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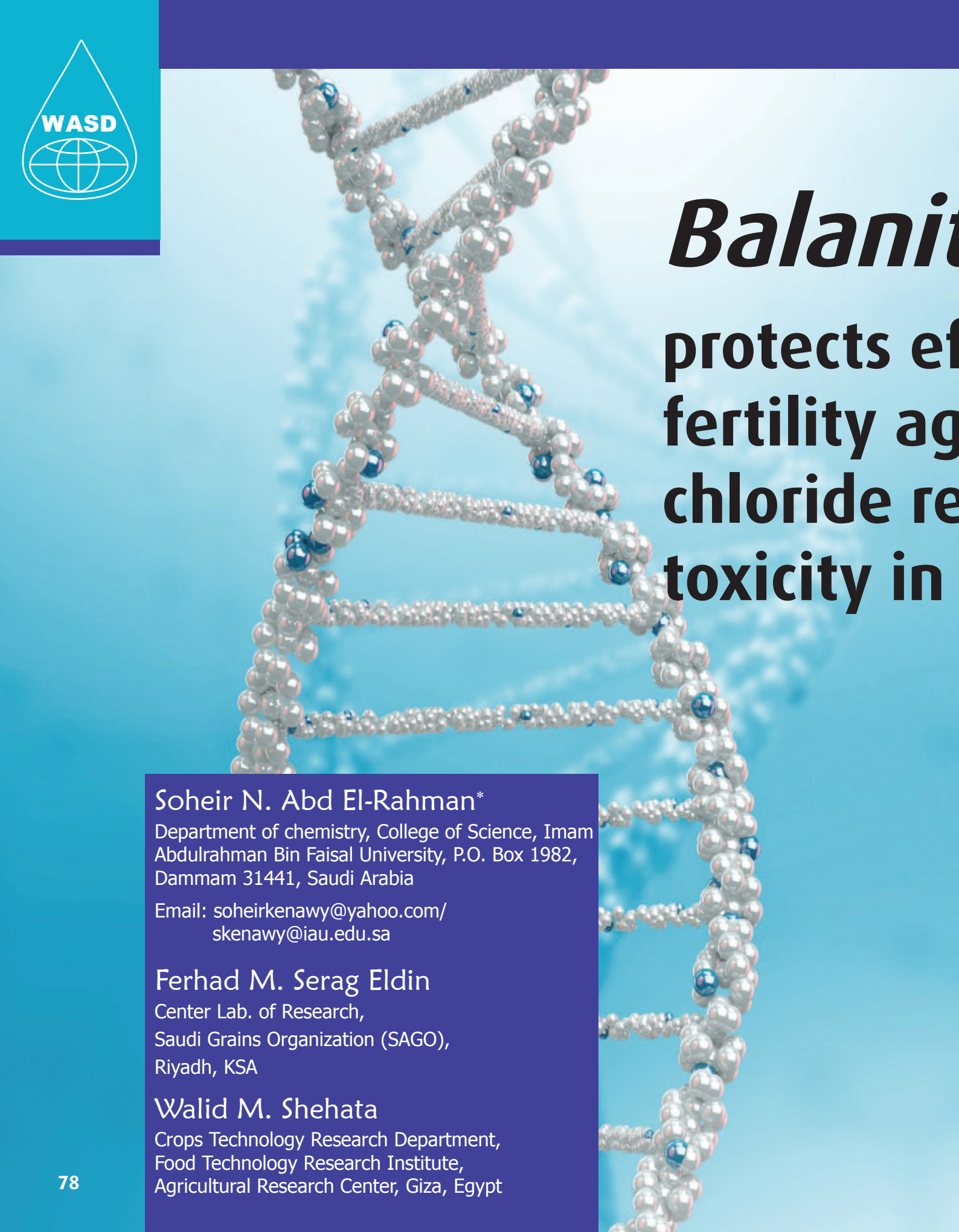

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BACKGROUND

Balanites aegyptiaca contains several steroidal saponins used in the industrial production of sexual hormones.

AIM

The present study aimed to investigate the protective effect of *Balanites aegyptiaca* aqueous extract (BAAE) against reproductive toxicity of aluminium chloride ($AlCl_3$) in male rats.

METHODS

The study was conducted on 50 male Wister rats weighing 191±2g; the animals were divided into 5 equal groups G1: Normal Control (NC) group that received distilled water (dw); G2: positive control (PC) group that received $AlCl_3$; G3: treated group that received $AlCl_3$ + 2ml/kg bw/day of BAAE (5g/100ml dw) orally; G4: treated group that received $AlCl_3$ + 2ml/kg bw/day of BAAE (10g/100ml dw) orally; and G5: treated group that received $AlCl_3$ + 2ml/kg bw/day of BAAE (15g/100ml dw) orally. $AlCl_3$ was administered through drinking water at dose 34mg/kg bw for 70 days.

