5 Aspects of male reproductive toxic effects of *Quassia amara* L.: Spermatological, histopathological and ultrastructural study in Swiss mouse

5.1 Introduction

*Quassia amara*, belonging to the family Simaroubaceae, is a small tropical tree, growing 2–6 m tall, distributed abundantly in Brazil (Cabral et al. 1993). This plant is commonly called amargo or Surinam wood. The bark and leaves of this plant are widely used as a traditional herbal medicine, as a tonic, digestion promoter, blood cleanser, insecticide and mild laxative, and it is also recommended for diarrhea, intestinal worms, dysentery, dyspepsia, excessive mucus, stomach-ache, anemia and gastrointestinal disorders. Amargo is a tribal remedy for debility, digestion problems, fever, liver problems, parasites, malaria, snakebite, and back spasms throughout South America (Amargo Database). A catalogue of pharmacological activities of this plant include anti-malarial (Trager & Polonsky 1981), anti-neoplastic (Kupchan & Streelman 1976), anti-viral (Apers et al. 2002), anti-feedant (Flores et al. 2008), amebicidal (Apers et al. 2002), anti-ulcerogenic (Toma et al. 2002) and insecticidal (Park et al. 1987, Mancebo et al. 2000;) properties. The key constituents of this plant bark are quassinoids (seco-triterpenoid compounds), including quassin, neoquassin, paraine, nigakilactone A and 18-hydroxyquassin (Grieco et al. 1988, Ohmoto et al. 1989, Diakanamwa et al. 1993) and also certain indole alkaloids (derivatives of canthin-6-one) (van Valkenburg & Bunyaphaphatsara 2002). *Quassia amara* and the major secondary metabolite in it, quassin, are extensively tested for anti-malarial activity (O’Neill et al. 1986, Ajaiyeoba & Krebs 2003, Vigneron et al. 2005, Bertani et al. 2007).

Male reproductive system, particularly spermatogenesis, sperm maturation and androgen biosynthesis, is highly sensitive to toxic inflictments (Boekelheide 2005). This is particularly relevant in the context of serious concerns in the recent past regarding deterioration of human and animal male reproductive health (Carlsen et al. 1992, 1993, Sharpe & Skakkebaek 1993, Veeramachaneni 2000, Handelsman 2001). The causative factors for this deterioration have been traced to environmental,
industrial and occupational chemicals, therapeutics, dietary toxins, lifestyle factors, etc. (Skakkebaek et al. 1998, Aruldhas et al. 2006, Slama et al. 2006, Winters et al. 2006). Herbal ingredients are readily used by millions of people without prescription under the belief that anything natural is safe. Like allopathic (prescription) drugs, herbal medicines also have different pharmacokinetic and pharmacodynamic properties which ultimately lead to produce therapeutic responses, but sometimes cause adverse actions and/or drug-herbal interactions (Capasso et al. 2000, Negi et al. 2008). Such adverse actions would affect aspects of male reproductive physiology (Akbarsha et al. 1998, 2000b, Akbarsha & Murugaian 2000). In fact the much talked about phytochemical approach to male contraception is a product of such toxic side effects since toxicology and contraception are two sides of the same coin.

Thus, in as much as *Q. amara* has extensive application as a medicinal plant, there are reports of preliminary studies implicating it in male reproductive toxicity. It was first reported in 1995 that a crude methanol extract of the stem wood of *Q. amara* inhibited both the basal and LH-stimulated testosterone secretion of rat Leydig cells in a dose-dependent manner, and quassin proved to be the bioactive agent (Njar et al. 1995). In another study in rat, the crude methanol extract of the stem wood caused a significant reduction in the weight of the testis, epididymis and seminal vesicles, but an increase in that of the anterior pituitary gland. Epididymal sperm counts and serum levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were significantly reduced (Raji & Bolarinwa 1997). In a third study, single daily intramuscular injections of the chloroform extract of *Q. amara* bark for 15 days resulted in a significant reduction in the weight of testis and epididymis but not that of the seminal vesicles and prostate, and a marked decrease in the counts, motility and viability was also observed in spermatozoa collected from the cauda epididymidis of the treated animals (Parveen et al. 2003). In a more recent paper we demonstrated that treatment of *Q. amara* crude extract to mouse can bring about generation of certain unusual sperm morphologies (Faisal et al. 2006). Taking leads from these reports, *Q. amara* was subjected to a more directed investigation and we tested the methanol extract as well as the major compound quassin in the Swiss mouse model, adopting gravimetric, sperm parametric, histo-pathological and ultrastructural tools.
5.2 Materials and Methods

