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Effect of sapogenin extract on anti-infertility induced by aluminium chloride in male rats

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ABSTRACT

Balanites aegyptiaca has been reported to be important saponins. Therefore, this study aimed at elucidating the protective effects of *B. aegyptiaca* sapogenin extract (BASE) against infertility caused by aluminium chloride in male rats. The first group served as control (NC). Group 2 received $AlCl_3$ (34 mg/kg bw) (1/25 LD_{50}) (PC). Groups 3, 4 and 5 were treated with $AlCl_3$ (34 mg/kg bw) plus BASE at different doses (25, 50 and 100 mg/kg bw) for 70 days. Luteinizing hormone (LH), estradiol, testosterone, sperm motility, sperm count (testicular and epididymal), daily sperm production, fructose in semen, semen quality and glucose were significantly ($P < 0.05$) increased. While, concentration of total cholesterol, sAST, sALT, urea and creatinine were significantly ($P < 0.05$) decreased in rats treated with $AlCl_3$ (34 mg/kg bw) plus BASE at levels 25, 50 and 100 mg/kg bw compared to PC. Also, follicle-stimulating hormone (FSH) and sperm transit rate (day) were decreased in rats treated with $AlCl_3$ (34 mg/kg bw) plus BASE at levels 25, 50 and 100 mg/kg bw compared to PC. The results obtained indicated that using 100 mg/kg bw of BASE was more beneficial than using 25 and 50 mg/kg bw for protection from infertility in male rats. It could be recommended to use BASE as natural source against infertility caused by $AlCl_3$ (34 mg/kg bw) and effectiveness of doses used to 100 mg/kg bw.

Keywords: *Balanites aegyptiaca*, sapogenin extract, anti-infertility, aluminium chloride, testosterone, sperm motility, fructose in semen.

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INTRODUCTION

Aluminium toxicity has been well documented in the pathogenesis of many disorders in patients undergoing long term dialysis including dialysis encephalopathy (Alfrey et al., 1976). Most of experimental studies on aluminium toxicity in an animal model have been pre-formed with the use of this metal in a soluble form (Alfrey, 1984) or with certain metal (Ebina et al., 1984). Aluminium compounds are widely used in medicine e.g. antacids, phosphate binders, buffered aspirins, vaccines and allergen injections (Cannata et al., 1983; Kaehny et al., 1997; Lione, 1985). It has recently been demonstrated that ingestion of aluminium compounds with either fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of Al in healthy subjects (Slanina et al., 1986; Weberg and Berstad, 1986). Aluminium has been tested for ant fertility in male rats and was found to show significant activity (Sharma et al., 2003). ATSDR (1990) reported that aluminium is distributed mainly in bone, liver, testis, kidneys and brain. Guo et al. (2002) observed that reduced testis acetyl cholinesterase (ACE) activity presumably plays an important role in oxidative damage of aluminium induced testicular toxicity. Chinoy et al. (2005) studied the effect of sodium fluoride together with aluminium chloride to male mice for 30 days and found some structural alteration in the testis with formation of giant cells. Krasovskii et al. (1979) studied the biological effects of lead and aluminium on rats and guinea pigs and observed that the lead and aluminium chloride caused gonad toxicity. Aluminium ingestion in excessive amount leads to 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). In addition, the suppressive effects of long-term oral $AlCl_3$ in drinking water on both sexual and aggressive behavior and fertility of male rats were also noted (Bataineh et al., 1998).

