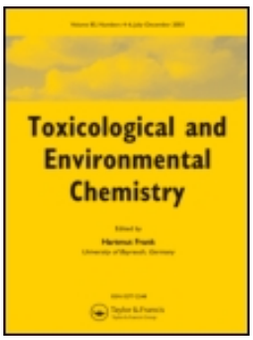


Toxicological & Environmental Chemistry

ISSN: 0277-2248 (Print) 1029-0486 (Online) Journal homepage: http://www.tandfonline.com/loi/gtec20

Potential histopathological and molecular changes in rat vas deferens inhaled by Boswellia papyrifera and Boswellia carterii

Mukhtar Ahmed, Ravindranath H. Aladakatti, Suliman Y. Al Omar, Daoud Ali, Shaban Rushdy Sayed, Mukhtar Ahmed Ghodesawar & Afrah Alkhuriji

To cite this article: Mukhtar Ahmed, Ravindranath H. Aladakatti, Suliman Y. Al Omar, Daoud Ali, Shaban Rushdy Sayed, Mukhtar Ahmed Ghodesawar & Afrah Alkhuriji (2014) Potential histopathological and molecular changes in rat vas deferens inhaled by Boswellia papyrifera and Boswellia carterii, Toxicological & Environmental Chemistry, 96:8, 1207-1220, DOI: 10.1080/02772248.2014.982937

To link to this article: http://dx.doi.org/10.1080/02772248.2014.982937

Accepted author version posted online: 03 Nov 2014.
Published online: 03 Dec 2014.

Toxicological & Environmental Chemistry, 2015
Vol. 96, No. 8, 1207–1220, http://dx.doi.org/10.1080/02772248.2014.982937

Potential histopathological and molecular changes in rat vas deferens inhaled by *Boswellia papyrifera* and *Boswellia carterii*

Mukhtar Ahmed[a]*, Ravindranath H. Aladakatti[b], Suliman Y. Al Omar[a], Daoud Ali[a], Shaban Rushdy Sayed[a], Mukhtar Ahmed Ghodesawar[c] and Afrah Alkhuriji[d]

[a]*Department of Zoology, College of Science, King Saud University, Riyadh, KSA;* [b]*Department of Central Animal Facility, Indian Institute of Science, Bengaluru, India;* [c]*Department of Zoology, Anjuman Arts, Sciences and Commerce College, Bijapur, India;* [d]*Department of Zoology (Female Campus), College of Science, King Saud University, Riyadh, KSA*

(*Received 19 September 2014; accepted 27 October 2014*)

Boswellia papyrifera and *Boswellia carterii* released from smoke contaminate indoor environment and consequently adversely affect humans as evidenced by respiratory disturbances. The aim of this study was to determine the effects of these plants on pathological and biochemical changes in vas deferens of albino rats. Animals were administered 4 g/kg body weight *B. papyrifera* and *B. carterii* daily for 120 days along with controls. Significant changes were observed in epithelial cell types and some cells showed signs of degeneration. The ultrastructural studies revealed marked changes in cytoplasmic organelles. Microvilli were missing and lysosomes were found in the cytoplasm. In addition, all treated groups plasma fructose and other biochemical parameters were decreased indicating reduced energy necessary for motility and contractility of spermatozoa. Many spermatozoa were disorganized and agglomerated. Data suggest that smoke from these plants adversely affects vas deferens.

Keywords: *B. papyrifera*; *B. carterii*; albino rat; vas deferens; ultrastructure; biochemical analysis

Introduction

The structural and integrity of sex accessory glands are influenced by androgens (Cavazos et al. 1975; Mann 1964) and contraction of the vas deferens is directly dependent on the adrenergic mechanisms modulated by a variety of local endogenous factors, notably adenosine triphosphate (ATP). Further, it is widely accepted that noradrenaline (NA) and ATP are stored and released together by adrenergic nerve endings in the rat vas deferens (Boselli and Govoni 2000; Brown et al. 1983).

