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Aluminium-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: Protective role of ascorbic acid

Mokhtar I. Yousef [a,*], Ahmed M.A. El-Morsy [a], Mervat S. Hassan [b]

[a] *Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, 163 Horreya Avenue, P.O. Box. 832, Alexandria 21526, Egypt*
[b] *Central Laboratory for Food and Feed (CLFF), Agriculture Research Center, Giza, Cairo, Egypt*

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Abstract

Aluminium (Al) has been proposed as an environmental factor that may contribute to some diseases, affect several enzymes and other biomolecules and induced free radical-mediated cytotoxicity. Also, Al induced reproductive toxicity and exerted a significant adverse effect on the steroidogenesis. The antioxidant ascorbic acid (AA) plays an important role in various physiological processes in the body including detoxification of different toxic materials. Therefore, the present investigation aimed to elucidate possible protective effects of AA in alleviating the toxicity of aluminium chloride ($AlCl_3$) on reproductive performance, lipid peroxidation and enzyme activities in seminal plasma of male New Zealand white rabbits. Six rabbits per group were assigned to one of four treatment groups: 0 mg AA and 0 mg $AlCl_3$/kg body weight (BW) (control); 40 mg AA/kg BW; 34 mg $AlCl_3$/kg BW; 34 mg $AlCl_3$ plus 40 mg AA/kg BW. Rabbits were orally administered their respective doses every other day for 16 weeks. Results obtained showed that $AlCl_3$ significantly ($P < 0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased ($P < 0.05$). Live body weight (LBW), feed intake (FI) and relative weights of testes (RTW) and epididymis (REW) were significantly ($P < 0.05$) decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly ($P < 0.05$) increased in seminal plasma of rabbits treated with $AlCl_3$ compared with control. While, activities of glutathione *S*-transferase (GST), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (AcP) were significantly ($P < 0.05$) decreased. Ascorbic acid alone significantly increased LBW, FI, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of free radicals. Also, the present study showed that ascorbic acid might be effective in the protection of aluminium-induced reproductive toxicity. It was suggested that $AlCl_3$ exerted a significant adverse effect on reproductive performance of male rabbits. Furthermore, AA could be able to antagonize the toxic effects of $AlCl_3$ and improved semen quality of male rabbit.

Keywords: Reproductive toxicity; Aluminium chloride; Ascorbic acid; Rabbits; Fertility; Free radicals; Enzymes

* Corresponding author. Tel.: +20 34 29 50 07; fax: +20 34 28 57 92.
E-mail address: mokhtar_yousef@yahoo.com (M.I. Yousef).

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1. Introduction

Aluminium (Al), the third most common element in the earth's crust has a significant toxic potential for humans. Aluminium compounds have many medical implications. They are widely used in antacids, phosphate binders, buffered aspirins, vaccine, antiperspirant and allergen injection (Exley, 1998). Aluminium is ubiquitous and its absorption/accumulation in humans can occur via the diet, drinking water. Aluminium, as aluminium chloride, was found to be embryotoxic and teratogenic when given parenterally to animals (Cranmer et al., 1986). Ingestion of excessive amount of Al leads to accumulation in target organs, which has been associated with damage of testicular tissue of both humans and animals. High concentrations of Al in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability (Dawson et al., 1998). Testicular Al accumulation, necrosis of spermatocytes/spermatids and a significant decrease in fertility were observed in male mice exposed to Al nitrate (Llobet et al., 1995). In addition, the suppressive effects of long-term oral Al chloride in drinking water on both sexual and aggressive behavior, and fertility of male rats were also noted (Bataineh et al., 1998). Guo et al. (2005) found that aluminium administration significantly increased nitric oxide (NO) production and decreased both testicular adenosine 3′,5′-cyclic monophosphate (cAMP) and testosterone levels. They demonstrated that excessive NO products, thus activated inducible NO synthase (NOS), may be involved in reproductive toxicity of aluminium.

Aluminium chloride is able to generate reactive oxygen species (ROS) (Yousef, 2004; Guo et al., 2005). The influence of ROS on fertility has become of increasing interest. In patients with asthenozoospermia, an elevated production of ROS in seminal plasma and increased ROS-mediated damage of sperm membranes has been detected. By altering membrane integrity, ROS may impair sperm motility as well as sperm viability. Therefore, protective agents against ROS may be useful therapeutic agents in the treatment of male infertility (Aitken, 1995).

Ascorbic acid is a water-soluble ROS scavenger with high potency. In human seminal plasma, ascorbic acid concentrations are 10-fold higher than in serum (Jacob et al., 1992). In semen samples exhibiting ROS activity, ascorbate concentrations in the seminal plasma are significantly reduced (Lewis et al., 1997). Moreover, ascorbic acid concentrations in seminal plasma are also positively related to the percentage of morphologically normal spermatozoa, and it has been suggested that ascorbic acid is a protective vitamin in the epididymis (Thiele et al., 1995). Furthermore, it has been shown that ascorbic acid protects human spermatozoa against endogenous oxidative DNA damage (Fraga et al., 1991). For rabbit AA is not essential since rabbits are able to synthesize a certain amount of AA starting from glucose.