Balanites aegyptiaca follow the zygophliace family popularly called the 'desert date' (Heiglige in Arabia), it is a highly drought-tolerant evergreen desert plant species. *B. aegyptiaca* has been used as various folk medicines in Africa and Asia where the fruit is used as fumigator in liver disease in Chad (Croach, 1962). The bark is used in treatment of Syphilis round worm infection and as fish poison (Bailey et al., 1962), the fruit and branches were lethal to snails, miracidiacercae of schistosoma (Watt and Bailey-Brandwijk, 1962). *B. aegyptiaca* is a widely distributed African plant of medicinal interest containing a number of cytotoxic and cytostatic compounds. A mixture of steroidal saponins: balanitin-6 (28%) and balanitin-7 (72%) isolated from *B. aegyptiaca* kernels have anti-cancer activity (Morsy, 2008). Also, the importance of *B. aegyptiaca* is back to presence of a steroidal sapogenin compound named diosgenin which is useful in pharmaceutical industries as a natural source of steroidal hormones. Studies have also revealed that diosgenin produces changes in the lipoxygenase activity of human erythroleukemia cells and is responsible for morphological and biochemical changes in megakaryocyte cells (Beneytout et al., 1995; Nappez et al., 1995). *B. aegyptiaca* protected the livers of treated mice against paracetamol. It hepatotoxicity as evidenced by a significant improvement of liver function tests. Also, *B. aegyptiaca* had a relatively modest hepato-protective activity (Ali et al., 2001). Extracts of *B. aegyptiaca* had a moderate biological activity on major promastigotes (Fatima et al., 2005). Therefore, the current study was carried out to investigate: (1) the different effects of $AlCl_3$ on blood indices of male rats, (2) the role of BASE in alleviating the negative effects of $AlCl_3$ and (3) the effect of $AlCl_3$ (34 mg/kg bw) + BASE on the tested parameters.

MATERIALS AND METHODS

Balanites aegyptiaca was obtained from, Agriculture Research Center, Egypt. Kits of TC; TG; ALT; AST; urea; creatinine; LH; FSH; estradiol and testosterone were obtained from Biodiagnostic Co., 29 El-Tahreer St., Dokki-Giza, Egypt.

Isolation of the major sapogenin from dry fruit

The powdered dry fruit, 170 g, was extracted for 24 h in a soxhlet with light petroleum (b.p. 40 to 60). The "defatted" powder was dried in a hot-air oven (80°C) to remove excess solvent. It was then extracted to exhaustion with MeOH in a soxhlet apparatus to afford 40 g of a dark-brown, hygroscopic crude saponin which was hydrolyzed by refluxing with 2N HCl (600 ml) for 2 h. The mixture was cooled, filtered and the acid-insoluble washed with H_2O before neutralizing with 20 ml of 10% NH_4OH. After it had drained, the acid-insoluble was dried in a hot-air oven (80°C) for 4 h. The dried residue was crushed in a mortar and extracted with light petroleum in a soxhlet for 2 days. Removal of the solvent, *in vucuo* (Hardman and Sofowora, 1970).

Biological methods

Male albino adult rats (50 animals weighing 180 ± 2 g) were obtained from the animal house in University of Dammam. Rats were housed in individual cages with screen bottoms and fed on basal diet (corn starch 70%, casein 10%, corn seed oil 10%, cellulose 5%, salt mixture 4% and vitamins mixture 1%) for two weeks. After equilibration, rats were weighted and divided into five groups (ten animals per each) everyone was assigned to one of the five diet groups, [Negative Control (NC)], Positive Control (PC) treated with $AlCl_3$ (34 mg/kg bw) and three groups treated with $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw). Total feed consumption was weighted, 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 mean of heparinized capillary glass tubes according to Schermer (1967). Each sample was placed into a dry clean centrifuge tube and centrifuged 1500 xg for 30 min at 4°C to obtain serum.

Serum chemistry

Total Cholesterol (TC) was determined according to the method described by Allain et al. (1974). Serum transaminases sAST and sALT (Aspartate transferase and Alanine transferase) were measured colorimetrically according to the method described by Reitaman and Frankel (1957). Serum urea was determined according to Fawcett and Soctt (1960) and creatinine was determined according to the method of Barthes et al. (1972).

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 by the method of Tietz (1995).

Sperm parameters

Sperm motility

Sperm motility was recorded and evaluated immediately after tissue isolation. Caudaepididimis was cut into the small pieces and transferred into the Petri dishes containing pre-warmed nutrition medium (RPMI). Sperm were allowed to swim out within the 5 min at 37°C. The analysis was carried out under the 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 by method of Akdag et al. (1999).

