Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats

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Abstract: Natural dietary antioxidants are studied for their ability to protect cells from miscellaneous damage. Grape seed extract (Vitis vinifera L., Vitaceae) is a potent antioxidant. The present study aimed to investigate the protective effect of grape seed extract (GSE) against the possible testicular dysfunction caused by aluminium chloride (AlCl3) in male rats. Twenty sexually mature male albino rats were divided into four equal groups, the first served as negative control, the second received AlCl3 (20 mg/kg bw, 1/20 LD 50), the third administered GSE (75 mg/kg bw), and the fourth received AlCl3 and treated with GSE. Doses were given once daily via gavage for 70 consecutive days. The results revealed that, AlCl3 induced significant decrease in final body weight, sex organs relative weight, sperm concentration, motility and viability, serum testosterone concentration and superoxide dismutase (SOD) activity, with significant increase in sperm abnormalities and thiobarbituric acid reactive substance (TBARS) concentrations. Moreover, AlCl3 induced apparent alteration in the histological structure of the testis. Treatment with GSE ameliorated the harmful effects of AlCl3, this was also proved histopathologically by the noticeable improvement in the testis tissues. It may be concluded that GSE may be promising as a natural therapeutic agent in AlCl3-induced reproductive toxicity and oxidative stress in the male rat testes.


Keywords: Grape seed extract- aluminium chloride- reproductive- experimental animals.

1. Introduction

Aluminium absorption and accumulation in humans can occur via the diet, 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). Different forms of aluminium are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive 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 (Yousef and Salama, 2009). AlCl3 showed reproductive toxicity on rabbit sperm in vitro Yousef et al. (2007). Testicular aluminium accumulation, necrosis of spermatocytes/spermatids and significant decrease in fertility were found in male mice (Guo et al., 2005 a,b).

Grapevine (Vitis Vinifera), is cultivated today in all temperature regions of the world (Gruenwald et al., 2004). Its seeds contain several active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol. The grape seed extract has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects. The seeds of the grape are used in herbal medicine and as a dietary supplement (Shenoy et al., 2007). Chedea et al. (2010) reported that GSE considered as a powerful antioxidant nutritive supplement that prevent premature ageing and diseases. Maier et al. (2009) stated that oil produced from grape seeds is considered a rich source of polyphenolics with strong antioxidant activity.

Grape seed extract is a natural extract from the seed of grape (Asl and Hosseinzeadeh, 2009). It is a rich source of one of the most beneficial groups of plant flavonoids and pro-anthocyanidins oligomers (El-Ashmawy et al., 2007). It has a protective effect on oxidant-induced production and deposition of extracellular matrix components (Delundu et al., 2007). GSE contains mainly flavonoids, which involved in ameliorating the oxidative stress in vitro and in vivo (Martinez-Florez et al., 2002). Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals, and that they have antimutagenic and anticarcinogenic
effects (Li et al., 2001 and Maier et al., 2009). Aysun et al. (2008) reported that GSE is widely consumed as a dietary supplement and could be useful in synergizing the efficacy of chemotherapeutic agents in cancer treatment. Therefore, the present study was designed to investigate the role of GSE against AlCl3-induced oxidative stress and reproductive toxicity in rat testes.

2. Material and Methods

Chemicals:
Aluminium chloride (AlCl3) was obtained from Sigma Chemical Co. (St Louis, Mo, USA). Casein (> 85% protein) was obtained from Misr Scientific Company, Dokki, Giza, Egypt. Cellulose and D-L methionine were purchased from Morgan Company, Cairo, Egypt. Minerals and vitamins constituent, sucrose, glucose and ethanol absolute were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt. Corn oil was obtained from the local market. Corn starch was obtained from Starch and Glucose Company, Helwan, Egypt.