Although the knowledge of aluminium toxicity has markedly improved in recent years, information concerning the reproductive toxicity of this element is still limited. Also, role of ascorbic acid against aluminium-induced deteriorations in reproductive performance of rabbits have not so far been studied. Therefore, it is relevant to evaluate reproductive toxicity of $AlCl_3$ and to study the influence of ascorbic acid against its deleterious effects.

2. Materials and methods

In this study, aluminium chloride ($AlCl_3$) and ascorbic acid (Vitamin C, liquid supplement) were used. Aluminium chloride was purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and ascorbic acid (AA, 20%) was purchased from Neofarma, Italy (Via Emilia Km 18, n.1854-47020 Longiano, Fo, Italy). The dose of AA was 40 mg/kg BW every other day.

Male New Zealand white rabbits (age of 7 months and initial weight of 2.871 ± 0.075 kg) were used. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were individually housed in stainless steel cages. Feed and water were provided ad libitum. Rabbits were fed pellets consisted of 30% berseem (*Trifolium alexandrinum*) hay, 25% yellow corn, 26.2% wheat bran, 14% whole soybean meal, 3% molasses, 1% CaCl2, 0.4% NaCl, 0.3% mixture of minerals and vitamins (0.01 g/kg diet of Vitamin E) and 0.1% methionine. The vitamin and mineral premix per kilogram contained the following vitamins: A, 4,000,000 IU; D3, 5000,000 IU; E, 16.7 g; K, 0.67 g; B1, 0.67 g; B2, 2 g; B6, 0.67 g; B12, 0.004 g; B5, 16.7 g; pantothinc acid, 6.67 g; biotein, 0.07 g; folic acid, 1.67 g; choline chloride, 400 g; minerals: Zn, 23.3 g; Mn, 10 g; Fe, 25 g; Cu, 1.67 g; I, 0.25 g; Se, 0.033 g; Mg, 133.4 g (rabbit premix produced by Holland Feed Int. Co.). The chemical analysis of the pellets (AOAC, 1990) showed that they contained 17.5% crude protein, 14.0% crude fiber, 2.7% crude fat and 2200 kcal digestable energy/kg diet.

Twenty-four mature male rabbits were randomly divided into four equal groups of six rabbits each. Group 1 served as control. However, groups 2–4 were given AA (40 mg/kg body weight (BW)), aluminium (34 mg/kg BW, 1/25 LD_{50}) or their combination every other day, respectively. The LD_{50} of aluminium when given orally

to rabbits was reported to be 400 mg/kg BW (Krasovskii et al., 1979). The doses of AA and aluminium were calculated according to the animal's body weight on the week before dosing. The proper doses of AA or aluminium for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oesopharyngeal region. The tested doses for AA and aluminium were given every other day for 16 week.

Daily feed intake and body weight were recorded weekly. Semen collection occurred weekly over the 16 weeks of the study, so 96 ejaculates obtained per treatment. Ejaculates collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. A weak eosin solution was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+ Co., Brandstwiete 4, 2000 Hamburg 11, Germany) (Smith and Mayer, 1955). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma carried out immediately after collection according to Mann (1948). Assessment of live and normal spermatozoa were performed using an eosin–nigrosine blue staining mixture (Blom, 1950). The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperm calculated by multiplying percentage of motile sperm and total sperm outputs. Reaction time for the buck is calculated as the time needed for mounting a doe until complete ejaculation; it measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) recorded. Total functional sperm fraction (TFSF) parameter was also calculated as the product of total sperm output by motility (%) by normal morphology (%) (Correa and Zavos, 1996). All rabbits were slaughtered at the end of the treatment period. The weight of testes and epididymis were recorded in the sacrificed rabbits.

Seminal plasma was obtained by centrifugation of semen samples at 860 × *g* for 20 min at 4 °C, and was stored at −60 °C until analysis. The activities of aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) activities were assayed by the method of Reitman and Frankel (1957). For assaying acid phosphatase (AcP; EC 3.1.3.2) activity, the method of Moss (1984) was used. *p*-Nitrophenyl phosphate is hydrolyzed in acid pH medium by the action of acid phosphatase. Liberated *p*-nitrophenyl is spectrophtometrically quantified. Seminal plasma glutathione *S*-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974), using *para*-nitrobenzylchloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in seminal at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M^{-1} Cm^{-1} was used for calculation (Tappel and Zalkin, 1959).

Data were analyzed as a completely randomized design (Steel and Torrie, 1981) using the general linear model procedure of SAS (1986). Means were statistically compared using least significant difference (LSD) test at 0.05 significance level (Steel and Torrie, 1981). The following model was used:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

where Y_{ijk}, experimental observation; μ, overall mean; a_i, treatment effect; b_j, week effect; ab_{ij}, interaction effect of treatment and week; e_{ijk}, random error.

