other day throughout the administration period using a syringe. The experiment consisted of two phases: 1) drug administration period for 8 wk; and 2) recovery period for another 8 wk without drug administration until complete drug withdrawal from the plasma.

Sample collection and analyses

The daily feed intake and body weight of each animal were recorded weekly throughout the experimental period before access to food or water. Blood samples were collected biweekly from the ear vein of each animal into heparinised vials throughout the experimental period in the morning before access to food or water and were centrifuged at 860 $\times g$ for 20 min. to obtain plasma, which was stored immediately at –20°C until analysis. Haemoglobin (Hb) concentration was determined using the cyanmethemoglobin procedure (Wintrobe, 1965). Red blood cell (RBC) and white blood cells (WBC) were counted on A0 Bright line haemocytometer using light microscope at 400 and 100 × magnifications, respectively. Micro Wintrobe haematocrit tubes and haematocrit centrifuge were used, respectively, to determine the packed cell volume (PCV). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulas proposed by Schalm *et al.* (1975).

Blood plasma total protein (TP) was measured by the Biuret method as described by Armstrong and Carr (1964). Total albumin concentration was determined by the bromocresol green method according to Doumas *et al.* (1977). Glucose was determined according to Barham and Trinder (1972). Blood plasma total lipids were determined as described by Frings *et al.* (1972) and total cholesterol concentration was measured calorimetrically as described by Watson (1960). Transaminase activities; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured calorimetrically as described by Reitman and Frankel (1957). Blood plasma electrolytes (Na^+, K^+ and Ca^{2+}) were determined using commercial kits (Quimica Clinica Aplicada S.A., Ampsta, Spain), then Na^+-K^+ ratios were calculated.

Free amino acid analysis in blood plasma

Free amino acids were extracted according to the method described by Hamilton (1962). One mL sample of blood plasma from pooled samples of each group at the end of each phase was mixed with 50 mg of sulfosalicylic acid and centrifuged at 1000 $\times g$ for 5 min. The supernatant was diluted with citrate buffer of 2.2 pH at a rate of 1:1. Individual free amino acids were then estimated by the method described by Spackman *et al.* (1958) using a Beckman 119 CL amino acids analyser (Spinco Division of Beckman Instruments, Inc., Palo Alto, CA, USA). The response of the amino acids analyser was checked by analysing a standard mixture of 17 commonly occurring amino acids in protein and ammonia, and the recoveries obtained were used to calculate the amounts of amino acids in various samples.

Total fatty acid analysis in blood plasma

Total fatty acids (TFA) of blood plasma were extracted according to the procedure described by Folch *et al.* (1957). Five mL sample of blood plasma from pooled samples of each group at the end of treatment was homogenised with 25 mL chloroform: methanol mixture (2:1, v/v) for 30 min in a 50 mL separation funnel. The lower layer containing chloroform and fats was decanted into a beaker at room temperature to evaporate chloroform. The extraction of the residual upper layer containing methanol and the rest of the sample were roentgenised with another 25 mL of the same solvent mixture and procedure.

Fatty acids methyl esters from blood plasma total lipids were prepared according to the procedure of Radwan (1978). They were then separated by gas liquid chromatography (GLC) using Shimadzu gas chromatograph GC-4CM (PFE) (Shimadzu Seisakusho Ltd., Analytical Instrument Plant, Kyoto, Japan) equipped with a flame ionisation detector (FID) under the following conditions: an analytical glass column (3 m×33 mm) packed with 5% diethylene glycol succinate (DEGS) on 80/100 chrom Q (SUPELCO, Inc., SUPELCO, S.A., Chemin du Lavasson 2, 1196 Gland, Switzerland) was used. Operating temperature was 1808°C for isothermal column and 2708°C for injector and detector. Gas flow rates (mL/min) were: nitrogen 30, hydrogen 1, air 0.5 and Chart speed was 0.5 mm/min.