Animals:
Twenty sexually mature male albino rats, Sprague Dawley strain, weighing (160 ± 10 gm) were purchased from the animal house of Ophthalmic Research Center, Giza, Egypt. The animals were housed in plastic cages, maintained on a natural light-dark cycle at room temperature of 26 ± 2ºC and fed standard diet according to Reeves et al. (1993) and water ad libitum. Rats were kept for one week as acclimatization period before the start of the experiment. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. The experiment was conducted at Faculty of Veterinary Medicine, Cairo University.

Preparation of grape seed extract:
Ripe grapes (Vitis vinifera L., Vitacease) were obtained from El-Behira government, Egypt. Undamaged and disease-free berries were snipped from clusters. Following manual separation of the seeds from whole berries, seeds were oven dried at 30 - 40 ºC. Dried grape seeds were ground to fine powder with a grinder. The ethanolic extract was prepared by soaking 100 gm of grape seeds powdered in 300 ml ethanol (95 %) with daily shaking and kept in refrigerator covered by a piece of aluminum foil. The infusion was filtered by a piece of double gauze and the filtrate was centrifuged at 3000 rpm for 10 minutes, then the ethanol was evaporated using a rotatory evaporator apparatus (Switzerland) attached with vacuum pump. The 100 gm of dried grape seeds powder yield 26.7 gm ethanol extract.

Experimental design:
After the acclimatization period, rats were divided into four equal groups, each of five rats. First group: was negative control administered 3 ml distilled water orally once daily. Second group: was positive control group (AlCl3 group) administered aluminium chloride (20 mg/kg bw, 1/20 LD50) dissolved in 3 ml distilled water, the LD 50 of (AlCl3) when administered orally to rats was reported to be (380 - 400 mg/kg bw (Krasovskii et al., 1979). Third group: was administered grape seed extract (GSE) (75 mg/kg bw) which dissolved in 3 ml distilled water orally once daily according to El-Ashmawy et al. (2007). Fourth group: was co-administered with AlCl3 and GSE in the same doses in 2nd and 3rd groups. Doses were given once daily via gavage for 70 consecutive days, for completion of the spermatogenic cycle and maturation of sperms in epididymis (Sarkar et al., 2003).

Blood sampling:
At the end of the experimental period, animals were fasted overnight, with free access to tap water. Rats in each group were anesthetized with diethyl ether for blood sampling by puncturing the inner canthus of the eye using heparinized microhematocrit tube. Collected blood was stored for 15 min at room temperature, then centrifuged with 3000 rpm for 20 min., and stored at -20ºC till analysis.

Sex organs weight:
The testes and accessory sex organs (seminal vesicles and prostate glands) were dissected out, trimmed off the attached tissues and weighed. The index weight of the organ was calculated by (Index weight) = organ weight/ body weight x 100.

Determination of testosterone assay:
Serum testosterone level was estimated using method of Ismail (1986).

Semen characteristics:
Seminal content of epididymis was obtained by cutting of cuda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted 10 times with 2.9 % sodium citrate dehydrate solution and thoroughly mixed to estimate the progressive motility and sperm concentration (Bearden and Fluquary, 1980). One drop of the suspension was smeared on a glass slide and stained by Eosin-nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities (Miller and Pass, 1952). Abnormal head and tails were evaluated according to (Nahas et al., 1989, Mori et al., 1991 and Okomura et al., 2005).
Determination of testicular antioxidant enzymes:
Preparation of testicular homogenate:
One testis of each sacrificed rat was used for estimation of oxidative enzymes and lipid peroxidation. One gram of testicular tissue was weighed after ice water washing of testes and homogenized in 9 volume buffered saline 0.9 %, centrifuged at 4000 rpm at 4 ° C for 15 min., the supernatant was collected and kept at -20 ° C till further investigation.

Determination of testicular thiobarbituric acid reactive substance formation (TBARS):
According to the method of Esterbauer and Cheeseman (1990), the extent of lipid peroxidation in terms of thiobarbituric acid reactive substances (TBARS) formation was measured. Tissue supernatant was mixed with 1 ml trichloroacetic acid (TCA) (20%), 2 ml thiobarbituric acid (TBA) (0.67 %) and heated for 1 h at 100 °C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. As 99% of TBARS was malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is 1.56 × 105 M−1 cm−1.