3. Results

Body weight (BW), feed intake (FI) and relative weight of testes and epididymis were significantly ($P < 0.05$) decreased in rabbits treated with $AlCl_3$ compared to control animals (Table 1). Ascorbic acid (AA) alone caused an increase ($P < 0.05$) in BW, FI and relative weight of testes and epididymis. In addition, the presence of AA with $AlCl_3$ caused increase in the reduction of these parameters (Table 1) and this means that the presence of AA minimized the hazardous effect of aluminium.

Rabbits quickly adapted themselves to semen collection procedure employed. Treatment of rabbits with $AlCl_3$ caused a decrease ($P < 0.05$) in the overall means of semen ejaculate volume (EV), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal sperm, initial fructose and libido (by decreasing the reaction time) compared to control group (Table 2 and Figs. 1–4). While, dead sperm and initial hydrogen ion concentration (pH) were increased in animals treated with $AlCl_3$ compared to control one. On the other hand, treatment with AA alone caused an increase ($P < 0.05$) in EV, sperm concentration, TSO, sperm motility (%), TMS, PSV, TFSF, normal sperm and initial fructose, while caused a decrease ($P < 0.05$) in dead sperm and pH compared to control group. AA also counteracted or

Table 1
The overall means (±S.E.) of body weight (BW), feed intake (FI), and relative testes (RTW) and epididymis weights (REW) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) and/or their combination

Parameter	Groups			
	Control	AA	$AlCl_3$	AA + $AlCl_3$
BW (g)	3207 ± 32^{b}	3407 ± 38^{a}	2561 ± 44^{d}	2969 ± 21^{c}
FI (g/(kg BW day))	50.7 ± 1.20^{b}	57.2 ± 1.40^{a}	40.0 ± 1.69^{d}	47.7 ± 1.27^{c}
RTW (g/100 g BW)	0.15 ± 0.013^{ab}	0.18 ± 0.010^{b}	0.11 ± 0.013^{a}	0.13 ± 0.013^{ab}
REW (g/100 g BW)	0.068 ± 0.009^{ab}	0.081 ± 0.006^{b}	0.046 ± 0.003^{a}	0.059 ± 0.008^{ab}

Within row, means with different superscript letters (a–d) differ significantly ($P < 0.05$).

Table 2
Effect of ascorbic acid (AA), aluminium chloride ($AlCl_3$) and/or their combination on semen characteristics of male rabbits (means ± S.E.)

Parameter	Groups			
	Control	AA	$AlCl_3$	AA + $AlCl_3$
Ejaculate volume (ml)	0.67 ± 0.140^{b}	0.80 ± 0.018^{a}	0.58 ± 0.012^{d}	0.64 ± 0.012^{c}
pH	7.66 ± 0.024^{b}	7.41 ± 0.033^{d}	8.08 ± 0.039^{a}	7.60 ± 0.018^{c}
Reaction time (s)	2.04 ± 0.103^{c}	1.73 ± 0.051^{c}	4.30 ± 0.273^{a}	2.70 ± 0.090^{b}
Packed sperm volume (%)	19.9 ± 0.20^{b}	22.4 ± 0.27^{a}	17.7 ± 0.29^{d}	19.1 ± 0.17^{c}
Sperm concentration ($\times 10^6$ ml^{-1})	236 ± 2.0^{b}	275 ± 4.0^{a}	205 ± 4.2^{c}	231 ± 2.3^{b}
Total sperm output ($\times 10^6$)	159 ± 3.7^{b}	221 ± 6.9^{a}	121 ± 4.3^{d}	148 ± 3.1^{c}
Sperm motility (%)	69.0 ± 0.7^{b}	78.2 ± 1.0^{a}	62.6 ± 1.2^{d}	66.0 ± 0.8^{c}
Total motile sperm ($\times 10^6$)	109 ± 2.7^{b}	175 ± 6.9^{a}	79 ± 3.8^{d}	100 ± 2.3^{c}
Dead sperm (%)	23.0 ± 0.36^{c}	16.2 ± 0.63^{d}	32.4 ± 0.96^{a}	25.6 ± 0.42^{b}
Normal sperm (%)	83.3 ± 0.2^{b}	88.0 ± 0.4^{a}	78.8 ± 0.5^{d}	82.6 ± 0.3^{c}
Total functional sperm fraction ($\times 10^6$)	92 ± 7.2^{b}	156 ± 6.8^{a}	79 ± 3.7^{c}	83 ± 1.9^{c}
Initial fructose (mg/dl)	234 ± 2.3^{b}	263 ± 3.8^{a}	202 ± 4.4^{d}	227 ± 2.2^{c}

The mean value represents 96 values for each treatment. Within row, means with different superscript letters (a–d) differ significantly ($P < 0.05$).

alleviated the harmful effects of $AlCl_3$ on these parameters (Table 2 and Figs. 1–4).

Results indicated that TBARS concentration was significantly increased in seminal plasma of rabbits treated with $AlCl_3$ (Table 3 and Fig. 5). While, the activities of GST, AST, ALT and AcP were significantly ($P < 0.05$) decreased in seminal plasma of rabbits treated with $AlCl_3$ (Table 3 and Figs. 5 and 6). Treatment with ascorbic acid alone caused significant ($P < 0.05$) decrease in seminal plasma TBARS, while GST, AST, ALT and AcP activities were significantly ($P < 0.05$) increased. In addition, the presence of AA with $AlCl_3$ caused reduction in the elevation of seminal plasma TBARS, and maintained the enzyme activities to the normal values compared to control group. This means that the presence of AA minimized the hazardous effect of aluminium.