A standard mixture of fatty acids methyl esters was analysed under identical conditions prior to running the samples. The retention time of the unknown sample of methyl esters was compared with those of the standard. The proportions of methyl esters were calculated by the triangulation method. The quantitative evaluation of chromatograms was based on the area under each peak, calculated from the height multiplied by the width at half height. The area method has been recommended for calculating the percentage of each fatty acid (Kates, 1972; Pomeranz and Meloan, 1978).

Statistical analyses

Data from the two experimental phases were analysed separately using MIXED procedure for repeated measurements of SAS (2004) according to the model:

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + e_{ijk},$$

where; Y_{ijk} = an observation of each trait recorded on animal k; μ = the overall mean; T_i = the fixed effect of the ith treatment (control, low dose, high dose); W_j = the fixed effect of the jth week; $(TW)_{ij}$ = the interaction between treatment and weeks; and e_{ijk} = a random error assumed to be independent normally distributed with mean = 0 and variance = σ^{2e}. Treatment means were compared by least significant difference procedure. Regression analysis was applied to detect the response of the amount of amino acids or fatty acids to the GOS concentration. The variation among the treatments was split to the sum of squares (SS) due to regression and SS deviation from regression. The latter was used to calculate the error variance with 1 degrees of freedom (d.f.) Because of the scarcity error d.f., a significant level of 0.34 was used to test if the regression coefficient "b value" is gretarer than zero by 1 standard deviation. (SAS, 2004).

RESULTS

Effect of level of gossypol on haematological parameters

Effects of GOS on haematological parameters during administration and recovery phases are shown in (Table 1). The biweekly mean values of these parameters are presented in (Figure 1). Low dose of GOS increased (P<0.01) Hb, MCH, MCHC and WBC compared to control, but RBC, PCV and MCV were not affected by administration of either dose of GOS compared to control. Withdrawal of GOS during the recovery phase caused full retrieval of all haematological parameters expect WBC, which decreased (P<0.01) and during the recovery phase of the high dose of GOS administration.

Effect of level of gossypol on biochemical parameters and electrolytes

Means of blood plasma biochemical parameters during administration and recovery phases as influenced by GOS treatments are shown in (Table 2). Treatment with the low dose of GOS increased (P<0.01) plasma TP concentration

Table 1: Mean (±standard error) of haemoglobin concentration (Hb, g/100 mL), red blood cell count (RBCs, $\times10^6$/mm^3), packed cell volume (PCV,%), mean corpuscular volume (MCV, μm^3), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/100 mL) and white blood cell count (WBCs, $\times10^3/mm^3$) concentrations of male rabbits during the gossypol administration and recovery phases.

	Haematological parameters						
Group	Hb	RBCs	PCV	MCV	MCH	MCHC	WBCs
Treatment (T)							
Control	13.58±0.43[a]	5.08±1.11	40.80±1.17	78.26±2.64	26.71±0.95[a]	33.39±1.05[a]	7.68±0.49[a]
GLD	15.42±0.45[b]	7.94±1.06	38.90±1.27	72.84±2.99	30.47±0.98[b]	37.61±1.08[b]	9.80±0.53[b]
GHD	13.30±0.43[a]	5.28±1.14	40.49±1.33	74.63±2.99	26.49±1.03[a]	33.54±1.05[a]	7.17±0.49[a]
Week (W)	**	NS	NS	NS	**	**	**
T x W	**	NS	NS	NS	NS	**	NS
Recovery (R)							
Control	15.54±0.55	5.13±0.13	41.79±0.74	81.20±1.30	30.75±0.92	37.91±1.26	6.34±0.35[b]
GLD	16.54±0.65	5.14±0.13	41.52±0.68	78.90±1.40	31.34±1.06	40.10±1.40	6.52±0.34[b]
GHD	15.30±0.62	5.24±0.14	41.25±0.88	78.79±1.45	29.34±1.07	36.77±1.41	5.18±0.38[a]
Week (W)	NS	NS	NS	*	NS	NS	**
R x W	NS	NS	NS	NS	NS	NS	NS

In both the treatment and recovery groups, n = 5. NS=non-significant; GLD=gossypol low dose (4 mg/kg live weight); GHD=gossypol high dose (20 mg/kg live weight). * P<0.05,** P<0.01. [a,b] means with different letters within columns, within phase, differ significantly (P<0.01).