Determination of testicular superoxide dismutase activity (SOD):
Superoxide dismutase (SOD) activity was measured using assay kit (Cayman, MI, USA) according to (Giannopolitis and Ries, 1977). This kit utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. The SOD assay measures all the three types of SOD (Cu/Zn, Mn, and Fe SOD). Enzyme activity was determined as the amount of the enzyme to reach an inhibition of 50 % nitro-blue tetrazolium (NBT) reduction rate.

Histopathological examination:
Sections were taken from testis tissues from different animals in each group immediately after sacrificed. The tissues were washed with the normal saline solution to remove blood, fixed in 10% neutral formalin for a period of at least 24 hrs, dehydrated in different grades of alcohol, and processed for paraffin embedding. Sections of 5 µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series, stained with Haematoxylin and Eosin, cleared in xylene and examined microscopically according to Bancroft et al. (1996).

Statistical analysis:
The data were analyzed statistically by analysis of variance, for statistical significance (p ≤ 0.05) using L.S.D. test, one way ANOVA, post hoc multiple comparisons according to Snedecor and Cochrion (1989). An IBM computer with a software system SPSS version 16 was used for these calculations.

3. Results

Body and sex organs weight:
The effect of grape seed extract (GSE) on final body weight and weight gain in AlCl₃ toxicity of male rats is represented in Table (1). Oral administration of GSE had no effect on body weight gain of rats, indicating its safe use under the experimental conditions, while there were highly significant decrease in final body weight and body weight gain (p <0.01) in AlCl₃ group (+ve) as compared with negative control group (-ve). On the other hand, there were highly significant elevation in final body weight and weight gain in AlCl₃ group treated with GSE as compared with untreated AlCl₃ group (+ve).

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (- ve)</td>
<td>165.6 ± 1.21</td>
<td>269.2 ± 1.77</td>
<td>103.6 ± 2.06</td>
</tr>
<tr>
<td>AlCl₃ (+ ve)</td>
<td>166.0 ± 1.41</td>
<td>202.0 ± 2.43 a**</td>
<td>36.0 ± 3.61 a**</td>
</tr>
<tr>
<td>GSE</td>
<td>167.8 ± 2.65</td>
<td>270.8 ± 3.31</td>
<td>103.0 ± 3.66</td>
</tr>
<tr>
<td>AlCl₃ + GSE</td>
<td>164.4 ± 1.63</td>
<td>253.0 ± 1.64 a*<strong>b</strong></td>
<td>88.6 ± 2.23 a*<strong>b</strong></td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 rats ± SE.
- a Significant difference from control group at p < 0.05* and highly significant difference from control group at p < 0.01**.
- b Significant difference between AlCl₃ group and AlCl₃ group treated with GSE at p < 0.05* and highly significant at p < 0.01**.
Results indicated significant decrease in the index weights of testes and prostate (p<0.01 and p <0.05, respectively), and non-significant decrease in index weights of seminal vesicle in AlCl₃ group as compared with negative control group (Table 2). Treatment with GSE ameliorated the toxic effect of AlCl₃, the index weights of testes recorded highly significant elevation as compared with untreated AlCl₃ group. While, oral administration of GSE alone did not cause any significant effect on the weights of the tested tissues as compared with negative control group.