Sperm count

Testicular sperm count: One testis of each rat was placed in 1 ml 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 using surgical blades in 1 ml of phosphate buffer saline. The testicular cell suspension was pipette several times to form a 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 by method of Fatma et al. (2009).

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 homogenized in saline Triton merthiolate solution (STM solution:17.5 g NaCl, 1 ml Triton X-100, and 0.2 g sodium ethylmercurithiosalicylate were dissolved in distilled water for 1 L of STM solution) with a Waring blender (Polytron, Kinematica, Littau/Luzern, Switzerland). After that, homogenization-resistant spermatids or sperm were counted using a hemocytometer by method of Omura et al. (1996).

Daily sperm production

After removing the tunica albuginea, both testis was minced and homogenized in 10 ml of 0.9% NaCl containing 0.5% Triton X-100 at medium speed in a POTTERS® tissuemizer for 1 min. After dilution, the number of homogenization-resistant spermatids was counted in a hemocytometer (Bürker, Germany). The number of homogenization-resistant spermatids obtained by summing the scores of right and left testis, 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 seminal vesicle was determined by the method of Foreman et al. (1973); semen quality was determined by the method of Reddy and Bordekar (1999).

Statistical analysis

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

RESULTS

Effect of feeding rats for 70 days on diets contains $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE at level 25, 50 and 100 mg/kg bw are recorded in Table 1. All rats significantly increased in their weight but the maximum increase was found in rats fed on NC. Rats treated with $AlCl_3$ (34 mg/kg bw) showed high significantly ($P < 0.05$) decreased in body weight gain compared to NC. While, rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at level 100 mg/kg bw showed high significantly increased in their weight compared to NC. Food intake show that rats fed on NC gave the highest body weight gain consumed the highest amount of their diet which reflected on their weight followed by rats fed on diet contained $AlCl_3$ (34 mg/kg bw) + BASE at level 100 mg/kg b.w. Daily food intake followed the same trend. For food efficiency and food efficiency ratio results indicated that NC and rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at level 100 mg/kg bw had maximum food efficiency and food efficiency ratio compared to rats treated with $AlCl_3$ (34 mg/kg bw) + BASE (50 and 100 mg/kg bw). On the other hand, the rats treated with $AlCl_3$ (34 mg/kg bw) obtained the lowest food efficiency and food efficiency ratio.

The obtain results in Table 2 indicated significantly ($P < 0.05$) increased in the serum cholesterol level and significantly ($P < 0.05$) decreased in serum glucose level in animals treated with $AlCl_3$ (34 mg/kg bw) compared to NC. On the other hand, the presence of $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw showed significantly decreased in serum cholesterol level and increased in serum glucose level, respectively compared to PC. No significantly ($P < 0.05$) effects of serum cholesterol level and serum glucose level in rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at level 100 mg/kg bw compared to NC.

Table 3 shows the results of sALT, sAST, urea and creatinine in rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw. Data show significantly ($P < 0.05$) increased in sALT, sAST, urea and creatinine (30.40, 29.80, 40.80 and 1.27 mg/dl), respectively, in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC (22.10, 24.70, 35.40 and 1.09 mg/dl), respectively. On the other hand, results indicated significantly ($P < 0.05$) decreased in sALT, sAST, urea and creatinine in rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw compared to PC. Also, no significant effect in serum creatinine in rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at level 25, 50 and 100 mg/kg bw compared to NC.