The bark of *Q. amara* was a gift from Late Dr. BMJ Pereira, IIT Roorkee, Uttarakhand, India. 200g of the powdered bark of the plant was extracted (20 cycles) in 1L of methanol using a Soxhlet apparatus and the solvent was evaporated using a rotary evaporator to about 50ml and further condensed by air drying at room temperature to yield approximately 2g of crude extract and this was stored at 0°C till use. Quassin was a gift from TRIFOLIO-M GmbH, Germany. The condensed methanol extract and quassin were dissolved in minimum quantity of ethanol and further diluted with phosphate buffered saline (PBS). Sixty 90 day old male Swiss albino mice, each weighing 30-35g, raised from a stock obtained from Indian Institute of Science, Bangalore, India, were divided into five groups of 10 each. A common control group was administered with PBS, and the remaining groups were injected, at a daily dose, with methanol extract (25 mg/kg, 50 mg/kg and 100 mg/kg body weight) or quassin (1mg/kg body weight), through intraperitoneal (*ip*) route for 35 days, the duration of one spermatogenic cycle in mouse (Oakberg 1956). At the end of the treatment, five mice in each group were dissected under mild anesthesia and the testicles and epididymides were removed. After a thorough wash in normal saline, 0.5 µl fluid from the cauda epididymidis was collected in a micropipette and diluted with 99.5 µl PBS, pH 7.4, according to Akbarsha *et al.* (2000b). The sperm numbers were assessed in a Neubauer counting chamber and expressed as number per ml of the original cauda epididymidal fluid. The number of motile spermatozoa was assessed in a hanging drop preparation and observed in phase contrast and/or dark field illumination in a Carl Zeiss Axioscope 2 Plus research microscope (Jena, Germany) at x400 magnification.

In order to find the viability and morphology of spermatozoa, fresh spermatozoa from the above preparation were stained with acridine orange (*AO*) and ethidium bromide (*EB*), according to Spector *et al.* (1997). A small drop of dilute semen was placed on a glass slide, and 10 µl each of AO (Sigma Chemical Co., USA; 100 µg/ml in normal saline, pH 7.4) and EB (Bio-Rad, USA; 100 µg/ml in normal saline, pH 7.4) were added separately. A cover slip was placed and the edges were sealed with fingernail polish. The preparations were observed in the same microscope, now with epifluorescent attachment. To calculate the *per cent* viable spermatozoa, 100 randomly selected spermatozoa from each slide were observed and categorized into viable...
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(spermatozoa with green fluorescence) and nonviable (spermatozoa with red fluorescence) and the data were expressed as per cent of viable spermatozoa. In all cases images were captured in a Pentium III computer via a Sony DXC-151AP CCD camera (Tokyo, Japan) using Carl-Zeiss Axiovision image-analysis software. For counts of spermatozoa with morphological abnormalities, 200 randomly selected spermatozoa from each slide were observed, assigned to the categories (Mortimer & Mortimer 2005) and expressed in per cent as in the case of viability. Giemsa’s stained smears were used to characterize the abnormal sperm morphologies.

Testicles and epididymides were excised from the remaining control and treated animals (five each), after perfusion of the male reproductive system with Karnovsky’s fluid (1965), cleared of fat and connective tissue, and weighed to nearest milligram in an electronic balance (Anamed, India) and then used for further histopathological and ultrastructural studies. Thin sections of the testis and epididymis were fixed in glutaraldehyde and post-fixed in osmium tetroxide (Hess & Thurston 1977). After a thorough wash in buffer (Hayat 1981), the sections were dehydrated in ethanol, cleared in propylene oxide and embedded in thin viscosity resin (Araldite CY212, SPI-Chem™, Switzerland). Semithin sections (1μm thick), obtained in a Leica (Jena, Germany) ultramicrotome, were stained in toluidine blue-O (TBO) (pH 4.4) for observation in the research microscope and photomicrographs were obtained. Ultrathin sections were obtained in the same microtome and stained with 0.1% lead citrate and 6% uranyl acetate. The sections were analyzed in a transmission electron microscope (Phillips 201C; Amsterdam, The Netherlands). The images of interest were photographed. Measurements were made in a computer using Axiovision (Carl-Zeiss, Jena, Germany) software. The height of the epididymal epithelium and diameter of the seminiferous tubules were calculated by measuring 25 randomly chosen tubules, circular in cross section, to find the mean ± SD. This study was approved by the Institutional Animal Ethics Committee (IAEC).