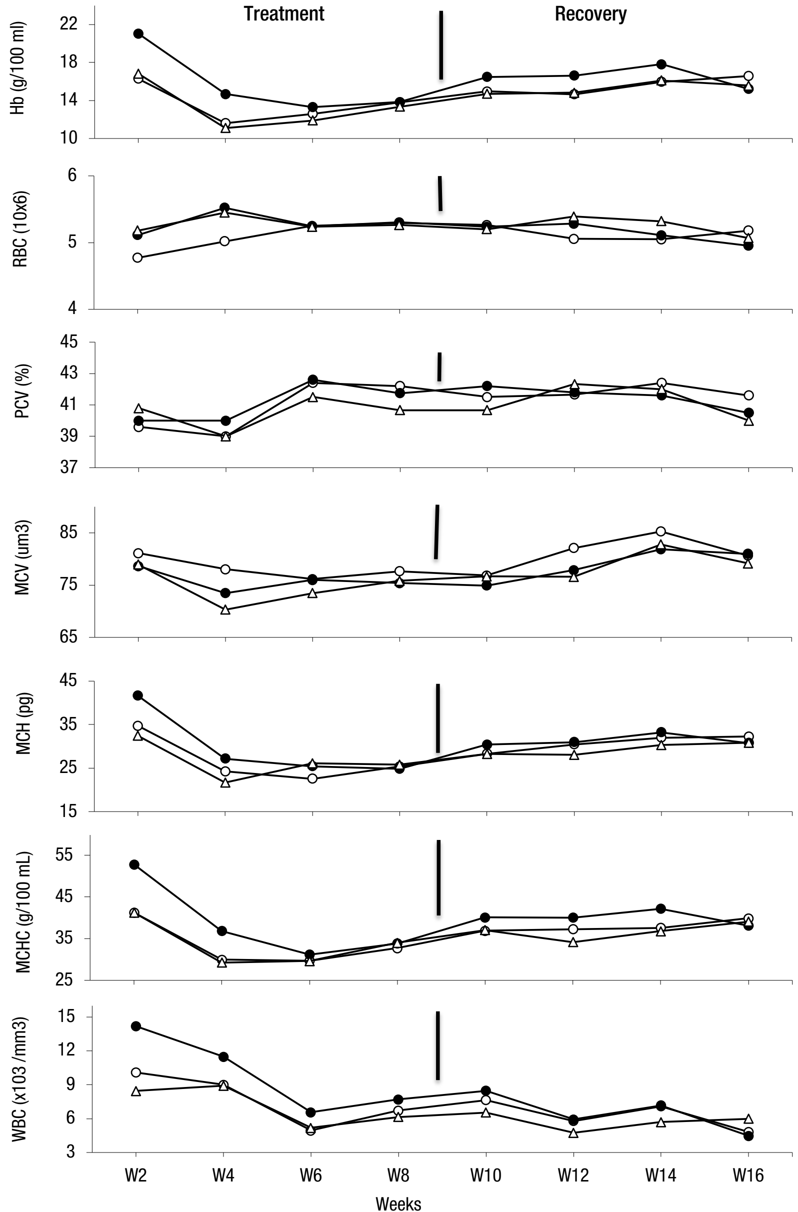

Figure 1: Changes in haemoglobin concentration (Hb), red blood cell count (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell count (WBCs) of male rabbits during gossypol administration and recovery phases. Control: ○; gossypol low dose: ● and gossypol high dose: △.

when compared to control. A similar trend was noted during the recovery phase. However, the concentrations of albumin, total lipids and cholesterol during the administration and recovery phases were not affected by presence of GOS doses compared to control. Treatments with low or high doses of GOS resulted in no difference in glucose

Table 2: Mean (±standard error of mean) of total protein (TP; g/100 mL), albumin (g/100 mL), glucose (mg/100 mL), total lipids (TL; g/L), cholesterol (mg/100 mL), aspartate aminotransferase (AST; U/mL) and alanine aminotransferase (ALT; U/mL) concentrations in blood plasma of male rabbits during the gossypol administration and recovery phases.