Data in Table 4 show significantly decreased in serum LH, estradiol and testosterone concentration ($P < 0.05$) and significantly increased in serum FSH concentration ($P < 0.05$) in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC. $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw significantly increased LH, estradiol and testosterone (1.94, 3.65, 6.12 IU/L), (13.00, 21.00, 35.00 pg/ml) and (316.00, 388.00, 432.00 ng/dl), respectively compared to PC (0.78 IU/L,

Table 1. Relative weights of initial body weight, final body weight, gain in body weight, food efficiency and food efficiency ratio of male rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	Initial body weight	Final body weight	Gain in body weight	Daily gain in body weight	Food intake	Daily food intake	Food efficiency	Food efficiency ratio
Negative control (NC)	180.16 ± 15.970^{a}	345.94 ± 59.710^{a}	165.78 ± 50.460^{a}	2.37 ± 0.721^{a}	716.10 ± 98.193^{a}	10.23 ± 1.566^{a}	0.2317 ± 0.035^{a}	23.17 ± 3.469^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	181.02 ± 16.010^{a}	299.19 ± 46.930	113.17 ± 34.170^{b}	1.62 ± 0.488^{b}	732.20 ± 48.759^{b}	10.46 ± 1.593^{b}	0.1549 ± 0.0231^{b}	15.49 ± 2.314^{b}
$AlCl_3$ (34mg/kg bw) + BASE (25 mg/kg bw)	182.16 ± 12.500^{a}	317.49 ± 32.350^{a}	135.33 ± 21.410^{ab}	1.93 ± 0.306^{ab}	767.20 ± 81.258^{bc}	10.96 ± 1.161^{bc}	0.1761 ± 0.017^{bc}	17.61 ± 1.488^{bc}
AlCl3(34 mg/kg bw) + BASE (50 mg/kg bw)	179.75 ± 12.340^{a}	333.75 ± 34.920^{a}	154.00 ± 24.190^{a}	2.20 ± 0.346^{a}	790.30 ± 83.545^{cd}	11.29 ± 1.193^{cd}	0.1949 ± 0.019^{cd}	19.49 ± 1.914^{cd}
AlCl3(34 mg/kg bw) + BASE (100 mg/kg bw)	180.36 ± 13.090^{a}	341.48 ± 36.850^{a}	161.12 ± 25.300^{a}	2.30 ± 0.361^{a}	750.40 ± 79.201^{ad}	10.72 ± 1.131^{ad}	0.2146 ± 0.021^{ad}	21.46 ± 2.114^{ad}
L.S.D. at 5%	14.92	51.55	39.07	0.5582	94.961	1.5993	0.0284	2.801

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

Table 2. Serum cholesterol (mg/dl) and glucose (mg/dl) of male rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	Cholesterol (mg/dl)	Glucose (mg/dl)
Negative control (NC)	161.70 ± 7.41^{a}	101.30 ± 5.64^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	254.80 ± 11.97^{b}	77.20 ± 3.52^{b}
$AlCl_3$ (34mg/kg b.w) + BASE (25 mg/kg bw)	212.50 ± 24.59^{c}	84.60 ± 7.24^{c}
$AlCl_3$ (34 mg/kg b.w) + BASE (50 mg/kg bw)	197.90 ± 11.16^{c}	91.40 ± 3.37^{c}
$AlCl_3$ (34 mg/kg b.w) + BASE (100 mg/kg bw)	188.70 ± 58.22^{ac}	98.90 ± 5.25^{a}
L.S.D. at 5%	34.95	6.20

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

7.00 pg/ml and 198.00 ng/dl), respectively. On the other hand, FSH serum concentration significantly ($P < 0.05$) decreased (13.02, 11.89 and 10.36 IU/L) in rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at levels (25, 50 and 100 mg/kg bw), respectively compared to PC (14.81 IU/L). Also, data in Table 4 indicated that no significant ($P < 0.05$) effects in rats treated with $AlCl_3$ (34 mg/kg bw) + BASE at level 100 mg/kg bw in all parameters