FINDINGS

$AlCl_3$ decreases sperm motility, sperm count (testicular and epididymal), daily sperm production, fructose in semen, semen quality, and serum LH, estradiol and testosterone content, while follicle-stimulating hormone (FSH) and sperm transit rate were increased. Also, testis showed histological changes, where it induced marked lesions in seminiferous tubules.

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CONCLUSIONS/RECOMMENDATIONS

BAAE at 5%, 10%, 15% were alleviated and controlled the toxic effect of $AlCl_3$. Our results concluded that BAAE has a preventative effect against the reproductive toxicity of $AlCl_3$.

KEYWORDS

Balanites aegyptiaca; reproductive toxicity; aluminium chloride; histological study; male rats

BIOGRAPHY

Soheir Nazmy Abd El-Rahman is a Professor in the Crops Technology Research Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. From 2004 to the present, she has worked in the Department of Chemistry, College of Science, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. She received her PhD in 2000 from the Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt. Her research interests include chronic diseases, food technology and environmental biotechnology. Her specific focus is on natural resources for diabetic, cholesterolemia, fertility, oxidative stress and celiac diseases. She is also working on the natural additives of food, environmental protection, nanoparticles, purification and recycling wastes to use in different industries.

Infertility affects 13–18% of couples, and growing evidence from clinical and epidemiological studies suggests an increasing incidence of male reproductive problems. The pathogenesis of male infertility can be reflected by defective spermatogenesis due to pituitary disorders, testicular cancer, germ cell aplasia, varicocele and environmental factors, or to defective sperm transport due to congenital abnormalities or immunological and neurogenic factors (Iammarrone et al., 2003).

Aluminium compounds have many medical implications, such as, antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants and allergen injection (Exley, 1998). Aluminium absorption/accumulation in humans can occur via the diet, food products, drinking water, ingestion with fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of aluminium in healthy subjects (Venturini-Soriano and Berthon, 2001). Ingestion in excessive amount leads to an accumulation in target organs, and has been associated with damage of testicular tissues of both humans and animals. High concentrations of aluminium in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability (Dawson et al., 1998). Testicular aluminium accumulation, necrosis of spermatocytes/spermatids and a significant decrease in fertility were found in both male mice and rats (Llobet et al., 1995; Sharma et al., 2003; Guo et al., 2005a, b). Aluminium chloride ($AlCl_3$) was found to be embryo toxic and teratogenic when given parenterally to animals (Cranmer et al., 1986). The suppressive effects of long-term oral $AlCl_3$ in drinking water on both sexual and aggressive behaviour and fertility of male rats were also noted (Bataineh et al., 1998).

There is evidence implicating androgenic hormones involved in mechanisms of aluminium toxicity on male reproduction (Sharpe, 1990). Llobet et al. (1995) and Memon and Chinoy (1998) noticed some histological changes, including necrosis of spermatocytes and spermatids in the testis of male mice treated with aluminium nitrate. A single dose of aluminium chloride (400mg/kg body weight) for 15 days of chronic treatment (200mg/kg body weight) for 60 days administered to male mice caused alterations in the metabolism of testis, epididymis, and vas deferens that led to poor sperm motility and a reduction in infertility rate (Chinoy and Bhattacharya, 1997; Chinoy et al., 2005).

Herbs have been used as food and for medicinal purposes for centuries. However, the use of medicinal herbs has increased in recent years. *Balanites aegyptiaca* Del (Zygophyllaceae), popularly called the 'desert date', is a highly drought-tolerant evergreen desert plant species; it is used for various folk medicines in Africa and Asia, fodder and charcoal and pesticides (Hall and Walker, 1991; Mohamed et al., 1999). Also, it is largely used as a component of many popular preparations for its abortive, antiseptic, anti-malarial, antisyphilitic and anti-viral (Herpes zoster) activity (Duke, 1983; Kokwaro, 1976). The bark aqueous extract is traditionally used to prevent jaundice, while the fruit mesocarps is administered as an oral hypoglycemic (Kamel et al., 1991); it also seems to be effective against the *Fasciola gigantic* (Koko et al., 2000). In addition, fruit is used as an antidiabetic in Egyptian folk medicine (Kamel et

al., 1991). Phytochemical investigations on *Balanites aegyptiaca* yielded the isolation of several classes of secondary metabolites, many of which expressed biological activities such as cumarins, flavonoids and steroidal saponins (Sarker et al., 2000). The roots, bark and plant tissues of *Balanites aegyptiaca* tree contains several steroidal saponins (saponins, sapogenins, diosgenins or its isomer yamogenin); these are used as raw material for the industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones (Liu and Nakanishi, 1982; Pettit et al., 1991; ONUDI, 1984).