5.3  Results

5.3.1  Body weight and reproductive organ weights

Body weight of the mice before and after treatment or between the groups after treatment did not differ significantly. However, weight of the testes and epididimides
decreased significantly in a manner dependent on the dose in the mice treated the extract (25 mg/kg <50 mg/kg < 100 mg/kg methanolic extract) (Table 1). In the quassin-treated mice also, these parameters decreased to significant levels (Table 1).

5.3.2 Sperm parameters

There was a drastic reduction in the sperm count, motility and viability in all the treated mice, in dose-dependent manner in the case of treatment with the methanol extract (Table 1). In the quassin-treated mice also the count decreased to 39%, progressive motility to 22% and viability to 41% compared to control animals. The percentage of spermatozoa with abnormal morphologies increased in the treated mice and this effect also followed the same pattern as above. The major head abnormalities included head alone, tail alone, macrocephali and kinked head. The tail abnormalities included retention of cytoplasmic droplet, and coiling of the tail at various levels and to various extents. A few spermatozoa had double tail and a few others remained fused in pairs. A few spermatozoa had the tail coiled around the head or pin head (Fig. 1).

5.3.3 Histopathological changes

The major histopathological changes in the testis included loss of germ cells, cytoplasmic vacuolation and breaking away of apical portions of Sertoli cells, formation of giant spermatids and vacuolation of Leydig cells. Both in the methanolic extract- (here, with indication of dose-dependence) as well as quassin-treated animals, germ cells immediately lining the lumen, were prematurely released to various degrees (Fig. 2a-c). Stage 12 tubules were the worst affected. In extreme cases, all germ cells except the dark and light spermatogonia reflected pathologies of one kind or another (Fig. 3a, b). In some cases, germ cells from intercalary region of the epithelium were missing (Fig. 2d). Sertoli cells invariably indicated pathological changes which included vacuolation of cytoplasm and accumulation of a dense material in large vacuoles (Fig. 3 c-f) and apical pinching of Sertoli cells either above or below the level of the nucleus (Fig. 2c). In about 5% of the tubules, the round spermatids were found as multinucleate giant cells or symplasts (Fig. 2e). In all treated animals invariably all the Leydig cells were thoroughly vacuolated (Fig. 2f). The vacuoles were either empty (Fig. 3g) or contained an electron-dense material (Fig. 3h). Though the epithelium of the ductus epididymidis at the five major segments, initial segment, intermediate
segment, caput, corpus and cauda, maintained height as in the control, there were small to severe histopathological changes which included the following:

- Vacuolation of epithelium (Fig. 4a).
- Appearance of pale vacuolated epithelial cells (Agnes & Akbarsha 2001), either empty (Fig. 4b) or containing a dense material (Fig. 4c).
- In the principal cells, particularly of the initial segment, the Golgi apparatus was hypertrophied and vacuolated (Fig. 5a) and the perinuclear cytoplasm contained an electron-dense material (Fig. 5b). Several principal cells were necrotic and opened up towards the lumen providing for access to entry of spermatozoa (Fig. 4d).
- The clear cells were either increased in granulation (Fig. 4e) or granulation as well as vacuolation (Fig. 4f, 5c) and in the latter case the vacuoles contained an opaque material (Fig. 4f).
- In a few clear cells the mitochondria became profuse (Fig. 5d).
- A few cells closer to the basement membrane were pycnotic and appeared rounded and detached from the surrounding cells (Fig. 4g).
- In the initial segment and intermediate zone the narrow cells and apical cells reflected hyperplasia and hypertrophy and these cells projected deep into the lumen (Fig. 6a-c, 5e).
- In a few tubules the principal cells were double the size of normal cells (Fig. 6d, e).
- Microcanalization in the epididymal epithelium was evident (Fig. 6f).
- The basal cells increased in the content of lipofuscin material and this material reflected different levels of condensation (Fig. 5f).
- One major observation was the tendency of principal cells of the initial segment to divide, as seen in the apical location of the nucleus, the mitotic figures and the binucleate cells representing cytokinesis (Fig. 7a-d). This was not observed in the epididymis of control mice.
- The lumen of the epididymis abounded with large cells which were undifferentiated germ cells arriving from seminiferous epithelium (Fig. 7e).
• Extensive macrophage activity in the lumen, phagocytosing spermatozoa, was evident (Fig. 7f-h).