Table 3
The overall means (±S.E.) of seminal plasma thiobarbituric acid-reactive substances (TBARS), glutathione *S*-transferase, (GST), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (AcP) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) and/or their combination

Parameter	Groups			
	Control	AA	$AlCl_3$	AA + $AlCl_3$
TBARS*	1.11 ± 0.013^{c}	0.93 ± 0.019^{d}	1.32 ± 0.019^{a}	1.13 ± 0.010^{b}
GST**	1.13 ± 0.012^{b}	1.33 ± 0.021^{a}	1.00 ± 0.012^{d}	1.09 ± 0.012^{c}
AST (U/L)	33.0 ± 0.16^{b}	38.0 ± 0.28^{a}	30.0 ± 0.31^{d}	32.0 ± 0.19^{c}
ALT (U/L)	23.0 ± 0.14^{b}	26.0 ± 0.25^{a}	20.2 ± 0.29^{d}	22.0 ± 0.13^{c}
AcP (IU)	32.0 ± 0.15^{b}	37.0 ± 0.41^{a}	29.0 ± 0.32^{d}	31.0 ± 0.13^{c}

The mean value represents 96 values for each treatment. Within row, means with different superscript letters (a–d) differ significantly ($P < 0.05$).
* TBARS is expressed as nmol/ml.
** GST specific activity, μmol/h.

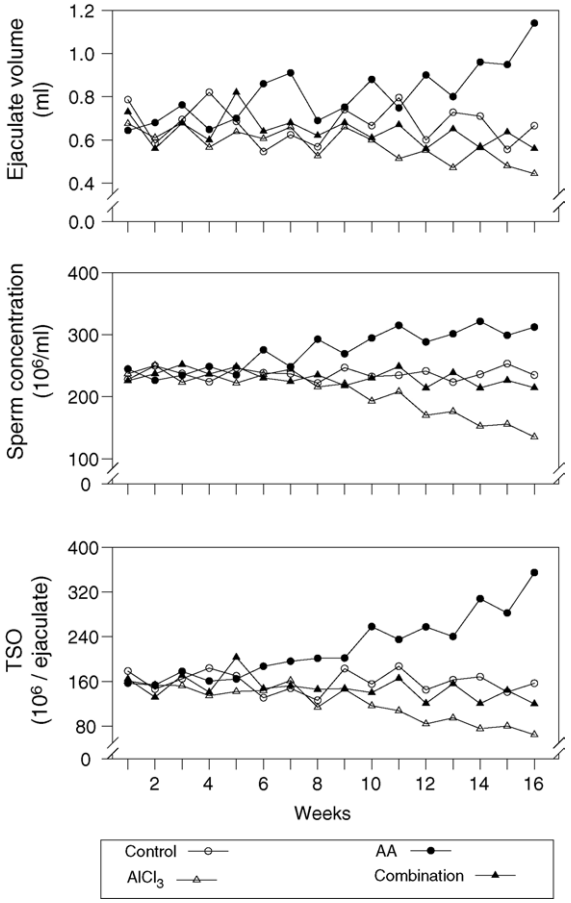

Fig. 1. Changes in ejaculate volume, sperm concentration and total sperm output (TSO) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

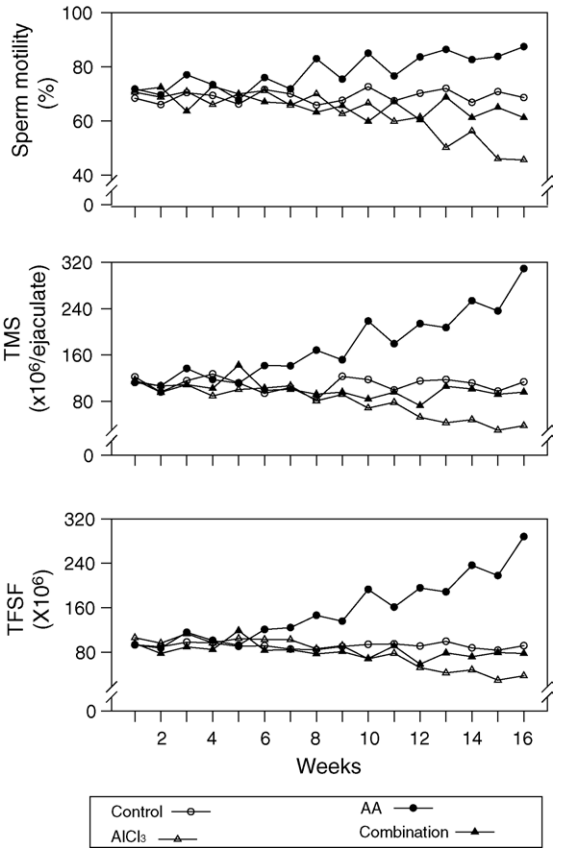

Fig. 2. Changes in motility, total motil sperm (TMS) and total functional sperm fraction (TFSF) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

4. Discussion

4.1. Aluminium

Body weight, feed intake, and relative testes (RTW) and epididymis (REW) weights were reduced by $AlCl_3$ treatment (Table 1). Similar results were obtained by Guo et al. (2001, 2005) and Llobet et al. (1995) in mice and Cherroret et al. (1995) in rats. Cherroret et al. (1995) suggested that the reduction in body weight of treated rats with aluminium chloride (100 mg/(kg day)), could be attributed to the decrease in food consumption, and this is coincided with the obtained results (Table 1).