Table (2): Effect of grape seed extract (GSE) on index weights (IW) of testes and sex organs of AlCl₃ intoxicated male rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Sex organs (g / 100 g body weight)</th>
<th>Testes</th>
<th>Seminal vesicle</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>0.922 ± 0.041</td>
<td>0.415 ± 0.023</td>
<td>0.130 ± 0.006</td>
</tr>
<tr>
<td>AlCl₃ (+ ve)</td>
<td></td>
<td>0.488 ± 0.018 a**</td>
<td>0.384 ± 0.013</td>
<td>0.118 ± 0.004 a*</td>
</tr>
<tr>
<td>GSE</td>
<td></td>
<td>0.918 ± 0.019</td>
<td>0.423 ± 0.012</td>
<td>0.124 ± 0.0036</td>
</tr>
<tr>
<td>AlCl₃ + GSE</td>
<td></td>
<td>0.68 ± 0.023 a** b **</td>
<td>0.404 ± 0.011</td>
<td>0.121 ± 0.002</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 rats ± SE.
- a Significant difference from control group at p < 0.05* and highly significant difference from control group at p < 0.01**.
- b Significant difference between AlCl₃ group and AlCl₃ group treated with GSE at p < 0.05* and highly significant at p < 0.01**.

Sperm characteristics:

Epididymal sperm concentration, sperm motility, viability and abnormal sperm are reported in Table (3) for AlCl₃ and/or GSE groups. GSE group did not differ significantly from the control (-ve) in terms of sperm motility, sperm viability and abnormal sperm rates, but had highly significant elevation in sperm count (p <0.01) as compared with negative control group (-ve). AlCl₃ group (+ve) had significantly lower sperm count, motility and viability than the control group (-ve).

Table (3): Effect of grape seed extract (GSE) on sperm character of AlCl₃ intoxicated male rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Sperm character</th>
<th>Count (10⁶/ml)</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
<th>Sperm abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>62.0 ± 3.63</td>
<td>82.4 ± 4.53</td>
<td>89.6 ± 4.31</td>
<td>6.6 ± 0.51</td>
</tr>
<tr>
<td>AlCl₃ (+ ve)</td>
<td></td>
<td>28.6 ± 1.29 a**</td>
<td>42.0 ± 3.73 a**</td>
<td>52.0 ± 3.65 a**</td>
<td>18.4 ± 0.93 a**</td>
</tr>
<tr>
<td>GSE</td>
<td></td>
<td>75.2 ± 3.84 a**</td>
<td>80.6 ± 3.92</td>
<td>90.0 ± 4.73</td>
<td>5.6 ± 0.24</td>
</tr>
<tr>
<td>AlCl₃ + GSE</td>
<td></td>
<td>43.2 ± 3.22 a** b **</td>
<td>58.4 ± 2.16 a** b **</td>
<td>68.8 ± 2.85 a** b **</td>
<td>12.0 ± 0.71 a** b **</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 rats ± SE.
- a Significant difference from control group at p < 0.05* and highly significant difference from control group at p < 0.01**.
- b Significant difference between AlCl₃ group and AlCl₃ group treated with GSE at p < 0.05* and highly significant at p < 0.01**.

Serum testosterone and testicular antioxidant enzymes:

Results in Table (4) showed highly significant decrease in serum testosterone concentration (p < 0.01) in AlCl₃ group compared to control group (-ve), while orally treatment with GSE induced highly significant elevation in serum testosterone concentration (p < 0.01) and alleviated the negative effects of AlCl₃ as compared with untreated AlCl₃ group.

Table (4): Effect of grape seed extract (GSE) on serum character of AlCl₃ intoxicated male rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Serum character</th>
<th>Count (ng/ml)</th>
<th>Antioxidant enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td></td>
<td>6.32 ± 0.73</td>
<td>SOD: 12.6 ± 0.23</td>
</tr>
<tr>
<td>AlCl₃ (+ ve)</td>
<td></td>
<td>2.9 ± 0.3 a**</td>
<td>TBARS: 10.8 ± 0.5 a**</td>
</tr>
<tr>
<td>GSE</td>
<td></td>
<td>6.8 ± 0.7 a**</td>
<td></td>
</tr>
<tr>
<td>AlCl₃ + GSE</td>
<td></td>
<td>4.2 ± 0.4 a**</td>
<td></td>
</tr>
</tbody>
</table>

- a Significant difference from control group at p < 0.05*.