Boswellia papyrifera and *Boswellia carterii* are used as natural medicines for arthritis and their by-products have long been used in perfume industries (Safayhi et al. 2000; Schweizer et al. 2000). In addition, boswellic acids are used as an anticancer, antimicrobial, and immunosuppressant agent (Huang et al. 2000; Hussein et al. 2000). These plants usually contain isoincensole and incensole acetate as their principal constituents and belong to family *Burseraceae* (Camarda et al. 2007). In addition to their medicinal value, these compounds produce marked changes in pulmonary function (Al-Arafi, Mubarak, and Alokail 2004; Alokail and Alarifi 2004). Alokail, Mohammad, and Al Arafi (2011a) noted decrease in activities of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, superoxide dismutase, catalase, and glutathione peroxidase as well as

*Corresponding author. Email: mahmed1@ksu.edu.sa

reduction in levels of glutathione and increased lipid peroxidation. Chronic exposure to incense smoke lowered body weight and high-density lipoprotein (HDL) cholesterol concentrations and increased triglycerides levels. Exposure to incense was also associated with a transient elevation in leptin levels (Alokail et al. 2011b).

Recently, Mukhtar et al. (2013) and Ahmed et al. (2013, 2014) reported that these plants also affect testis, cauda epididymis, and gonadal hormones in rats. Data suggested that incense smoke influenced metabolism and structural changes in rats. Information related to incense smoke on vas deferens and its internal milieu is limited. The aim of this investigation was to explore potential changes on rat vas deferens produced by plant incense smoke containing these compounds.

Materials and methods

Animals and incense

Wistar albino male rats (*Rattus norvegicus*), aged 8−9 weeks and weighing 200−210 g, were obtained from the Animal Care Center, College of Science, King Saud University; Riyadh, Saudi Arabia. The Ethical Committee of the Experimental Animal Care Center approved the study. Animals were housed in a temperature-controlled room on a 12-hr light/dark cycle and had access to water and normal chow diet *ad libitum*. *B. papyrifera* and *B. carterii* were obtained locally. All chemicals and solvents of analytical grade were obtained from local dealers.

Exposure to B. papyrifera *and* B. carterii *smoke*

After one-week acclimatization, rats were randomly divided into three groups, I, II, and III, with each group consisting of 11 animals. Each group of rats was housed separately from the other groups to avoid cross exposure. Rats in group I served as control and were kept under normal conditions, while rats in groups II and III were exposed to *B. papyrifera* and *B. carterii* smoke, respectively, in a smoking chamber (measuring 24 × 24 × 18 inches) as described by Wang et al. (1999). Rats were exposed daily to the smoke emanating from the burning of 4 g of each incense material for four months. Smoke exposure duration lasted for 30−40 min/day. Dose and duration of incense exposure followed in this study was based on the optimized conditions from our studies (Mukhtar et al. 2013; Ahmed et al. 2013b, 2014). At the end of exposure duration, all animals were killed by cervical dislocation.

Evaluation of biochemical parameters

Plasma was collected from vas deferens and thereafter centrifuged at 12000 × *g* for 30 min in an Eppendorf centrifuge 5415 C, then plasma was stored at −40 °C until specific spectrophotometric determination of proteins, sialic acid, carnitine, and carbohydrates. The biochemical parameters were estimated according to Huacuja, Puebla, and Carranco (1997).

Measurement of fructose levels

Fructose levels of vas deferens fluid were measured according to Bauer, Ackermen, and Toro (1974). Absorbance of our test and standard samples was measured at 490 nm. The

average fructose content of fluid was calculated and expressed as mg fructose/100 ml (absorbance of samples/absorbance of standard X 200).

Morphometric analysis

The mean height of epithelium, principal cells, and basal cells and nuclear diameter of vas deferens were estimated using a computer image analysis system, Leica IM500, coupled with image manager software (version: 5 Release 247). Data were analyzed using the Statistical Package SPSS for Windows, version 16.0. Fifty cells per animal were chosen from the testicular side. Data were expressed as the standard error means. The statistical analysis was based on the Student's *t*-test, taking $p < 0.05$ as the criterion for significance.

Histological study

Immediately after sacrifice, vas deferens was fixed in 10% buffer formalin and subsequently preserved in 70% alcohol. Histological sections were made at intervals of 4 μm and stained with hematoxylin and eosin.