Table 3. Serum AST, ALT, urea and creatinine of male rats treated with $AlCl_3$ (34 mg/kg b.w) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	sAST (mg/dl)	sALT (mg/dl)	Ure (mg/dl)	Creatinine (mg/dl)
Negative control (NC)	22.1 ± 1.04^{a}	24.7 ± 1.62^{a}	35.4 ± 3.65^{a}	1.09 ± 0.10^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	30.4 ± 1.68^{b}	29.8 ± 1.95^{bd}	40.8 ± 4.32^{b}	1.27 ± 0.06^{b}
$AlCl_3$ (34 mg/kg bw) + BASE (25 mg/kg bw)	27.9 ± 1.52^{c}	29.1 ± 1.51^{cd}	39.2 ± 1.67^{bc}	1.19 ± 0.07^{ab}
$AlCl_3$(34 mg/kg bw) + BASE (50 mg/kg bw)	26.1 ± 1.52^{d}	27.6 ± 1.54^{ce}	36.1 ± 1.88^{acd}	1.12 ± 0.08^{a}
$AlCl_3$(34 mg/kg bw) + BASE (100 mg/kg bw)	24.7 ± 1.33^{d}	26.2 ± 1.19^{ae}	35.7 ± 2.01^{ad}	1.1 ± 0.13^{a}
L.S.D. at 5%	1.71	1.88	3.46	0.11

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

Table 4. Serum LH (IU/L), FSH (IU/L), Estradiol (pg/ml) and Testosterone (ng/dl) of male rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	LH (IU/L)	FSH (IU/L)	Estradiol (pg/ml)	Testosterone (ng/dl)
Negative control (NC)	6.61 ± 0.536^{a}	7.93 ± 0.889^{a}	38 ± 4.243^{a}	450 ± 50.339^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	0.78 ± 0.064^{b}	14.81 ± 1.663^{b}	7 ± 0.894	198 ± 22.235^{b}
$AlCl_3$ (34 mg/kg bw) + BASE (25 mg/kg bw)	1.94 ± 0.171^{c}	13.02 ± 1.461^{c}	13 ± 1.549^{c}	316 ± 35.710c
$AlCl_3$ (34 mg/kg bw) + BASE (50 mg/kg bw)	3.65 ± 0.316^{d}	11.89 ± 1.337^{cd}	21 ± 2.366^{d}	388 ± 43.607^{d}
$AlCl_3$ (34 mg/kg bw) + BASE (100 mg/kg bw)	6.12 ± 0.792^{a}	10.36 ± 1.164^{ad}	35 ± 3.688^{ad}	432 ± 48.469^{ad}
L.S.D. at 5%	0.544	1.580	3.381	49.195

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

Table 5. Change in sperm motility (%), sperm count ($\times 10^6$/ml) (testicular and epidydemal), daily sperm production (per gram testicular parenchyme) and sperm transit rate (day) of male rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	Sperm motility (%)	Sperm count ($\times 10^6$/ml)		Daily sperm (*) production	Sperm transit rate (day)
		Testicular	Epididymal		
Negative control (NC)	79.6 ± 6.647^{a}	169.8 ± 19.06^{a}	254.7 ± 28.59^{a}	21.4 ± 2.402^{a}	6.4 ± 0.718^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	43.1 ± 3.599^{b}	104.1 ± 11.69^{b}	166.8 ± 18.72^{b}	16.7 ± 1.875^{b}	13.8 ± 1.549^{b}
$AlCl_3$ (34 mg/kg bw) + BASE (25 mg/kg bw)	57.8 ± 4.826^{c}	126.8 ± 14.23^{c}	191.6 ± 21.51^{bc}	17.8 ± 2.00^{bc}	11.2 ± 1.257^{c}
$AlCl_3$ (34 mg/kg bw) + BASE (50 mg/kg bw)	64.3 ± 5.369^{d}	147.2 ± 16.52^{d}	213.7 ± 23.90^{cd}	19.5 ± 2.19^{ac}	9.5 ± 1.066^{d}
$AlCl_3$ (34 mg/kg bw) + BASE (100 mg/kg bw)	74.9 ± 6.254^{a}	158.6 ± 17.8^{ad}	232.8 ± 26.13^{ad}	20.4 ± 2.29^{ac}	7.7 ± 0.864^{a}
L.S.D. at 5%	6.479	19.123	28.59	2.568	1.344

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

compared to NC.