	Biochemical parameters						
Group	Total protein	Albumin	Glucose	Total lipids	Cholesterol	AST	ALT
Treatment (T)							
Control	7.78±0.15[a]	5.91±3.06	101.91 ±5.34	1.70±0.93	41.08±2.30	11.10±0.6[a]	15.62±0.95[a]
GLD	8.59±0.18[b]	11.97±3.48	103.70±5.67	3.49±0.95	40.60±2.69	12.57±0.6[a]	17.93±0.98[a]
GHD	7.92±0.17[a]	6.01±3.83	117.14±6.22	1.89±1.00	46.05±2.57	14.84±0.6[b]	20.96±1.06[b]
Week (W)	*	NS	NS	NS	**	**	NS
T x W	NS	NS	NS	NS	NS	NS	NS
Recovery (R):							
Control	7.94±0.16[a]	6.20±0.12	127.92±4.94[a]	1.63±0.09	31.75±1.97	13.47±0.65	16.48±0.86
GLD	8.21±0.15[b]	6.13±0.10	124.25±3.95[a]	1.62±0.07	30.79±1.97	13.39±0.70	16.42±0.84
GHD	7.43±0.20[a]	6.08±0.13	143.61±4.77[b]	1.87±0.09	35.19±2.38	15.36±0.79	18.33±0.94
Week (W)	**	**	NS	**	NS	**	NS
R x W	NS	NS	NS	NS	NS	NS	NS

In all groups, n = 5. NS=non-significant; GLD = gossypol low dose (4 mg/kg live weight); GHD = gossypol high dose (20 mg/kg live weight). * $P<0.05$,** $P<0.01$. [a,b] means with different letters within columns, within phase, differ significantly ($P<0.01$).

concentration during the administration phase. However, plasma glucose under the high dose of GOS increased ($P<0.01$) during the recovery phase compared to other treatments. In addition, treatment with the high dose of GOS increased ($P<0.01$) AST (14.84 U/mL) and ALT (20.96 U/mL) concentrations compared to the control, but withdrawal of GOS during the recovery period resulted in complete retrieval of their normal concentrations.

Mean values of blood plasma Na^+, K^+, and Ca^{2+} and Na^+-K^+ ratio during the administration and recovery phases as influenced by GOS are shown in (Table 3). GOS administration did not affect any of the electrolyte concentrations during the administration of high or low doses of GOS as compared to the control, except Na^+ concentration, which increased ($P<0.01$) under the low dose of GOS during the two experimental phases compared to control.

Table 3: Mean (±standard error of mean) of Na^+, K^+, Na^+-K^+ ratio and Ca^{2+} concentrations in blood plasma of male rabbits during the gossypol administration and recovery phases.

Group	Blood plasma parameters			
	Na^+ (mmol/L)	K^+ (mmol/L)	Na^+- K^+ ratio	Ca^{2+} (mmol/L)
Treatment (T)				
Control	84.66±13.20	3.25±1.64	25.33±3.38	14.77±5.37
GLD	54.49±14.26	7.67±1.77	14.53±3.66	27.08±5.80
GHD	70.20±14.26	3.81±1.64	18.09±3.66	13.94±5.37
Week (W)	**	NS	*	NS
T x W	NS	NS	NS	NS
Recovery (R)				
Control	75.03±1.89[a]	3.92±0.17	19.64±1.08	13.99±0.34
GLD	88.50±1.89[b]	4.10±0.17	21.80±1.08	13.94±0.34
GHD	80.26±2.04[a]	4.13±0.18	19.71±1.17	13.59±0.37
Week (W)	NS	NS	NS	**
R x W	NS	NS	NS	NS

In all groups, n = 5. NS= non-significant; GLD = gossypol low dose (4 mg/kg live weight); GHD = gossypol high dose (20 mg/kg live weight). * $P<0.05$,** $P<0.01$.
[a,b] means with different letters within columns, within phase, differ significantly ($P<0.01$).