The cauda epididymidal spermatozoa, as observed in TEM sections, indicated several abnormal morphologies which included the following:

• Coiling of the flagellum around the head (Fig. 8a-c). This is the section corresponding to the spermatozoa in Figure 2.
• Retention of cytoplasmic droplet (Fig. 8d) accompanied by folding of the flagellum (Fig. 8e).
• Several spermatozoa retaining the CD possessed abnormal inclusions in the cytoplasm (Fig. 8f, g).
• Several spermatozoa exhibited bizarre morphologies like single head and several flagella in section and fusion of flagella of two or more sperm (Fig. 8h-i).

5.4 Discussion

The present study shows that treatment of *Quassia amara* methanolic extract and its major secondary metabolite, quassin, can potentially affect the male reproductive mechanisms as revealed in the gravimetric, histometric, spermatological, histopathological and ultrastructural changes in the treated mice, strengthening the inferences in the previous preliminary studies (Njar *et al.* 1995, Raji & Bolarinwa 1997, Parveen *et al.* 2003, Faisal *et al.* 2006).

The decreased sperm counts may be explained in the light of the histological evidence indicating impact of *Q. amara* / quassin on spermatogenesis and bringing about premature loss of germ cells. It is a clear indication of direct or indirect toxic effect of the extract or quassin treatment to the spermatogenic compartment. Although a direct effect of *Q. amara* / quassin on male germ cells may not be excluded, Sertoli cell can be one of the principal targets. Male germ cells are held in position or translocated along the seminiferous epithelium by the activity of inter-Sertoli cell tight junctions. Premature release of germ cells from the Sertoli cell is thought to be a consequence of disruption of inter-Sertoli cell junctions (Nakai & Hess 1994, Akbarsha *et al.* 2000, Richburg 2000).
The aspects of the spermatogenesis, including the structure and function of the Sertoli cells, are under the control of testosterone produced by the Leydig cell (Bremner et al. 1994, De Gendt et al. 2004). We have obtained histological evidence of pathological changes in the Leydig cell in the present study. Similar pathological changes due to the treatment of dimethane sulfonate (Jackson et al. 1986) and vincristine (Akbarsha et al. 1995) have been shown to result in a hypo-androgen status. Njar et al. (1995) have reported inhibition of steroidogenesis in crude stem wood methanol extract of *Q. amara* treated rats. Raji & Bolarinwa (1997) have also reported significant reduction of serum levels of LH, FSH and testosterone when treated with this extract. Though we did not measure the testosterone level in our study, in the light of the previous studies on the effect of methanolic extract of *Q. amara* bringing about reduced steroidogenesis and loss of germ cells, and in the light of Leydig cell pathology observed in this study the spermatogenic effects may be correlated with the Leydig cell effect of *Q. amara*. Regression of testicular and epididymis weight can also be interpreted in terms of impaired steroidogenesis.