Results indicated in Table 5 significantly ($P < 0.05$) decreased in sperm motility, sperm count (testicular and epididymal) and daily sperm production (43.1%, 104.1 (10^6/ml), 166.8 (10^6/ml) and 16.7*), respectively. While, the data showed significantly increased in the sperm transit rate (day) (13.8) in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC (79.6%, 169.8 (10^6/ml), 254.7 (10^6/ml), 21.4* and 6.4), respectively. Rats treated with $AlCl^3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw indicated significantly increased in the sperm motility, sperm count (testicular and epididymal) and daily sperm production (57.8, 64.3 and 74.9%), [126.8, 147.2 and 158.6 (10^6/ml)], [191.6, 213.7 and 232.8 (10^6/ml)] and (17.8, 19.5 and 20.4*), respectively and decreased in the sperm transit rate (day) (11.2, 9.5 and 7.7), respectively compared to PC.

The fructose in semen and semen quality in rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw obtained in Table 6. The results show that fructose in semen and semen quality significantly ($P < 0.05$)

Table 6. Changes in fructose in semen and semen quality of male rats treated with $AlCl_3$ (34 mg/kg bw) and $AlCl_3$ (34 mg/kg bw) + BASE (25, 50 and 100 mg/kg bw) for 70 days.

Treatments	Fructose in semen (mg/dl)	Semen quality (%)
Negative control (NC)	310 ± 25.67^{a}	1.81 ± 0.20^{a}
Positive control (PC) ($AlCl_3$ 34 mg/kg bw)	134 ± 10.99^{b}	1.07 ± 0.12^{b}
$AlCl_3$ (34 mg/kg bw) + BASE (25 mg/kg bw)	206 ± 17.20^{c}	1.41 ± 0.16^{c}
$AlCl_3$ (34 mg/kg bw) + BASE (50 mg/kg bw)	221 ± 18.45^{c}	1.59 ± 0.18^{cd}
AlCl3(34 mg/kg b.w)+ BASE (100 mg/kg b.w)	249 ± 20.79^{d}	1.67 ± 0.19^{ad}
L.S.D. at 5%	22.870	0.204

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

decreased in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC. While, animals treated with $AlCl_3$ (34 mg/kg bw) + BASE at levels 25, 50 and 100 mg/kg bw significantly ($P < 0.05$) increased in semen and semen quality compared to PC.

DISCUSSION

Aluminium chloride

The present study was carried out to investigate the protective effect of *B. aegyptiaca* sapogenin extract on aluminium-induced infertility and biochemical alterations in male rats. Serum Luteinizing hormone (LH), estradiol and testosterone concentration ($P < 0.05$) significantly decreased. While, serum Follicle-stimulating hormone (FSH) concentration ($P < 0.05$) significantly increased in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC. Data show significantly ($P < 0.05$) increased in cholerterol, sALT, sAST, urea and creatinine and decreased in serum glucose in rats treated with $AlCl_3$ (34 mg/kg bw) compared to NC. These observations are similar to the data reported by Abdel Aziz and Zabut (2011), they indicated that $AlCl_3$ decreased serum glucose levels by 30%, and increased triglyceride (28%) and cholesterol (20%) levels; neither vitamin treatments restored the levels of these components. Our findings also revealed a decrease in serum glucose levels in response to aluminium. Indirectly, aluminium is known to play a specific role in carbohydrate metabolism. Concerning lipid metabolism, our results demonstrated that triglycerides and total cholesterol levels increased in response to aluminium, consistent with increasing lipogenesis in the liver (Thirunavukkarasu and Sakthisekaran, 2003). The increased glucose production and decreased glucose utilization would lead to hyperglycemia. Oxidative stress (OS) has been suggested as a major pathogenic link to both insulin resistance and β-cell dysfunction. Oxidative stress causes structural damages to the pancreatic islets with the formation of amyloid proteins, which not only prevents the release of insulin into the circulation, but also destroys the insulin-secreting β-cells (Hayden, 2002). The present data indicated that plasma total lipids, cholesterol, triglycerides and LDL-c were significantly increased by aluminium chloride ($AlCl_3$) treatment, while HDL-c levels were decreased (Newairy et al., 2009). Also, Wilhelm et al. (1996) reported that $AlCl_3$ exposure resulted in aluminium accumulation in the liver and this may lead to disturbance of lipid metabolism and an elevation of serum cholesterol.