Two furostanol glycosides and 6-methyl-diosgenin were also obtained from the fruit (Hosny et al., 1992; Kamel, 1998). Diosgenin is generally used as starting material for the partial synthesis of oral contraceptives, sex hormones, and other steroids (Zenk, 1978). The partial synthesis of steroids from plant-based precursors has been a boon because of the increasing demand for corticosteroids, contraceptives, sex hormones, and anabolic steroids since about 1960 (Hall and Walker, 1991). Therefore, the current study aimed to determine the reproductive toxicity of aluminium chloride and evaluate the protective effect of *Balanites aegyptiaca* aqueous extract against the possible testicular dysfunction caused by aluminium chloride.

MATERIALS AND METHODS

Balanites aegyptiaca was obtained from the Agriculture Research Center, Giza, Egypt. Kits of LH, FSH, estradiol and testosterone were obtained from Biodiagnostic Co., 29 El-Tahreer St., Dokki-Giza, Egypt (email: Biodiagonsticeka@Lycos.com).

BIOLOGICAL METHODS

Male albino adult rats (50 animals weighing 191g ± 2g) were obtained from Vaccination Center, Helwan, Giza, Egypt, then transported to the Animal House of Ophthalmology Research Institute, Giza, Egypt. Rats were housed in individual cages with screen bottoms and fed on a basal diet (corn starch 70%, casein 10%, corn seed oil 10%, cellulose 5%, salt mixture 4% and vitamins mixture 1%) for ten days. After equilibration, rats were weighed and divided into five groups (10 animals per group); everyone was assigned to one of the five diet groups:

- G1: Normal Control (NC) group that received distilled water (dw).
- G2: positive control (PC) group that received $AlCl_3$ (34mg/kg bw) in drinking water.
- G3: treated group that received $AlCl_3$ (34mg/kg bw) in drinking water plus 2ml/kg bw/day of BAAE (5g/100ml dw) orally.
- G4: treated group that received $AlCl_3$ (34mg/kg bw) in drinking water plus 2ml/kg bw/day of BAAE (10g/100ml dw) orally.
- G5: treated group that received $AlCl_3$ (34mg/kg bw) in drinking water plus 2ml/kg bw/day of BAAE (15g/100ml dw) orally.

BIOGRAPHY CONT.

Ferhad Mohamed Serag Eldin has been a Quality Expert at The Saudi Grains Organization (SAGO) Riyadh, Saudi Arabia since 20 November 2012. He was a researcher in the Crops Technology Research Department, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt from 1999 to 2012. He received his PhD in 2011 from the Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt. His research interests include chronic diseases, food technology and environmental biotechnology. His specific focus is on natural resources for diabetic, cholesterolemia, fertility, oxidative stress and celiac diseases. He is also working on the natural additives of food, bakery industry, bread improver, environmental protection, purification and recycling wastes to use in different industries.

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Total feed consumption was weighed, fresh feed was provided every day, and total body weight of the animals was recorded at the beginning and during the experimental period. Blood samples were collected from the orbital plexus by means of heparinised capillary glass tubes according to Schermer's (1967) method. Each sample was placed into a dry clean centrifuge tube and centrifuged at 1500xg for 30 min at 4°C to obtain serum.

SERUM HORMONAL ASSAY

The concentrations of serum testosterone were measured according to standard methods (Ekins, 1998); LH and FSH were measured by the method of Uotila et al. (1981), and serum levels of estradiol were measured using Tietz's (1995) method.

SPERM PARAMETERS

Sperm Motility

Sperm motility was recorded and evaluated immediately after tissue isolation. Cauda epididymis was cut into small pieces and transferred to Petri dishes containing pre-warmed nutrition medium (RPMI). Sperm were allowed to swim out within five min at 37°C. The analysis was carried out under a light microscope magnification of 400 fold. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, both motile and non-motile. The sperm cells that were not moving at all were considered to be non-motile, while the rest, which displayed some movement, were considered to be motile using Akdag et al.'s (1999) method.

Sperm Count

Testicular Sperm Count

One testis of each rat was placed in 1ml of phosphate buffer saline immediately after dissection. The tunica albuginea was cut by surgical blades and removed, and the remaining seminiferous tubules were mechanically minced by surgical blades in 1ml of phosphate buffer saline. The testicular cell suspension was pipetted several times to form an homogenous cell suspension. One drop of the suspension was placed on a Makler Counting Chamber and the testicular sperm concentration was determined under a phase contrast microscope at 200x magnification and expressed as million sperm cells per ml of suspension using Uzun et al.'s (2009) method.