Although aluminium has been implicated as a factor in a number for pathological disorders, and several hypotheses have also been proposed for its toxicity, the precise mechanisms have not been clearly defined. While, nitric oxide (NO) has been suggested to play multiple roles in aluminium intoxication (Guo et al., 2001, 2005). Studies of Guo et al. (2001, 2005) demonstrated that excessive NO products, thus activated inducible NO syntheses, may be involved in reproductive toxicity in aluminium-treated mice. The present study showed that semen quality (Table 2 and Figs. 1–4) deteriorated following treatment with $AlCl_3$. Also, the gonadotoxic effect of Al was also found. Necrosis of spermatocytes/spermatids was observed in testes of mice exposed to 100 and 200 mg/kg of Al nitrate. Also, significant decreases in testicular and spermatid counts, and epididymal sperm counts were also noted at 200 mg/kg. In addition, testicular function/spermatogenesis was adversely affected (Llobet et al., 1995).

The decline in ejaculate volume, sperm concentration, total sperm output and semen initial fructose concentration (Table 2 and Figs. 1–4) can be partly attributed to the aluminium-induced reduction in testosterone (Guo et al., 2001, 2005). Guo et al. (2001) suggested that Al-induced NO might be a suppressor of testosterone. Also, Dobashi et al. (2001) presented an observation of the inhibition of LH-stimulated steroidogenesis by NO

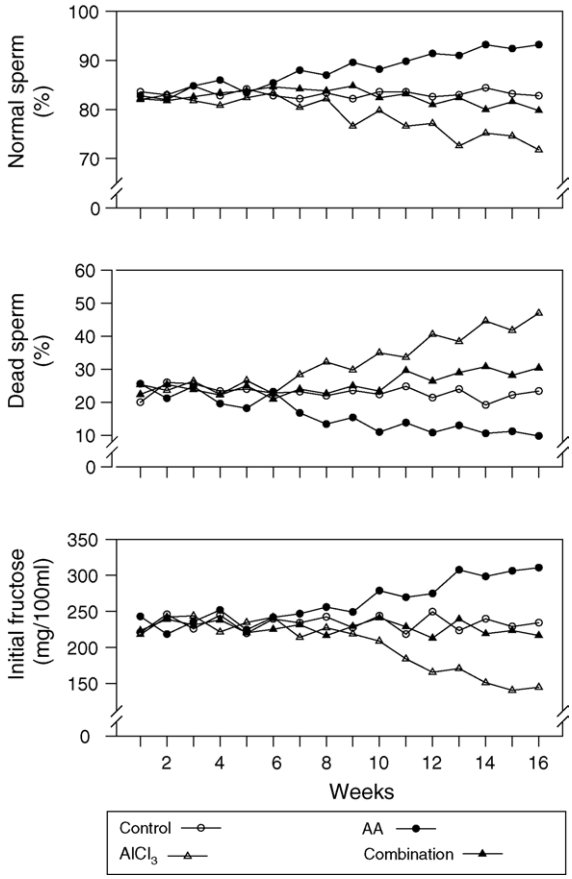

Fig. 3. Changes in normal sperm, dead sperm and initial fructose during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

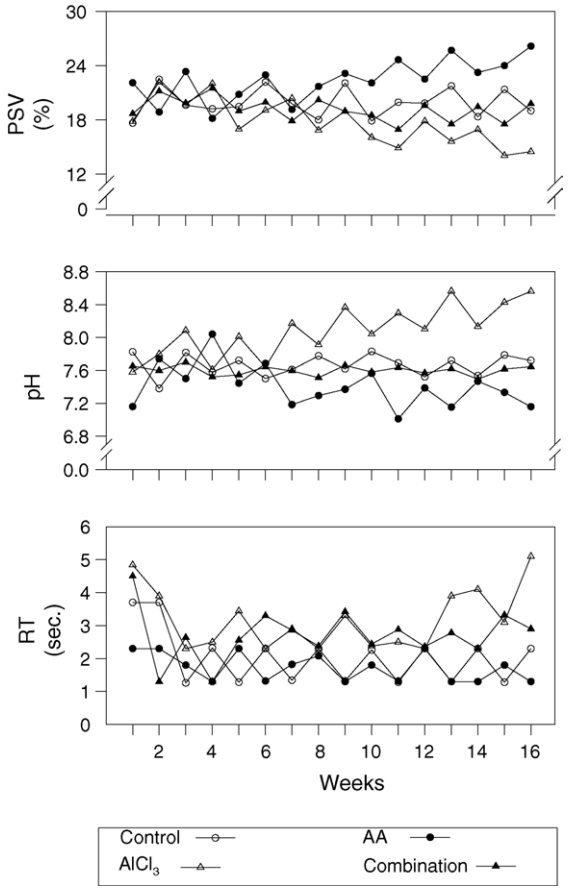

Fig. 4. Changes in packed sperm volum (PSV), initial hydrogen ion (pH) and reaction time (RT) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