On the other hand, AlCl₃ caused highly significant decline in SOD accompanied with highly significant elevation in TBARS compared to control group (-ve). While co-administration with GSE and AlCl₃, improved the toxic effect of AlCl₃ as manifested by highly significant increase in SOD accompanied with highly significant decrease in TBARS as compared with untreated AlCl₃ group.
Table (4): Effect of grape seed extract (GSE) on serum testosterone level and testicular oxidative state of AlCl₃ intoxicated male rats.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Serum testosterone (ng/ ml)</th>
<th>Testicular oxidative (µ/mg protein)</th>
<th>TBARS (n mol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>1.86 ± 0.049</td>
<td>0.068 ± 0.003</td>
<td>0.074 ± 0.002</td>
</tr>
<tr>
<td>AlCl₃ (+ve)</td>
<td>0.936 ± 0.046**</td>
<td>0.031 ± 0.001**</td>
<td>0.116 ± 0.001**</td>
</tr>
<tr>
<td>GSE</td>
<td>1.846 ± 0.043</td>
<td>0.073 ± 0.003</td>
<td>0.066 ± 0.002</td>
</tr>
<tr>
<td>AlCl₃ + GSE</td>
<td>1.356 ± 0.05**</td>
<td>0.044 ± 0.001**</td>
<td>0.103 ± 0.004**</td>
</tr>
</tbody>
</table>

- Each value represents the mean of 5 rats ± SE.
- * Significant difference from control group at p < 0.05* and highly significant difference from control group at p < 0.01**.
- b Significant difference between AlCl₃ group and AlCl₃ group treated with GSE at p < 0.05* and highly significant at p < 0.01**.

**Histopathological results:**

Microscopical examination of the testis of normal negative control group (-ve) revealed the normal histopathological structure of seminiferous tubules (Fig 1). Meanwhile, testis of rat group orally administered AlCl₃ (+ve) showed congestion of interstitial blood vessel (Fig 2), marked degeneration and necrosis of germ cells lining seminiferous tubules (Fig 3), as well as interstitial edema and testicular degeneration with complete absence of germ cells (Fig 4). Testis of rats group received GSE showed no histopathological changes (Fig 5). In rats group received AlCl₃ and treated with GSE, improvement in histopathological examination was noticed in testis sections as shown in (Fig 6), where examined sections revealed no histopathological changes.

Fig (1): Testis of control group (-ve) showing normal histological structure of seminiferous tubules. (H&E x 200)

Fig (2): Testis of AlCl₃ group (+ve) showing congestion of interstitial blood vessel as well as marked degeneration and necrosis of germ cells lining seminiferous tubules. (H&E x 200)

Fig (3): Testis of AlCl₃ group (+ve) showing marked degeneration and necrosis of germ cells lining seminiferous tubules. (H&E x 200)

Fig (4): Testis of AlCl₃ group (+ve) showing interstitial edema and testicular degeneration with complete absence of germ cells. (H&E x 200)
4. Discussion:

Grape seed extract is an extract by-product obtained from the seed of Višnjevina, it contains a variety of biologically active species used for protection against oxidative stress induced by free radicals and reactive oxygen species (ROS) (Sharma et al., 2004). Aluminium is considered to be a non-redox active metal, it promotes biological oxidation both in vitro and in vivo because of its pro-oxidant activity (Turner and Lysiak, 2008). Testicular oxidative stress appears to be a common feature in much of what underlies male infertility, which suggests that there may be benefits to develop better antioxidant therapies for relevant cases of hypospermatogenesis (Turner and Lysiak, 2008 and Yousef and Salama, 2009). GSE has antioxidant and free radical scavenging activity (Jayaprakasha et al., 2003 and Caillet et al., 2006). Most of the beneficial health effects of GSE are attributed to their antioxidant and free radical scavenging properties (Faria et al., 2006).