Epididymal Sperm Count

The left testis was decapsulated and the left epididymis was divided into two portions (head and body plus tail). Each part was homogenised in saline Triton merthiolate solution (STM solution: 17.5g NaCl, 1mlTriton X-100, and 0.2g sodium ethyl mercuri thiosalicylate were dissolved in distilled water for 1 litre of STM solution) with a Waring blender (Polytron, Kinematica, Littau/Lucerne, Switzerland). Homogenisation resistant spermatids or sperm were then counted using a hemocytometer using Omura et al.'s (1996) method.

Daily Sperm Production

After removing the tunica albuginea, both testes were minced and homogenised in 10ml of 0.9% NaCl containing 0.5% Triton X-100 at medium speed in a POTTER'S® tissuemiser for 1 min. After dilution, the number of homogenisation-resistant spermatids was counted in a hemocytometer (Bürker, Germany). The number of homogenisation resistant spermatids obtained by summing the scores of the right and left testes, was divided by 6.1, the number of days these spermatids were present in the seminiferous epithelium, to convert them to daily sperm production per testis (Robb et al., 1978).

Sperm Transit Rate

The epididymal sperm transit rate was estimated for each male rat by dividing the epididymal sperm number by the daily sperm production (Amann, 1982).

Determination of Fructose in Semen and Semen Quality

Fructose concentration in the seminal vesicle was determined using Foreman et al.'s (1973) method; semen quality was determined using Reddy and Bordekar's (1999) method.

Preparation of Histopathological Tissues

The testis were cut into small pieces about 1mm thick. The tissues were immersed for 3 to 5 mins in cold (4°C) phosphate buffered, then fixed in 2% Para formaldehyde and 2% glutaraldehyde in 0.02M phosphate 0 buffer, and then after placed in fresh cold fixative for 24 hrs in a refrigerator. The tissue was rinsed in cold phosphate buffer (pH 7.4) and post fixed in 2% aqueous osmium tetroxide for 2 hrs at 4°C. The samples were then washed in 0.1M phosphate buffer (twice), then dehydrated in ethanol series and embedded in epoxy resin. Semithin sections (1um) were cut by RMC ultratome, stained with 0.5% toludine blue in borax and examined under a light microscope.

Statistical Analysis of Data

Data collected from the biological evaluation were statistically analysed using one-way ANOVA with a *post hoc* Newman Keuls test: $p \leq 0.05$ was considered significant. All data were expressed as mean ±S.D. LSD was used to compare the significant differences between means of treatment (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Food and nutritional security are key issues for human wellbeing. Fentahun and Hager (2009) reported that wild edible fruit contribute to nutrition and health security of rural people as they contain proteins, vitamins and minerals. Feyssa et al. (2015) studied *Balanites aegyptiaca* as a multipurpose species in semi-arid areas. They found that fruit is rich in P, Ca, Fe, Zn, Cu, Na, K, Mg and Mn. The mean calculated energy value of lipids ranged from 0.09–0.27kcal to 4.2–7.68 for *B. aegyptiaca,* and the total energy from carbohydrates was 342.2–354.24kcal. Therefore, the fruit of *B. aegyptiaca* is promising for the diversification of human's diets in terms of

TABLE 1 Relative Weights of Testis and Epididymis of Male Rats Treated with $AlCl_3$ and $AlCl_3$ + BAAE (5, 10 and 15%) for 70 Days

Treatments	Testis weight / 1g of body weight	Epididymis weight / 1g of body weight
G1 (NC)	$0.0105^{ab} \pm 0.0004$	$0.0092^{a} \pm 0.0011$
G2 (PC)	$0.0089^{a} \pm 0.0040$	$0.0145^{b} \pm 0.0009$
G3: $AlCl_3$ + BAAE 5%	$0.0123^{b} \pm 0.0014$	$0.0118^{c} \pm 0.0011$
G4: $AlCl_3$ + BAAE 10%	$0.0117^{b} \pm 0.0015$	$0.0109^{c} \pm 0.0010$
G5: $AlCl_3$ + BAAE 15%	$0.0120^{b} \pm 0.0014$	$0.0108^{c} \pm 0.0016$
L.S.D. at 5%	0.0024	0.0015

Each value is mean ± SD for 10 rats in each group. Significantly different from controls ($p \leq 0.05$) by ANOVA multiple rage test.