in Leydig cells. The stress-induced testicular NO also caused the decrease of steroidogenic enzyme activities (Kostic et al., 2000). The results obtained by Guo et al. (2005) suggested that exposure to Al-induced excessive NO compounds might directly inhibit the main second messenger cAMP that mediates gonadotropin action in the conversion of cholesterol to pregenolone in Leydig cell steroidogenesis, thus less testosterone was produced.

The present study showed that there were decreases in body weight and feed intake in aluminium-treated rabbits (Table 1) (despite the unlimited access to food) and this is coincided with the decline in semen characteristics (Table 2 and Figs. 1–4). Thus, the reduction in both body weight and feed intake (about 20%) would likely be a factor in the deterioration of semen quality. Moreover, our previous study (Yousef, 2004) showed that aluminium chloride was able to generate reactive oxygen species in different tissue organs and blood plasma, also the present study demonstrated that TBARS induced in seminal plasma (Table 3 and Fig. 5). Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999). Thus, the spermatoxic effect of $AlCl_3$ might be due to induced free radicals.

The effect of aluminium chloride on sperm motility parameters and semen fructose was observed in this study (Table 2 and Fig. 2). Also, Dawson et al. (1998) found that high concentrations of Al in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability. Motility is critical in enabling the sperm to ascend the female reproductive tract to the site of fertilization and also is necessary to achieve fertilization (Aitken, 1990). Thus, high quality semen should contain a high percentage of vigorous and active sperms and should have higher glycolytic or fructolytic rates than do weak immobile sperms (Mann, 1964a). Fructose synthesis and secretion by the accessory glands is dependent upon the secretion of testosterone by the testis (Mann, 1964b). The present study showed decline

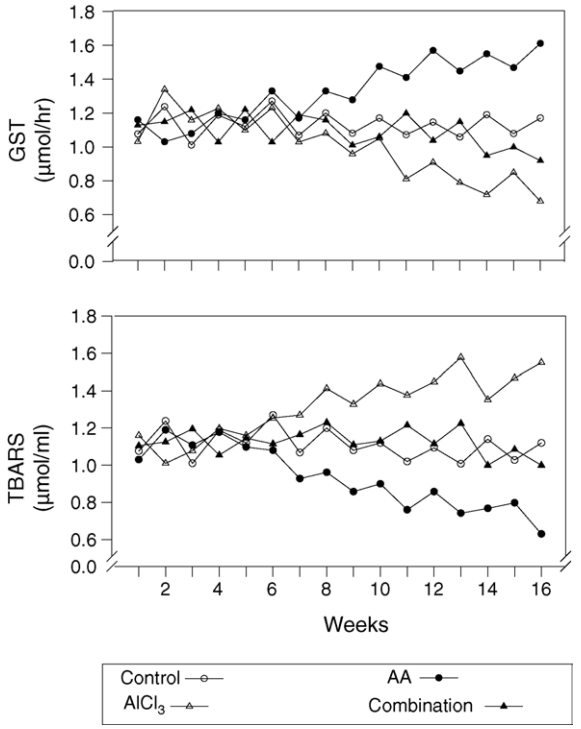

Fig. 5. Changes in seminal plasma glutathione *S*-transferase (GST) and thiobarbrituric acid-reactive substances (TBARS) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

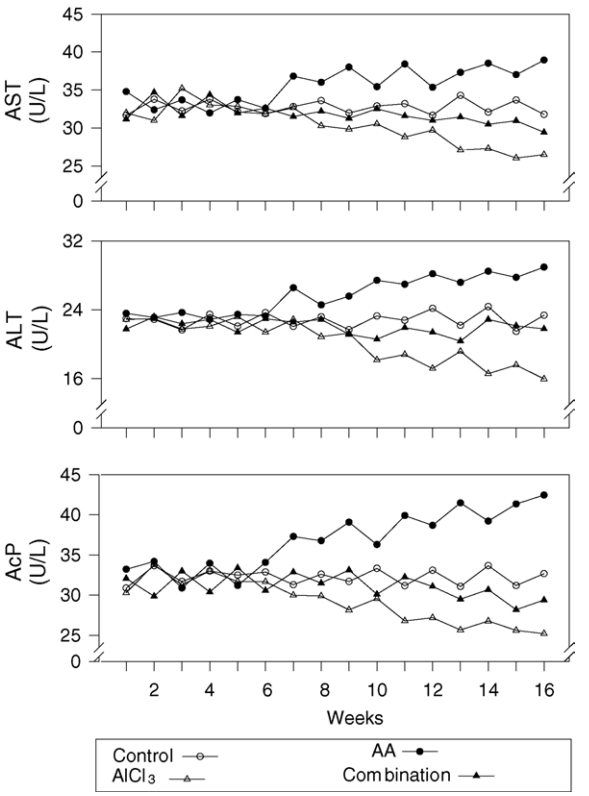

Fig. 6. Changes in seminal plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (AcP) during treatment of male rabbits with ascorbic acid (AA), aluminium chloride ($AlCl_3$) or their combination.