The present results showed that oral administration of GSE had no effect on body weight gain and index weights of sex organs of rats, this confirmed its safe use and agreed with Clifton (2004) who mentioned that GSE is widely marketed as a dietary supplement and is considered safe for human consumption. Moreover, GSE- containing flavonoids are currently used as nutritional supplements, and have been shown to exert antioxidant, chemopreventive and anticancer effects (Singletary and Meline, 2001, Shi et al., 2003 and Mosaad et al., 2006). On the other hand, our results indicated significant decrease in the body weight gain and the index weights of testes and prostate (p< 0.01, p <0.01 and p < 0.05, respectively) in rats group received AlCl₃ as compared with control, and highly significant decrease in the body weight gain and the relative weights of testes (p < 0.01) compared with AlCl₃ treated with GSE.

The majority of studies that utilized chronic doses of aluminium reported significant reduction in weight gain, particularly in studies initiated in male animals, Kowalczyk et al. (2004) found that during three months observation of rats receiving aluminium chloride, decrease in water and food intake and transient diarrhea occurred, which resulted in lowering of final body mass of animals in comparison to the controls (differences statistically significant). The physiologic basis for this outcome is unclear, but it was reported that animals exposed to chronic doses of aluminium consumed less food. Whether general effects of aluminium on metabolic processes depress metabolism or reduce nutritional efficiency remains to be resolved. The obtained results were in agreement with Yousef et al. (2005) and Guo et al. (2005 a,b). In addition, (Bataineh et al., 1998) found decrease in absolute and relative testes weights and seminal vesicles weights after aluminium chloride ingestion. The decrease in the reproductive organs weights could be due to the decrease in testosterone level which was observed in the current study, that may be resulted from the oxidative damage induced in rat testes (El-Ashmawy et al., 2007). The amelioration effect of GSE may be due to grape seeds which are rich sources of monomeric phenolic compounds such as catechin, epicatechin, dimeric, trimeric and tetrameric proanthocynidins (Escríban-Bailón et al., 1992). These molecules possess a structure that confers on them an antioxidant property, which has been demonstrated to exert a novel spectrum of biological, pharmacological, therapeutic, and chemo-protective effects against oxygen free radicals and oxidative stress (Bagchi et al., 1997).

AlCl₃ induced highly significant decrease in sperm count, motility (%) and viability (%), with increase in dead and abnormal sperm count as compared to both control group (-ve) and AlCl₃
group treated with GSE, this means that GSE minimized the toxicity of AlCl₃. Moreover, rats orally administered GSE alone showed highly significant increase in sperm count, but had no effect on the other sperm character determined. Previous studies showed that, sexual behavior of male rats was suppressed after ingestion of aluminum chloride (Bataineh et al., 1995). Necrosis of spermatocytes/spermatids was observed in the testes of mice exposed to aluminium (Llobrt et al., 1995). Yousef et al. (2007) showed also that AlCl₃ declined semen quality in vivo and vitro, and induced significant decrease in ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, packed sperm volume, normal and live sperm, while dead and abnormal sperm were increased. The current results may be explained by aconitase, a protein that binds citrate and catalyzes its isomerization to isocitrate via the intermediate cis-aconitate in Krebs cycle, which showed decreased activity in the presence of aluminium, thus influence mitochondrial enzymes, consequently, changes in mitochondrial functions may be reflected in sperm motility and viability (Yousef et al., 2007). Moreover, the observed decrease in sperm motility could be attributed in part to the concomitant reduction in testosterone production following aluminium treatment (Guo et al., 2005a and Yousef et al., 2005).

Testosterone is a key hormone that regulates spermatogenesis. AlCl₃ induced significant (p < 0.01) decrease in serum testosterone concentration and SOD activity, with significant increase in TBARS levels compared to control rats. However, it was found that, GSE was capable of restoring the SOD activity and serum testosterone level in rats group administered AlCl₃ and treated with GSE, there were significant differences as compared with untreated AlCl₃ group (+ve). Also, GSE significantly reduced TBARS levels compared to AlCl₃ group, this means that GSE increased the process of steroidogenesis and hence testosterone production and improved sperm production and the process of fertility. These results were in line with the results reported by Yousef (2004) who showed that aluminium chloride was able to generate reactive oxygen species in rabbit's testes. Yousef et al. (2005) found that aluminium enhanced lipid peroxidation in plasma, testes and liver. Guo et al. (2005a) demonstrated also that, exposure to aluminum lowered plasma and testicular testosterone levels in mice. The authors suggested that the severe reduction in male fertility following aluminium administration might result from excessive aluminium accumulation in the testes and low testosterone concentrations. Moreover, Turner and Lysiak (2008) found over productive of ROS, which can be detrimental to sperm and associated with male infertility, and thus spermatoxic effect might be due to AlCl₃ induced free radicals.