TABLE 2

Serum LH, FSH, Estradiol and Testosterone of Male Rats Treated with $AlCl_3$ and $AlCl_3$ + BAAE (5, 10 and 15%) for 70 days

Treatments	LH (IU/L)	FSH (IU/L)	Estradiol (pg/ml)	Testosterone (ng/dl)
G1 (NC)	$6.51^{(a)} \pm 0.517$	$7.88^{(a)} \pm 0.869$	$39^{(a)} \pm 4.163$	$462^{(a)} \pm 49.809$
G2 (PC)	$0.81^{(b)} \pm 0.061$	$15.01^{(b)} \pm 1.703$	$6.99^{(b)} \pm 0.798$	$201^{(b)} \pm 21.975$
G3: $AlCl_3$ + BAAE 5%	$6.71^{(ac)} \pm 1.477$	$7.85^{(a)} \pm 0.964$	$40^{(a)} \pm 4.733$	$504^{(a)} \pm 56.367$
G4: $AlCl_3$ + BAAE 10%	$7.22^{(acd)} \pm 1.491$	$7.72^{(a)} \pm 1.096$	$46^{(c)} \pm 5.586$	$618^{(c)} \pm 69.354$
G5: $AlCl_3$ + BAAE 15%	$8.14^{(d)} \pm 1.191$	$7.04^{(a)} \pm 1.047$	$53^{(d)} \pm 6.033$	$710^{(d)} \pm 79.307$
L.S.D. at 5%	1.315	1.385	5.548	69.970

Each value is mean ± SD for 10 rats in each group. Significantly different from controls ($p \leq 0.05$) by ANOVA multiple rage test.

Source: Devised by authors

TABLE 3

Change in Sperm Motility, Sperm Count (Testicular and Epididymal), Daily Sperm Production (Per Gram Testicular Parenchyme) and Sperm Transit Rate (Day) of Male Rats Treated with $AlCl_3$ and $AlCl_3$ + BAAE (5%, 10% and 15%) for 70 Days

Treatments	Sperm motility (%)	Sperm count(**) ($\times 10^6$/ml)		Daily sperm production(**) (***)	Sperm transit rate (day)
		Testicular	Epididymal		
G1 (NC)	$79.7^{(a)} \pm 6.701$	$168.9^{(a)} \pm 18.90$	$256.1^{(a)} \pm 27.49$	$21.2^{(a)} \pm 2.402$	$6.3^{(a)} \pm 0.708$
G2 (PC)	$41.9^{(b)} \pm 3.609$	$104.1^{(b)} \pm 11.71$	$167.1^{(b)} \pm 17.92$	$15.8^{(b)} \pm 1.386$	$15.6^{(b)} \pm 1.448$
G3: $AlCl_3$ + BAAE 5%	$79.8^{(ab)} \pm 6.663$	$165.4^{(ab)} \pm 18.57$	$257.9^{(ab)} \pm 28.95$	$20.5^{(ab)} \pm 2.301$	$6.4^{(a)} \pm 0.718$
G4: $AlCl_3$ + BAAE 10%	$80^{(ab)} \pm 6.680$	$169.8^{(ab)} \pm 19.06$	$260.3^{(ab)} \pm 29.22$	$21.2^{(ab)} \pm 2.380$	$6.3^{(a)} \pm 0.707$
G5: $AlCl_3$ + BAAE 15%	$80.2^{(ab)} \pm 6.697$	$170.8^{(ab)} \pm 19.17$	$262.5^{(ab)} \pm 29.47$	$21.5^{(ab)} \pm 2.414$	$6.2^{(a)} \pm 0.696$
L.S.D. at 5%	42.550	21.113	32.478	2.716	1.118

(*) the count is calculated per gram of testicular parenchyma. Each value is mean ± SD for 10 rats in each group. Significantly different from controls ($p \leq 0.05$) by ANOVA multiple rage test.

Source: Devised by authors

nutrient content. Balanites kernel is a source of highly regarded edible and medicinal oil. It contains four major fatty acids; linoleic (39.85%), oleic (25.74%), stearic (19.01%) and palmitic (15.40%) were found in Balanites oil. This gives a high percentage of the nutritionally beneficial unsaturated fatty acids (65.6%) (Okia et al., 2013).

Data in TABLE 1 show the relative weight of testis and epididymis of male rats. The results indicated a significant ($p \leq 0.05$) decrease in relative testis weight and an increase in relative epididymis weight, respectively, in rats treated with $AlCl_3$ compared to NC. In addition, the presence of BAAE with $AlCl_3$ alleviated the $AlCl_3$ toxic effect and the weight of these tissues almost reached those of NC values; these results are similar to previous studies (Yousef and Salama, 2009) in rats. Also, Bataineh et al. (1998) found decreased absolute and relative testis weights after aluminium chloride ingestion. Moreover, ammonium molybdate treatment resulted in a significant decrease in the relative weight of testis (Pandey and Jain, 2015).