The increase in plasma lipids due to aluminium administration indicates a loss of membrane integrity. Enhanced protein catabolism and accelerated amino acid deamination in response to low glucose levels caused by aluminium ion administration is the best interpretation for the elevated levels of urea. The presence of toxic compounds can increase blood urea and decrease plasma protein (Berne and Levy, 1998). The observed increase in uric acid concentration might be due to extra degradation of purines in the liver, or an inability to excrete uric acid by the kidneys (Varely, 1987). An increase in creatinine has been seen, interpreted as caused by a decrease in muscle mass (Pevicharova et al., 1997) or abnormal glomerular function of the kidneys induced by $AlCl_3$ administration (Berne and Levy, 1998). The activity of AST is significantly increases in such cases and escapes to the plasma from the injured hepatic cells. In addition, ALT level is of value also indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver. It increases in serum when cellular degeneration or destruction occurs in this organ (Hassoun and Stohs, 1995). In the present study, the activities of AST, ALT and LDH were significantly increased in plasma of rats treated with aluminium chloride ($AlCl_3$) (Yousef and Salam, 2009). This may be due to the leakage of these enzymes from the liver cytosol into the blood stream and/or liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place. Also, Wilhelm et al. (1996) reported that aluminium exposure can result in aluminium accumulation in the liver and this metal can be toxic to

the hepatic tissue at high concentrations. Mahieu et al. (2005) reported that alterations in serum urea may be related to metabolic disturbances (e.g. renal function, cation–anion balance). In addition, Katyal et al. (1997) reported that aluminium has been implicated in the pathogenesis of several clinical disorders, such as renal dysfunction. The increase in urea concentrations in plasma of animals treated with aluminium may be due to its effect on liver function, as urea is the end-product of protein catabolism and this is confirmed by the decrease in plasma proteins and/or referred to liver dysfunction as proven by the increase in plasma AST, ALT and LDH activities. This was further confirmed when $AlCl_3$ treatment resulted in a significant effect on the various membrane-bound enzymes in terms of increased activities of plasma AST, ALT and LDH (Newariy et al., 2009). $AlCl_3$ increased levels of urea (12%), uric acid (77%) and creatinine (25%) compared to the controls, and vitamin E separately or together with vitamin C restored the levels of these nitrogen compounds. The activities of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were also increased by the $AlCl_3$ treatment (Abdel Aziz and Zabut, 2011).

The study of Guo et al. (2005a) demonstrated that exposure to aluminium lowered plasma and testicular testosterone levels in mice. The authors suggested that the severe reduction in male libido and fertility following the aluminium administration might be a result from excessive aluminium accumulation in the testes and low testosterone concentrations. However, they reported that the high levels of aluminium in aluminium-treated mice were apparent at week 3 before the effects on male libido and fertility proliferated. 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. However, the present study showed that $AlCl_3$ caused significant decline in the activity of 17-ketosteroid reductase after 70 days treatment. 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 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. (2005a) 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.