in seminal plasma fructose and increase in pH, and this could be due to the low energetic metabolism of spermatozoa. Thus, the observed decrease in sperm motility could be attributed in part to the concomitant reduction in semen fructose (Table 2 and Fig. 3), the decrease in body weight and feed intake (Table 1), and/or the reduction in testosterone production (Guo et al., 2001, 2005) follows the aluminium treatment. Also, the formation of thiobarbituric acid-reactive substances in seminal plasma was significantly ($P < 0.05$) increased by $AlCl_3$ treatment (Table 3 and Fig. 5). The increase in TBARS can bring negative effects on motility, midpiece abnormalities and sperm–oocyte fusion (Kim and Parthasarathy, 1998).

The production of reactive oxygen species is a normal physiological event in various organs including the testis. But, there is a positive correlation between overproduction of ROS and male infertility (Akiyama, 1999). Sharma and Agarwal (1996) reported that human spermatozoa exhibit a capacity to generate ROS and initiate peroxidation of the unsaturated fatty acids in the sperm plasma membrane, which plays a key role in the etiology of male infertility. Also, the present results showed a significant increase in thiobarbituric acid-reactive substances in seminal plasma of aluminium-treated rabbits (Table 3 and Fig. 5), and this suggests participation of free radicals induced oxidative cell injury in mediating the reproductive toxicity of aluminium.

Sharma and Agarwal (1996) reported that seminal plasma confers some protection against ROS damage because it contains enzymes that scavenge ROS, such as catalase, superoxide dismutase and glutathione *S*-transferase. GSTs are a family of proteins that catalyze the conjugation of glutathione with various electrophils, many of which are toxic. In addition, GSTs bind to a variety of hydrophobic compounds which do not undergo metabolism and binding of these non-substrate ligands to GST results in inhibition of the catalytic activity of GST. GST functions both as an enzyme that catalyzes glutathione conjugation and an intracellular binding protein of various non-substrate ligands (Arita et al., 1998). The present results showed a decrease ($P < 0.05$) in GST activity in seminal plasma of animals treated with (Table 3 and Fig. 5). Thus, the observed increase in free radicals could be attributed in part to the concomitant

reduction of GST activity following aluminium treatment.

The transaminases and phosphatases in semen play an important role in transamination and phosphorylation processes in sperm metabolism (Dhami et al., 1994). The present results revealed a significant ($P < 0.05$) decrease in the activities of seminal plasma AST, ALT and AcP of rabbits treated with $AlCl_3$ (Table 3 and Fig. 6). The decrease in the activities of these enzymes may be due to the decrease in the secretory activity of male accessory sex glands. Dhami et al. (1994) reported that the activities of AST, ALT, AcP, AlP and LDH were lower in static than in motile ejaculates of bulls. Also, our results revealed that the decrease in the activities of seminal plasma enzymes (Table 3) was coincided with the decrease of semen quality of treated rabbits with $AlCl_3$ (Table 2).

4.2. Ascorbic acid

In the present study, 40 mg AA/kg BW was used because the previous studies showed that 20 or 40 mg AA/kg BW afforded comparatively more significant amelioration against pesticides, but the lower dose (10 mg/(kg BW day)) of AA (quantitatively equivalent to the human therapeutic dose according to body weight) was least efficacious (Khan and Sinha, 1996). Also, our pervious study showed that treatment with 20 mg/kg BW every other day against the toxicity of sublethal doses of aflatoxin B_1 (AFB_1) was effective with the low dose of AFB_1 not with the high dose (Yousef et al., 2003a). While, 40 mg/kg BW every other day was effective against the toxicity of aluminium (Yousef, 2004) and stannous chloride (Yousef, 2005). Therefore, in this study we suggest to use 40 mg AA not 20 mg AA/kg BW every other day.

Ascorbic acid increased ($P < 0.05$) BW, FI and relative weight of testes and epididymis and alleviated the negative effects of $AlCl_3$ (Table 1), and this is in accordance with the previous study of Salem et al. (2001), Yousef et al. (2003b) and Yousef (2005) in rabbits.

Ascorbic acid is an essential component in the diet of humans and a small range of other mammals. It has been associated with fertility for many years and may have evolutionary significance (Millar, 1992). Ascorbic acid has three biological actions of particular relevance to reproduction, each dependent on its role as a reducing agent: it is required for the biosynthesis of collagen, steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules. It is frequently involved in mixed-function oxidation, resulting in the incorporation of oxygen from molecular oxygen into a substrate (Luck et al., 1995). Many enzymatic functions of Vitamin C are essential for the normal integrity and function of the testes, i.e. the synthesis, development and maintenance (Dawson et al., 1990). The present study showed that administration of AA caused significant improvements in rabbit sperm characteristics (Figs. 1–4). Such improvements agree with the previously reported benefits of AA supplementation on sperm quality and male fertility (Dawson et al., 1990; Salem et al., 2001; Yousef et al., 2003b; Yousef, 2005). Also, high level of ascorbic acid is necessary for steroid hormone production and possibly for follicle grown and integrity (Luck et al., 1995). According to Luck et al. (1995) steroidogenesis is dependant on ascorbic acid, especially at the hydroxylation step. It is possible that the reason that steroidogenesis is enhanced by ascorbic acid due to its antioxidant properties.