TABLE 4 Changes in Fructose in Semen and Semen Quality of Male Rats Treated with $AlCl_3$ and $AlCl_3$ + BAAE (5, 10 and 15%) for 70 Days

Treatments	Fructose in semen	Semen quality test
G1 (NC)	312(a)±24.57	1.83(a)±0.10
G2 (PC)	139(b)±11.00	1.03(b)±0.14
G3: $AlCl_3$ + BAAE 5%	321(ac)±26.80	1.78(a)±0.20
G4: $AlCl_3$ + BAAE 10%	349(cd)±29.14	1.85(a)±0.21
G5: $AlCl_3$ + BAAE 15%	358(d)±29.89	1.9(a)±0.21
L.S.D. at 5%	30.282	0.228

Each value is mean ± SD for 10 rats in each group. Significantly different from controls ($p \leq 0.05$) by ANOVA multiple rage test

Source: Devised by authors

Data in TABLE 2 show a significant decrease in serum LH, estradiol and testosterone concentration ($p \leq 0.05$), and a significant increase in serum FSH concentration ($p \leq 0.05$) in rats treated with $AlCl_3$ compared to NC. $AlCl_3$ + BAAE at levels 5%, 10% and 15% significantly increased LH, estradiol and testosterone (6.71, 7.22, 8.14 IU/L), (40.00, 46.00, 53.00 pg/ml) and (504.00, 618.00, 710.00 ng/dl), respectively compared to PC (0.81 IU/L, 6.99pg/ml and 201.00 ng/dl), respectively. On the other hand, FSH serum concentration significantly ($p \leq 0.05$) decreased (7.85, 7.72 and 7.04IU/L) in rats treated with $AlCl_3$ + BASE at levels (5%, 10% and 15%), respectively compared to PC (15.01 IU/L). These results were similar to those obtained by Yousef and Salama (2009) and Abd El-Rahman and Al-Ahmary (2013) in rats, and Guo et al. (2005b) in mice.

The severe reduction in male libido and fertility following the administration of aluminium might be a result of excessive aluminium accumulation in the testes and low testosterone concentrations. The discrepancy was reasoned such that aluminium accumulation failed to immediately affect the enzymes for androgen biosynthesis or produce a possible disturbance in hypothalamic-pituitary-gonadal axis (Guo et al., 2005b). However, $AlCl_3$ caused a significant decline in the activity of 17-ketosteroid reductase after 70 days treatment. Al-induced NO might be a suppressor of testosterone and an observation of the inhibition of LH stimulated steroidogenesis in Leydig cells (Dobashi et al., 2001; Guo et al., 2005b). The stress-induced testicular NO also caused the decrease of steroidogenic enzyme activities (Kostic et al., 2000). Moreover, Guo et al. (2005b) 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. Yakubu and Afolayan (2009) found that the aqueous extract of *Bulbinen atalensis* stem at doses of 25 and 50mg/kg body weight enhanced the success rate of mating and fertility due to increased libido as well as the levels of reproductive hormones in male rats.

Results indicated in TABLE 3 show significantly ($p \leq 0.05$) decreased in sperm motility, sperm count (testicular and epididymal) and daily sperm production, respectively. While, the spermtransit rate (day) was significantly increased in rats treated with $AlCl_3$ compared to NC. Rats treated with $AlCl_3$ + BAAE at levels 5%, 10% and 15% indicated significantly increased sperm motility, sperm

count (testicular and epididymal) and daily sperm production, respectively, and a decrease in the sperm transit rate (day) compared to PC.

Yousef and Salama (2009) stated that necrosis of spermatocytes/spermatids was observed in the testes of mice exposed to aluminium nitrate. Also, the decrease demonstrated that aconitase, a protein that binds citrate and catalyses its isomerisation to isocitrate via the intermediate cis-aconitate in Krebs cycle, showed decreased activity in the presence of aluminium, suggesting that aluminium may influence mitochondrial enzymes. Consequently, changes in mitochondrial functions may be reflected in sperm motility and viability (Yousef et al., 2007). Also, Dawson et al. (1998) found that high concentrations of aluminium in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability. Thus, the observed decrease in sperm motility could be attributed in part to the concomitant reduction in testosterone production (Guo et al., 2005b; Yousef et al., 2005).