Motility is an important requisite for fertilizing ability of spermatozoa. Here, in our study, motility assessment was made on the cauda epididymal spermatozoa. The factors affecting motility are to be looked at among the endogenous factors like the machinery for motility such as flagellum, and exogenous factors, such as contribution of the epididymis to physiological maturation of spermatozoa (Cooper 1998). We found severe histopathological changes in the epididymis of *Q. amara* / quassin treated mice. Such changes are reflections of altered epididymal function, particularly in respect of protein secretion. Epididymal secretory proteins contribute a lot to the physiological maturation of spermatozoa (Cooper 1998). Here again, the epididymis is dependent on androgen for maintenance of its structure and function (Robaire & Viger 1995). As discussed above, *Q. amara* / quassin treatment brings about pathological changes in the Leydig cell that in turn would imminently result in a hypo-androgen status. Thus, we presume that *Q. amara* / quassin treatment, through a direct effect on the epididymis or indirectly through Leydig cells, would affect the epididymal physiological maturation of sperm, leading to an impairment of sperm motility.
Though normal ejaculates can have small percentage of abnormal spermatozoa (Turner 1995), a semen sample with high incidence of abnormal spermatozoa is an indication of impaired fertility (Meistrich et al. 1985). The wide array of abnormalities we observed reflects aberrant spermatogenesis and/or spermiogenesis caused by Q. amara / quassin treatment. The results of the present study further revealed that Q. amara / quassin treatment causes considerably increased abundance of cauda epididymal spermatozoa which retain the CD, compared to control mice. This can be attributed to the toxic effect of Q. amara / quassin treatment on spermatogenesis and/or epididymal physiological maturation of spermatozoa.

The apical opening (fistula formation) of epididymal epithelial principal cells, entry of spermatozoa from the lumen into the fistula, appearance of pale vacuolated epithelial cells (PVEC) and the hypo-androgen status have been correlated in an earlier study of pathological manifestations as responses in mouse to aflatoxin treatment (Agnes & Akbarsha 2001). PVEC have been earlier reported in the epididymis of normal mice (Toshimori et al. 1990), 20-30 day old mice which were subjected to ligation of epididymal duct (Abe et al. 1982), androgen-deprived goat (Goyal et al. 1994), high dose testosterone implanted mice (Itoh et al. 1999) and aflatoxin-treated mice (Agnes & Akbarsha 2001). It has been suggested that fistula formation in epididymal epithelial principal cells would result due to a direct effect of a toxicant on the epididymis or indirectly as a consequent of the toxicant-induced hypo-androgen status. This would provide for entry of sperm from the lumen into the fistula which might provoke an autoimmune response to sperm antigens. To circumvent this, the spermatozoa are closeted in vacuoles enclosed in very special cells called PVEC. The vacuole in the cell is lined by a membrane which produces microvilli into the lumen and contains the spermatozoa or the product of their disintegration in the form of a dense PAS-positive material (Agnes & Akbarsha 2001). In the present study of Q. amara / quassin treatment we found evidence for all histopathological manifestations mentioned above. Thus, Q. amara extract and quassin would be as much toxic to male reproductive system as aflatoxin B1 (AFB1), and the principal mechanism appears to be the hypo-androgen status produced due to the Leydig cell toxicity (discussed vide supra) as has been suggested for AFB1 treated mice (Agnes & Akbarsha 2001).
Apical and narrow cells are restricted in distribution to the initial segment, intermediate zone and caput of the epididymis (Robaire & Hermo 1988). The apical cell is a funnel-like cell widening towards the lumen and has an apically located goblet-shaped nucleus, numerous Golgi stacks and several mitochondria. Apical cell is known to play role in endocytosis of proteins from the lumen and their degradation in the lysosomes (Adamali & Hermo 1996, Hermo et al. 1997, Adamali et al. 1999a, 1999b). It may also be concerned with some aspect of detoxification so as to protect the cell and the epididymal lumen from electrophilic attack (Adamali & Hermo 1996). Narrow cell has an expanded apical nucleated portion contacting the lumen and extends basally as a narrow stalk to end in a basal peduncle (Adamali & Hermo 1996). This cell also is believed to be concerned with detoxification (Veri et al. 1994). Clear cells are limited to the corpus and cauda. They are characterized by numerous electron-lucent apical vesicles, vacuoles, multivesicular bodies and lysozyme-like structures (Robaire & Hermo 1988, Robaire & Viger 1995). The clear cells are concerned with two major roles, endocytosis of proteins and cell debris and recognition and internalization of remnants from cytoplasmic droplets shed by the sperm (Hermo et al. 1988, Robaire & Hermo 1988). The hypertrophy and hyperplasia of these three cell types of epididymis of Q. amara / quassin treated mice matches the situation in AFB1 treated mice (Trasler et al. 1988) and might suggest a physiological response to the toxic inflictment signifying heightened endocytosis and detoxification or a pathological response.