Also, Counsell and Hornig (1986) reported direct effects of ascorbate deficiency on male fertility in laboratory and farm species. Low levels of ascorbate in bull semen were associated with poor breeding performance, while scorbutic guinea pigs experienced degeneration of the testicular germinal epithelium. In rabbits, the gonadal growth-enhancing effects of gonadotropins could be significantly enhanced by simultaneous treatment with ascorbic acid. These studies suggest that ascorbate affects both the integrity of the tubular structure and functionality of sperm (Luck, 1994). At the endocrine level, ascorbate stimulates the secretion of an oxytocin-like peptide by guinea pig Leydig cells, presumably by facilitation of peptide amidation (Kukucka and Misra, 1992). Also, low or deficient ascorbate levels have been associated with low sperm counts, increased numbers of abnormal sperm, reduced motility and agglutination (Dawson et al., 1990). The beneficial effects of ascorbate on sperm (Table 2) may result from the decrease in the levels of TBARS (Table 3), present as a consequence of environmental pollution and cellular metabolism, which would otherwise cause oxidative damage to DNA (Dawson et al., 1990). Dawson et al. (1990) concluded that male fertility in general would be improved by an increased dietary Vitamin C intake.

Castellini et al. (2003) found that Vitamin C inhibited the oxidative processes and imporoved the characteristics of fresh and stored rabbit semen. Also, Yousef et al. (2003b) and Yousef (2004, 2005) showed that AA reduced the formation of radicals in seminal and blood plasma, and different tissues of rabbits. The evidence that ascorbic acid acts as an important antioxidant in many body tissues is convincing (Jacob and Sotoudeh, 2002). Moreover, Sierens et al. (2001) demonstrated that antioxidant species may act in vivo to decrease oxidative

damage to DNA, protein and lipids. This finding suggested that antioxidant AA may be needed to protect sperm against reactive oxygen species. Therefore, the improved in semen quality of rabbits treated with AA (Table 2 and Figs. 1–4) can be attributed to the fact that these compounds are efficient antioxidant, and a scavenger of oxygen free radicals which are toxic biproducts of many metabolic processes (Fran et al., 2000). A second explanation to the obtained results could be that AA either prevent Al absorption in the gut (since AA and $AlCl_3$ were administered simultaneously allowing chemical interactions between them), or facilitate Al excretion through urine. So, AA could cause a decrease in the amount of Al available to reach the testis, and therefore, a lower ROS-mediated damage. Therefore, AA is important in maintaining the physiological integrity of testis, epididymis and accessory glands. Diets deficient in AA were demonstrated to cause the rapid degeneration of the entire reproductive system of male guinea pig (Chinoy et al., 1986). These biological roles of AA in other species may explain the significant improvement in RTW of male rabbits (Table 1).

The present results showed a significant increase ($P < 0.05$) in the activity of GST levels in seminal plasma of animals treated with AA (Table 3 and Fig. 5). Thus, the observed decrease in free radicals could be attributed in part to the concomitant in the induction of GST activity following AA treatment. Also, Yousef et al. (2003b) reported that semenal plasma GST increased with AA administration in rabbits.

Concurrent administration of Vitamin C to $AlCl_3$-treated animals ameliorates the induced sperm quality damage, improves the sperm parameters and reduces the induction of seminal plasma free radicals. Possibly, AA acts by preventing the aluminium-induced oxidative damage to sperm DNA. Even in small amounts AA can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (Carr and Frei, 1999). Two major properties of Vitamin C make it an ideal antioxidant. First are the low one-electron reduction potentials of both ascorbate and its one-electron oxidation product, the ascorbyl radical. These low reduction potentials enable ascorbate and the ascorbyl radical to react with and reduce basically all physiologically relevant radicals and oxidants. The second major property that makes Vitamin C such an effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scavenges a reactive oxygen or nitrogen species (Tsao, 1997).

The present results showed that exposure to aluminium caused deterioration in semen quality, induced free radicals and decreased the enzyme activities in seminal plasma. Thus, the increasing awareness of the dangers associated with aluminium generates more and more concerns of unsafe exposure to human (Dawson et al., 2000; Guo et al., 2001, 2005). Therefore, the present study demonstrates therapeutic effects of ascorbic acid administered in combination with aluminium to minimize its hazardous effects. In addition, ascorbic acid alone had beneficial effect in improving the reproductive performance of male rabbits. Consequently, we have to reduce our exposure to aluminium chloride and pay attention to sources of it in our foods, water and personal products. Also, using diet rich in Vitamin C could be a beneficial way to overcome the toxicity of aluminium.

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