Rabbits orally administered with $AlCl_3$ at 34mg/kg bw every other day for 16 weeks showed a significant decrease in sperm concentration and sperm motility (Yousef et al., 2005). Also, Yousef et al. (2007) reported that $AlCl_3$ showed reproductive toxicity on rabbit sperm *in vitro*. Moreover, Yousef (2004) showed that aluminium chloride was able to generate reactive oxygen species in rabbit testes; overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Guo et al., 2005b; Turner and Lysiak, 2008). Thus, the spermatoxic effect of $AlCl_3$ might be due to induced free radicals. Abd El-Rahman and Al-Ahmary (2013) reported that sperm motility, sperm count (testicular and epididymal) and daily sperm production significantly ($p < 0.05$) decreased and sperm transit rate (day) significantly increased in rats treated with $AlCl_3$ (34mg/kg bw) compared to NC. However, rats treated with $AlCl_3$ (34mg/kg bw) + BASE at levels 25, 50 and 100mg/kg bw indicated a significant increase in sperm motility, sperm count (testicular and epididymal) and daily sperm production, and a decrease in sperm transit rate (day) compared to PC. Significantly lower testosterone levels in rats exposed to aluminium sulphate (AS) and in generations F_1 and F_2 compared to the parental one, luteinising hormone (LH) fluctuations in F_0, and a significant LH decrease in F_2 and F_3 generations, testis weight decrease, increased immobile and abnormal sperm, and histo-architecture alterations in the testes were observed by Muselin et al. (2016).

Fructose in semen and semen quality in rats treated with $AlCl_3$ and $AlCl_3$ + BAAE at levels 5%, 10% and 15% are shown in TABLE 4. The results show that fructose in semen and semen quality significantly ($p \leq 0.05$) decreased in rats treated with $AlCl_3$ compared to NC, while animals treated with $AlCl_3$ + BAAE at levels 5%, 10% and 15% significantly ($p \leq 0.05$) increased in semen and semen quality compared to PC. The decline in semen quality of rats treated with $AlCl_3$ was similar to the results obtained by Yousef and Salama (2009), and Abd El-Rahman and Al-Ahmary (2013). Yousef and Salama (2009) stated that the aluminium chloride caused testicular dysfunction, and deterioration in testosterone and semen quality levels. Also, other studies reported that $AlCl_3$ lowered semen quality *in vivo* and *in vitro* (Yousef et al., 2005, 2007). Abd El-Rahman and Al-Ahmary (2013) showed that treatment with BASE at levels 50 and 100mg/kg bw significantly ($p < 0.05$) increased fructose in semen compared to NC.

In this study, the light microscopy of NC testis showing normal histological structure of testis, seminiferous tubules (SM) interstitial cells (IC) and narrow lumina (arrow). Testis surrounded by a dense fibrous tissue capsule and normal thick connective tissue capsule (tunica albuginea). The seminiferous tubules are two types of cells: sertoli cells, resting on the tin basal lamina (basement membrane), and the spermatogenic cells. Spermatogenic cells include many layers, namely, the spermatogonia, primary and secondary spermatocytes, spermatoids, and finally mature spermatozoa (FIGURE 1A).

After rats were administered with $AlCl_3$ for 70 days, testis tissue showed severely damaged in seminiferous tubuless cells (arrow), exfoliated germ cells (E) and congestion of interstitial blood vessel (BV), in the general cells and more exaggerated features of focal areas of spermatogenesis. Maturation arrest in some tubules and the germinal cells spermatogenesis arrest at the spermatoids level with reduction in sperm density was also noted (FIGURES 1B and 1C). These results are in agreement with Mahran et al. (2011) who stated that after 70 days of treatment of rats with aluminium chloride, the testes revealed congestion of interstitial blood vessels, disorganised germinal epithelium, appearance of many focal areas of degenerative changes in the form of degenerative germinal cells together with few fragmented spermatozoa in the lumen of the seminiferous tubules, which appeared to have acquired a thick, irregular basement membrane (Khattab and Khattab, 2007). Also, Buraimoh et al. (2012) reported that aluminium chloride showed seminiferous