Effect of level of gossypol on amino acids and fatty acids

Changes in blood free amino acid concentrations at the end of treatment periods and their regression coefficients on GOS concentration are shown in (Table 4). At the end of the treatment period, all blood plasma concentrations of free amino acids revealed no dependency on GOS dose except proline (b= 0.05509), which revealed positive regression coefficient. In addition, total non-essential amino acids (non-EAA) showed significant positive regression coefficients on GOS concentration at the end of the treatment period. On the other hand, regression coefficients of blood plasma total amino acids (TAA), EAA and (EAA/non-EAA) ratios, on GOS dose were not significant. The individual blood plasma fatty acid concentrations (% of the total peak area) at the end of GOS treatment and their regression coefficients on dose are presented in (Table 5). Blood plasma concentrations of total saturated fatty acids (SFA), total unsaturated fatty acids (USFA) and the (SFA/USFA) ratio showed no significant regression coefficients on the dose of GOS.

DISCUSSION

Effect of level of gossypol on haematological parameters

Gossypol exhibited advantageous effects on haematological parameters expressed as increases of Hb, MCH, MCHC and WBC under low administered dose. Lack of effects of GOS on RBC and WBC counts were observed in goats by Solamin *et al.,* (2009), but the WBC count of the low dose-treated rams was low compared to those treated with a high dose of GOS (El-Mokadem *et al.,* 2013). On the contrary, a toxic effect of GOS on haematological parameters detected by reductions in Hb and PCV was reported previously in goats by Solamin *et al.* (2009) as well as in MCV and MCH, in consistency with that reported by Danke and Tillman (1965). These results are consistent with previous researches dealing with haematological toxicity of GOS in bulls (Wyse *et al.,* 1991) and heifers (Colin-Negrete *et al.,* 1996). However, the contradiction between the current and previous results could be attributed to the increased non-

Table 4: Free amino acid concentrations (mg/100 mL) at the end of treatment period in blood plasma of gossypol male rabbits.GLD = gossypol low dose; GHD = gossypol high dose.

	Treatment			
	Control	GLD	GHD	b
Essential amino acids				
Threonine	11.75	8.347	11.426	0.04341
Methionine	2.554	2.221	2.132	–0.01666
Lysine	3.579	4.576	4.647	0.03941
Valine	3.89	3.401	3.89	0.00873
Isoleucine	2.087	1.789	2.087	0.00532
Leucine	1.902	1.859	2.557	0.03586
Arginine	2.48	2.903	1.366	–0.06723
Histidine	7.401	4.769	6.591	0.00361
Phenylalanine	1.386	0.973	1.266	0.00094643
Non-essential amino acids:				
Glutamic	3.094	3.101	3.743	0.03464
Proline	3.305	3.526	4.407	0.05509**
Tyrosine	2.274	1.941	2.867	0.03771
Serine	5.115	5.193	6.149	0.05400
Glycine	11.439	11.779	12.029	0.02554
Cysteine	0.11	0.329	0.439	0.01371
Aspartic	1.723	2.523	1.827	–0.00871
Alanine	7.071	6.528	8.462	0.08421
Ammonia	2.72	2.805	2.975	0.01214
Total amino acid	71.16	65.758	75.885	0.34959
Essential amino acid	37.029	30.838	35.962	0.05339
Non-essential Amino Acid	34.131	34.92	39.923	0.29620*
Essential/Non-essential	1.08	0.88	0.9	–0.00607

*P<0.05, **P<0.01. b: regression coefficient.

Table 5: Fatty acid concentrations (mg/100 mL) at the end of the gossypol treatment period in blood plasma of male rabbits.