Yousef and Salama (2009) stated that necrosis of spermatocytes/spermatids was observed in the testes of mice exposed to aluminium nitrate. Also, the decrease in testicular and epididymal weights, testicular and spermatid counts, and epididymal sperm counts were noted (Llobet et al., 1995). Zattaet et al. (2000) demonstrated that aconitase, a protein that binds citrate and catalyzes its isomerization to isocitrate via the intermediatecis-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. 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, 1995). Thus, the observed decrease in sperm motility could be attributed in part to the concomitant reduction in testosterone production (Guo et al., 2005a; Yousef et al., 2005). Results obtained from Yousef et al. (2005) revealed that rabbits orally administered $AlCl_3$ at 34 mg/kg bw every other day for 16 weeks showed significant decrease in ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, packed sperm volume, total functional sperm fraction, normal and live sperm, while dead and abnormal sperm were increased. 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., 2005a; Turner and Lysiak, 2008). Thus, the spermatoxic effect of $AlCl_3$ might be due to induced free radicals. Possible mechanisms of aluminium interference with male reproductive systems have not been revealed (Guo et al., 2005a).

The decline in semen quality of rats treated with $AlCl_3$ were similar to the results obtained by Yousef and salama (2009) they told that the present results showed that aluminium chloride caused testicular dysfunction, and deterioration in semen quality and testosterone levels. Our previous results also showed that $AlCl_3$ declined semen quality in vivo and in vitro (Yousef et al., 2005, 2007).

Balanites aegyptiaca sapogenin extract

B. aegyptiaca has been found to have several medicinal properties. However, the conducted to evaluate the curative effect of its sapogenin extract against $AlCl_3$ induced infertility and desfunction in testes has not been investigated. There is no previous study carried out with *B. aegyptiaca* sapogenin extract and aluminium. This mechanism belongs to first line therapies in anti-infertility treatment. The demonstrated results might be a base for further studies with *B. aegyptiaca* sapogenin. In addition, the *B. aegyptiaca* fruit can be

used to decrease the level of total cholesterol (HDL and LDL-cholesterol) and triglycerides.

The results of the present study agree with Morsy et al. (2010) who stated that the *B. aegyptiaca* had an effect on decreasing cholesterol level in the blood compared to that of the control group and the recommended dose was that dose of G3 (5.4 g/week/rat). Kameswara et al. (1997) stated that *B. aegyptiaca* fruit part lowered blood glucose with a simultaneous decrease in triglyceride and total cholesterol blood.

Soheir et al. (2008) they told that, urea and creatinine significantly decreased in rats given *B. aegyptiaca* aqueous extract (ABAE) and rats fed diet contained *B. aegyptiaca* cake (BAC) at 5, 15 and 25% (24.31,23.13, 22.31 mg/dl and 23.31, 22.71, 21.73 mg/dl for urea and 1.31, 1.21, 1.01 mg/dl and 1.11, 1.01, 0.99 mg/dl for creatinine), respectively in comparison with alloxan diabetic rats (67.22 mg/dl for urea and 4.51 mg/dl for creatinine). The activities of AST and ALT ranged from 32.01 to 30.07 IU/l and from 28.88 to 26.09 IU/l with group given ABAE and group fed on BAC 5, 15 and 25% relative to their control (82.72 and 62.33 IU/l), respectively. Ali et al. (2001) who found that treatment of mice with the plant extract (*B. aegyptiaca* extract) followed by the vehicle of paracetamol did not affect the liver adversely, as the liver weight, appearance and histology, AST, ALT and GGT (γ-glutamyl transferase) activities, and pentobarbitone – sleeping time were all unaffected.

Samir et al. (2000) stated that *B. aegyptiaca* extract induced significant reduction in serum glucose, glucagons, total lipids, total cholesterol, triglycerides level and transaminases (AST, ALT and γGT) activities.

Soheir and Haya (2013) studied the effect of *B. aegyptiaca* sapogenin extract (BASE) on the fertility of male rats and found that BASE at levels 25 and 50 mg/kg bw is safer for inducing fertility in male rats.

Conclusion

This study clearly showed the protective effect of *B. aegyptiaca* sapogenin extract on infertility induced by aluminium chloride in male rats. The obtain results indicated that the *B. aegyptiaca* sapogenin at 100 mg/kg bw would be a good choice natural source for protective against infertility in male rats. Also, it can be used to decrease the level of total cholesterol, sALT, sAST, urea and creatinine in male rats were treated with $AlCl_3$.

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