There is evidence implicating androgenic hormones involved in mechanisms of aluminium toxicity on male reproduction. Guo et al. (2005a) found that aluminium administration significantly increased nitric oxide (NO) production and decreased both testicular adenosine 3′, 5′-cyclic monophosphate (cAMP) and testosterone levels. Excessive NO activated inducible NO synthase (NOS) which may be involved in reproductive toxicity of aluminum, hence reducing rate and motility of sperm cells, increasing their morphological abnormalities, and suppressing testosterone secretion in male rats. Moreover, these effects of AlCl₃ may be attributed to aluminium ability to cross the blood-testis barrier, after inducing oxidative stress and lipid peroxidation that damages the biological membranes in the testes, this in turn causes the degeneration of the spermatogenic, which disrupts spermatogenesis and reduces sperm counts (Latchoumycandane et al., 2002). Also, the increase in TBARS can bring negative effects on motility and sperm-oocyte fusion (Kim and Parthasarathy, 1998), which was found in the present study. Moreover, increased ROS subsequently attack almost all cell components including lipid membrane and producing lipid peroxidation (Flora et al., 2003). The protective effect of GSE treatment agreed with Aysun et al. (2008) who reported that oral intake of GSE reduced the oxidative stress. In addition, GSE treatment considerably increased the formation of antioxidant products which may be regarded to the phenolic constituents of GSE and its antioxidant activity. Flavonoids have been shown to alleviate the oxidative stress by increasing the endogenous antioxidant status, protecting cells against free-radical damage by increasing resistance to oxidative stress (Perez et al., 2002).

Histopathological examination of rats group orally administered AlCl₃ (+ve) showed apparent alteration in the testes, where it induced marked degeneration and necrosis of germ cells lining seminiferous tubules, as well as interstitial oedema and complete absence of germ cells. Meanwhile, treatment of AlCl₃ group with GSE showed noticeable improvement in histopathological changes induced by AlCl₃ in testis sections. The histological changes in testes of rats administered AlCl₃ are in agreement with Khattab (2007) who studied the effect of AlCl₃ on the rat's testes. Also, Guo et al. (2005b) observed deleterious effects and histopathological changes in testicular tissues after 2 weeks of aluminium treatment, as well as noticeable spermatogenetic loss as necrosis in the spermatids.
and spermatozoa at the 5th week of aluminium treatment. This damage effect may be explained by Yousef and Salama (2009) who reported that oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity of the stressed tissue. Many conditions or events associated with male infertility are inducers of oxidative stress, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis, such stress condition, can cause changes in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell apoptosis. Moreover, reactive oxygen species and oxidative damage of bimolecular may contribute to male infertility by reducing sperm function (Atessahin et al., 2005). Minimizing the hazard effects of AlCl₃ by GSE treatment may be due to the flavonoids in GSE, which exert many health-promoting effects, including the ability to increase intercellular antioxidant levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals (Singth et al., 2004).

In conclusion, AlCl₃ caused fertility disturbances and testicular dysfunction, decrease in antioxidant enzymes and increase in lipid peroxidation in testes. Treatment with GSE showed protective effect against its reproductive toxicity and this may be attributed to the activity of GSE as a natural antioxidant. Oxidative stress is one of the mechanisms of AlCl₃ reproductive toxicity and GSE has protective effect against such oxidative damage. Additional studies are needed to demonstrate GSE efficacy in humans.

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References