Fatty acids	Treatment			
	Control	GLD	GHD	b
Myristic (C14:0)	2.434	1.863	2.516	0.01459
Palmitic (C16:0)	32.459	25.408	22.368	–0.41468
Palmitoleic (C16:1)	1.623	1.27	2.349	0.04520
Stearic (C18:0)	9.467	8.893	9.395	0.00639
Oleic (C18:1)	17.771	16.938	23.207	0.30609
Linoleic (C18:2)	32.459	40.864	37.075	0.09720
Arachidic (C20:0)	1.623	2.114	1.342	–0.02382
Erucic (C22:1)	2.164	2.647	1.748	–0.03091
Total SFA	45.983	38.278	35.621	–0.41752
Total USFA	54.017	61.719	64.379	0.41757
SFA/USFA ratio	0.85	0.62	0.55	–0.01184

Individual fatty acid concentrations (% of the total peak area) in blood plasma at the end of treatment of male rabbits with gossypol and their regression coefficients. GLD = gossypol low dose; GHD = gossypol high dose; SFA = saturated fatty acids; USFA = unsaturated fatty acids.
b: regression coefficient.

degradable protein intake associated with consuming rations containing high levels of GOS such as cottonseed meal (McDonald *et al.,* 1995). Owing to the protein-binding property of GOS, its cytotoxicity can be diminished (Haspel *et al.,* 1984), resulting in high animal tolerance to concentration of GOS.

The adverse effect of increasing dietary GOS is a result of reduction in Hb formation and PCV (Velasquez-Pereira *et al.,* 1999). Gossypol reacts with iron in the intestine (Muzaffaruddin and Saxena, 1966), forming an insoluble non-absorbed complex excreted in faeces (Herman and Smith, 1973). Iron bonding to GOS makes it unavailable for utilisation and interferes with the normal biosynthesis of Hb (Barraza *et al.,* 1991). It also prevents iron retention, chelates liver and causes iron deficiency (Lindsey *et al.,* 1980).

Effect of level of GOS on biochemical parameters and electrolytes

The present increase in plasma protein concentration of GOS-treated rabbits could be attributed to a biological reaction intended to counteract the cytotoxic effect of GOS. Because of the ability of GOS to cross the blood-testis barrier (Wang *et al.,* 1992), the aldehyde groups present in GOS molecules may bind readily to the free amine groups of the protein molecules to form a Schiff's base (Wu and Reidenberg, 1990). The covalent linkage formed in the Schiff's base could not be separated unless the protein molecule is hydrolysed. Due to the protein-binding property of GOS, its cytotoxicity can be diminished by serum protein (Haspel *et al.,* 1984). So, increasing the protein concentration in GOS-treated animals can be considered a biological response to counteract the cytotoxicity of GOS. This view may explain the present results, in which the blood plasma of GOS-treated rabbits revealed hyperproteinaemia, which was characterised by a proportionate increase in albumin. This finding is partly in agreement with that of Lindsey *et al.* (1980), who reported that the hyperproteinaemia in GOS-fed lactating cows was characterised by a disproportionate increase in plasma globulin. Other studies, however, showed that GOS treatment had no adverse effect on either total protein or albumin concentrations in lambs (Nikokyris *et al.,* 1991).

However, only the present high GOS dose revealed no effect on blood plasma total lipids and cholesterol, which reflected an altered hepatic function detected by the concomitant marked increase in AST and ALT concentrations. Several studies have previously shown that high doses of GOS increase transaminase activity (Braham and Bressani, 1975; Abou-Donia, 1976; Lohiya *et al.,* 1990). However, the marked discrepancy between the effects of low and high doses of GOS on transaminase activities could be explained by several mechanisms. Concerning the high dose of GOS, the increased activity of transaminases was consistent with results elsewhere (Abou-Donia, 1976). This view is supported by the finding of Nomeir and Abou-Donia (1985) that GOS may alter the activity of transaminases by reacting with the substrate and blocking the action of the enzyme and/or by combining with the enzyme itself.