The epididymis is fully differentiated to the adult status by the time of sexual maturity i.e., 45-60 days in rat (Sun & Flickinger 1979) and 29-49 days in mouse (Bronson et al. 1975) and, thereafter, mitosis of the epithelial cell is noticed only very rarely (Clermont 1961; Clermont & Flannery 1970, Martan & Risley 1963). Abe & Takano (1989) noticed mitotic figures in the principal cells of initial segment as an aspect of cell renewal rather than expansion. In other words, adult epididymal epithelium is only a renewing cell population and not an expanding cell population (Robaire & Fan 1998). Thus, the prevalence of dividing principal cells in Q. amara / quassin treated mice, and not in the control mice, in this study may be interpreted as an indication of renewal of cells which undergo degeneration (discussed elsewhere). Alternatively, and more plausibly, the Q. amara principle / quassin might be mitogenic
and can thus lead to tumorogenesis, as is known for AFB1 (Wang & Groopman 1999). If it is the latter case, there is need for extreme caution in the application of *Q. amara* / quassin as a therapeutic.

Thus, the evidences presented herein strongly implicate *Quassia amara* and the major secondary chemical in it, quassin, in male reproductive toxicity when applied as an herbal remedy.
Table 1: Effect of *Q. amara* methanolic extract / quassin treatment on the sperm parameters, reproductive organ weight and seminiferous tubule (ST) diameter of Swiss mouse. Mean ± SD., n=5 animals / group.

<table>
<thead>
<tr>
<th></th>
<th>Sperm count (10⁶/ml)</th>
<th>Motile sperm (%)</th>
<th>Viable sperm (%)</th>
<th>Sperm with abnormal morphologies (%)</th>
<th>Testis weight (mg)</th>
<th>ST diameter (µm)</th>
<th>Epididymis weight (mg)</th>
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<tbody>
<tr>
<td>Control</td>
<td>63.5 ± 4.0</td>
<td>92.5 ± 4.5</td>
<td>88.6 ± 4.7</td>
<td>04.2 ± 0.6</td>
<td>113.5 ± 5.4</td>
<td>215.1 ± 14.7</td>
<td>82.82 ± 3.6</td>
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<tr>
<td>QA25</td>
<td>47.7 ± 4.9***</td>
<td>70.4 ± 4.5***</td>
<td>75.7 ± 3.3***</td>
<td>48.9 ± 5.1***</td>
<td>104.2 ± 5.1</td>
<td>198.4 ± 10.7</td>
<td>74.9 ± 3.9**</td>
</tr>
<tr>
<td>QA50</td>
<td>43.6 ± 3.1***</td>
<td>58.8 ± 3.0***</td>
<td>67.2 ± 3.4***</td>
<td>59.6 ± 5.1***</td>
<td>95.4 ± 3.4***</td>
<td>197.0 ± 07.2*</td>
<td>69.9 ± 2.7***</td>
</tr>
<tr>
<td>QA 100</td>
<td>35.9 ± 3.7***</td>
<td>46.6 ± 2.0***</td>
<td>51.9 ± 2.6***</td>
<td>65.3 ± 5.4***</td>
<td>88.3 ± 3.2***</td>
<td>193.2 ± 05.1**</td>
<td>67.1 ± 2.8***</td>
</tr>
<tr>
<td>Q 1mg</td>
<td>24.5 ± 3.3***</td>
<td>22.4 ± 1.3***</td>
<td>41.5 ± 2.5***</td>
<td>71.9 ± 5.5***</td>
<td>74.3 ± 2.7***</td>
<td>184.4 ± 06.0**</td>
<td>61.7 ± 2.5***</td>
</tr>
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</table>