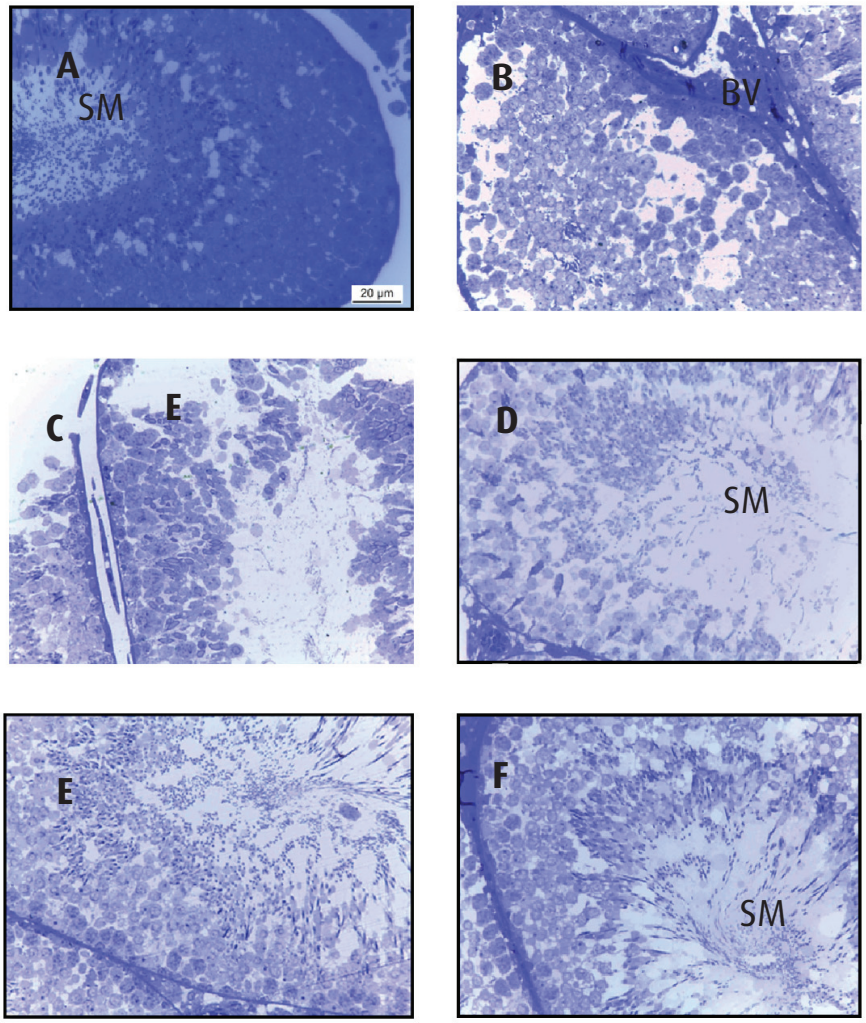

FIGURE 1

Photomicrographs of Testis Sections in [A] NC group, [B, C] PC group [C] $AlCl_3$ + BAAE 5% group, [E] $AlCl_3$ + BAAE 10% group Showing Germ Cells (G) and [F] BAAE 15% group (blue stain 400)Source: Authors' own compilation from data

Source: Devised by authors

tubules that attained different shapes and lost the normal distribution of epithelial lining and vacuolar cytoplasm, which are indications of testes degeneration that could affect sperm cell production.

Pandey and Jain (2015) showed marked degeneration in spermatogenic and Leydig cells, shrunken seminiferous tubules, thinner germinal epithelium, exfoliation of germ cells and depletion of spermatozoa from lumen of seminiferous tubules in testis of rats that received 50, 100 and 150mg/kg b. wt./day ammonium molybdate orally for 60 days. Testes from those treated with aluminium chloride revealed markedly shrunken seminiferous tubules with severe sperm cell aplasia and basement membrane thickening, as well as rupture, vacuolisation and fibrosis in interstitial and peritubular tissue (Arhoghro and Sule, 2017).

A section of testis of rats administrated with $AlCl_3$ + BREE 5% showed less improvement in histological structure of seminiferous tubules (SM), it is less similar to the normal control (NC) section (FIGURE 1D). Some of seminiferous tubules were normal, also, some germ cells appeared regular in shape and most nuclei became vesicular (FIGURE 1D).

FIGURE 1E shows the light microscope of the group of rats administrated with $AlCl_3$+BREE 10%. The light microscope showed an improvement in histopathological toxicity of testis sections and regained nearly its normal structure; blue was attained.

A light microscope showed that the group of rats administrated with $AlCl_3$+BREE 15% showed recovery of histological features of germ cells lining of the seminiferous tubules (SM) as well as the interstitial cells of leydig and normal nuclei, normal basement membrane of those tubules, as well as normal interstitial cells of Leydig. There was also a lack of congestion of interstitial blood vessels, normal seminiferous tubules epithelium with distinct nuclei, and sperm bundles in their lumen (FIGURE 1F).

CONCLUSIONS

The results of the present study showed that aluminium chloride caused testicular damage and deterioration in testosterone levels. *B. aegyptiaca* aqueous extract (BAAE) at 10% and 15% would be a good choice of a natural source for protection against infertility in male rats. Also, it can protect testis against $AlCl_3$ reproductive toxicity; this may be attributed to the activity of *Balanites aegyptiaca* as an antioxidant. Therefore, using a diet rich in *Balanites aegyptiaca* could be a beneficial way of overcoming the reproductive toxicity of aluminium, but other studies are needed in the future to apply these results to humans.

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