Being highly lipophilic, GOS can interact directly with the phospholipid bilayer of biological membranes to alter their structure (De Peyster *et al.,* 1986), electrostatic charge (Reyes *et al.,* 1986) and transmembrane ion fluxes (Huang and Urthaler, 1986). A major side effect of GOS administration is the change in electrolyte status (Xue, 1985). The high Na^+ retention observed in rabbits treated with low dose of GOS during the recovery period coincided with results of a study conducted on monkeys (Lohiya *et al.,* 1990), but contradicts those of Xue (1985) who reported that a major side effect of GOS administration is the change in electrolyte status hypokalaemia; the reduction in serum K levels (Liu *et al.,* 1988). Enzyme 11β-hydroxysteroid dehydrogenase (11 β-HSD) in the kidney is known to oxidise the active hormone cortisol to the inactive cortisone (Stewart and Krozowski, 1999). Thus, the active cortisol lowers apparent mineralocorticoid activity by inactivating cortisol and leaving aldosterone only as the active mineralocorticoid, whereas inhibition of this enzyme produces the effects of mineralocorticoid excess, hypokalaemia (Stewart *et al.,* 1987) associated with the increase in Na^+. Comparison between the two levels of GOS treatments showed no significant differences in concentrations of plasma glucose. However, plasma glucose under the high dose of GOS increased ($P<0.01$) during the recovery phase compared to other treatments. However, Kanwar *et al.* (1990) and Fornes *et al.* (1993) reported that GOS can induce inhibition of glucose uptake and inhibit glucose 6-phosphate dehydrogenase (Hadley *et al.,* 1981).

Effect of level of GOS on amino acids and fatty acids

Blood plasma free amino acids play an important role in semen quality and quantity. It has been reported that free amino acids may serve as oxidisable substrate for aerobic metabolism by spermatozoa (Mann, 1964), create a favourable condition for nucleic acids synthesis (Setchell *et al.,* 1967) or enhance sperm survival (Tyler and Rothschild, 1951). Additionally, amino acids have been implicated in spermatozoa motility (Gassner and Hopwood, 1952), where the addition of any amino acids and peptides to spermatozoa extends their motility duration (Tyler and Tanabe, 1952).

The function of free amino acids and the factors affecting their contents in rabbit blood plasma have not yet been fully determined. Furthermore, no information is available on comparing the amino acid contents of rabbit blood plasma with other species. Such comparison might provide insight into the biological role of these amino acids in male reproduction. Moreover, studies on effects of GOS administration on the amino acid content of rabbit blood plasma have not been reported. Accordingly, this discussion focuses on some free amino acids of interest and offers some insight into their possible biological effect and their possible imbalance resulting from GOS administration. Taha *et al.,* (2008) reported that GOS administration reduced rabbit seminal plasma concentrations of TAA, total EAA and total non-EAA in a dose-dependent manner, and this was associated with poor-quality semen.

Lipids are a basic component of semen that contribute to the membrane structure of spermatozoa, the metabolism of the sperm cells and their ability to capacitate and fertilise the female gamete (Mann and Lutwak-Mann, 1981). Functional roles of lipids in overall fertility may be related to their general effects on the biophysical properties of membranes, such as fluidity and permeability (Hammerstedt, 1993). The endogenous respiration of spermatozoa depends largely on oxidation of the hydrolysed acyl groups of plasmalogens (Hartree and Mann, 1961); endogenous fatty acids may also be utilised and are readily incorporated into the lipid of spermatozoa (Mills and Scott, 1969). Taha *et al.* (2008) reported that rabbit GOS administration caused decreases in total USFA and increases in SFA and SFA/USFA ratio in a dose-dependent manner and was also associated with poor-quality semen. To our knowledge, no other studies dealing with the effect of GOS on blood plasma concentration of TFA are available.

CONCLUSION

Sublethal doses of GOS induced changes in rabbit haematological properties, expressed as increases in most haematological parameters and rabbit blood plasma TP, glucose, AST, ALT and Na^+ concentrations that were reversible after GOS withdrawal. No effects on albumin, total lipids, cholesterol, K^+, Na^+- K^+ ratio and Ca^{2+} of blood plasma were found. In addition, GOS administration did not affect rabbit blood plasma concentrations of AA in a dose-dependent manner, except for proline and non-EAA, which revealed a positive regression coefficient on dose. On the other hand, the blood plasma individual fatty acid concentrations showed non-significant regression coefficients on GOS dose at the end of treatment.

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