***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05
Fig. 1. AO-EB (A-O) / Giemsa’s (P-T) stained spermatozoa of control (A) and *Q. amara* methanolic extract (QAE)/ quassin treated (B-V) mice. A, normal sperm; B, head alone; C, tail alone; D, macrocephali; E, H, kinked head; I-J, retention of cytoplasmic droplet (arrowheads); K, bent tail; L, folded tail; M, double tail; N, O, fusion of flagella. P-T, Abnormal head morphologies, wherein the midpiece is coiled around the head (P-S) and pin head (T). Scale bar (A-O): 15 µm; (P-T): 12 µm.
Fig. 2. TBO-stained semi-thin sections of testis of control (A) and Quassin treated mice (B-F). B, premature loss of germ cells from the adluminal compartment of Stage 12 tubule (note absence of step 12 spermatids); C, premature loss of germ cells accompanied by apical fragmentation of Sertoli cell (SC); D, a large empty space in seminiferous epithelium (asterisk); E, multinucleate giant spermatids (arrowheads); F, vacuolated Leydig cell (LC). Scale bar (A): 2 µm; (B-C, E-F): 10 µm; (D): 5 µm.
Fig. 3. TEM of testis of QAE / quassin treated mice. A, pathological germ cells of post -spermatogonial stages around an intact dark spermatogonium (DS); B, disintegrated male germ cells around an intact light spermatogonium (LS); C, a Sertoli cell with swollen mitochondria (arrowheads) and endoplasmic reticulum, reflecting as vacuolation; D, detachment of elongating spermatids (ES) from Sertoli cells (SC) and accumulation of a dense material in the Sertoli cell cytoplasm (arrowheads); E, a magnified view of disrupted mitochondria (MI) and endoplasmic reticulum (ER) of Sertoli cell; F, a large electron dense body in the cytoplasm of Sertoli cell (arrowhead); G, vacuolated Leydig cell (asterisk, vacuoles); H, vacuoles in the Leydig cell containing a dense material (asterisks). Scale bar (A, B, F, G): 1 µm; (C, D): 0.5 µm; (E, H): 0.2 µm.
Fig. 4. TBO-stained semi-thin sections of epididymis of QAE / quassin treated mice. A, B, large vacuoles (asterisks) in the cytoplasm of principal cells of caput (A) and intermediate zone (B); C, a pale vacuolated epithelial cell containing a dense material (PVEC); D, fistula formation in the principal cell of intermediate zone (arrowhead); E, increase in the abundance of clear cells (CC) of corpus (the clear cells are increased in vacuolation as well as granulation); F, an opaque material in the vacuoles of clear cells (arrowheads); G, detachment and rounding of cells in epididymal epithelium (arrowheads). Scale bar (A-C, E-F): 10 µm, (D): 20 µm.
Fig. 5. TEM of epididymal epithelium of QAE/quassin treated mice. A, hypertrophy of Golgi apparatus of principal cell of initial segment (GA); B, accumulation of a dense material in principal cells of initial segment (arrowheads); C, vacuolated and granulated clear cell; D, abundance of mitochondria (MI) in the apical cytoplasm of clear cells; E, hypertrophied apical cells (AC); F, accumulation and condensation of lipofuscin material (arrowheads) in the basal cells. Scale bar (A): 0.2 µm; (B): 3 µm; (C): 1 µm; (D): 0.4 µm; (E): 5 µm; (F): 0.6 µm.
Fig. 6. TBO-stained semi-thin sections of epididymal duct of QAE / quassin treated mice. A, hypertrophy of narrow cells (NC) in initial segment; B, deep lumen-ward projection of narrow cell (NC) in initial segment and their apical pinching into the lumen (arrowheads); C, hypertrophy and apical pinching of apical cell (AC) in initial segment; D, an unusual giant principal cell in the corpus (arrowhead); E, same as D, in caput (arrowheads); F, a microcanal (MC) in the epithelium of intermediate zone. Scale bar (A-C, F): 20 µm; (D-E): 10 µm.
Fig. 7. TBO-stained semi-thin sections of initial segment of epididymis of **QAE** / quassin treated mice. A-D, showing mitotic activity in the principal cells (arrowheads), metaphase (A, B); cytokinesis (C-D). E, prematurely released testicular germ cells in the lumen of epididymis (arrowheads); F-H, luminal macrophages (MA) active in phagocytosing sperm (F) and cell debris (G, H). Scale bar (A): 20 \( \mu m \), (B-H): 10 \( \mu m \).
Fig. 8. TEM of spermatogonia of QAE / quassin treated mice showing unusual morphologies. A-C, coiling of flagellum around the head (NU, nucleus; FL, flagellum); D, retention of cytoplasmic droplet (CD); E, coiling of flagellum (FL, flagellum) in the region of cytoplasmic droplet (CD); F, G, abnormal inclusions (arrowheads) in the retained CD; H, I, spermatozoa with bizarre morphologies. Scale bar (A-H): 0.5 µm, (I): 0.2 µm.