Ultrastructural study

Immediately after the removal of vas deferens from the dissected rats, tissues were sliced from the testicular side into small size (1 mm^3) and fixed in 3% buffered glutaraldehyde for 4 hr at 4 °C. Tissue specimens were then post-fixed in 1% osmium tetraoxide (OsO_4) for 1.3 hr. Dehydration of the fixed tissue was performed using ascending grades of ethanol and then tissue was transferred to resin via propylene oxide. After impregnation with the pure resin, tissue specimens were embedded in the same resin mixture (Reynolds 1963). Ultra-thin sections were (70−75 nm) cut on an ultra-microtome (Leica, UCT) with a diamond knife (Diatome, Switzerland) and stained with uranyl acetate and lead citrate. Stained sections were observed under TEM (JEOL 1100) operating at 80 Kv.

Statistical analysis

Data were expressed as mean values ± SE. Student's *t*-test was applied to compare significant differences. A probability level of $p < 0.05$ was considered to be significant.

Results

Metabolic parameters

Plasma biochemical composition of vas deferens showed a significant decrease in proteins, total carbohydrate, sialic acid, and carnitine induced with *B. papyrifera* and *B. carterii* groups compared to controls (Table 1).

Fructose levels

Plasma fructose of vas deferens which is a major source of energy to transport motile sperm was significantly reduced threefold in both treated groups (Table 2).

Table 1. Biochemical parameters of vas deferens plasma exposed to *B papyrifera* and *B. carterii*.

Groups	Protein	Total carbohydrate	Sialic acid	Carnitine
I. Control	3.61 ± 0.40	1.70 ± 0.21	2.20 ± 0.13	0.53 ± 0.11
II. *B. papyrifera*	1.13 ± 0.12*	0.53 ± 0.13*	1.31 ± 0.15*	0.19 ± 0.13*
III. *B. carterii*	1.10 ± 1.31*	0.47 ± 0.21*	1.34 ± 0.17*	0.21 ± 0.11*

Note: Values are expressed in g/100 ml of plasma of 11 determinations and are indicated as mean ± standard deviation.
*Significant compared to control $p < 0.05$.

Table 2. Fructose content of whole vas deferens of *B. papyrifera* and *B. carterii* exposed rats.

Group	Plasma fructose levels (mg/100 ml)
I Control	70.32 ± 10.13
II *B. papyrifera*	30.17 ± 15.11*
III *B. carterii*	31.63 ± 10.21*

Note: Values are expressed as SEM of 11 animals.
*Significant from control $p < 0.05$.

Morphometric analysis

Morphometric data of treated groups showed a marked fall in the height of epithelium, principle cells, basal cells, and nuclear diameter of vas deferens (Table 3).

Histopathology

Vas deferens of control group showed normal histological features with an outer serous coat with blood vessels, thick muscular coat, and inner mucous layer (Figure 1(a)). The vas deferens of treated groups displayed disturbances in epithelium with loss of ciliated microvilli and inner epithelium lining became wavy (zigzag). There was a modest reduction in diameter of vas deferens, and there were fewer agglomerated spermatozoa in lumen (Figure 1(b) and 1(c)). Further, treated groups showed vacuolization as well as exfoliation of principal and basal cells (Figure 1(b) and 1(c)). The most affected regions of either side were found to be regions adjacent to testes, contralateral side being more damaged than ipsilateral side.

Table 3. Effect of inhalation of *B. papyrifera* and *B. carterii* on the epithelial height (μm), principal cells, basal cells, and the nuclear diameter (μm) of the vas deferens.

	100 × (μm)			
Group	Epithelial height	Nuclear diameter	Principal cell	Basal cells
I Control	33.40 ± 0.21	13.11 ± 0.23	19.12 ± 0.9	14.11 ± 1.2
II *B. papyrifera*	21.37 ± 0.12*	5.21 ± 0.19*	10.15 ± 0.5*	08.03 ± 8.3*
III *B. carterii*	20.566 ± 0.17*	5.17 ± 0.16*	11. 21 ± 0.8*	07.07 ± 7.9*

Note: Values are expressed as SEM of 11 animals.
*Significant from control $p < 0.05$.

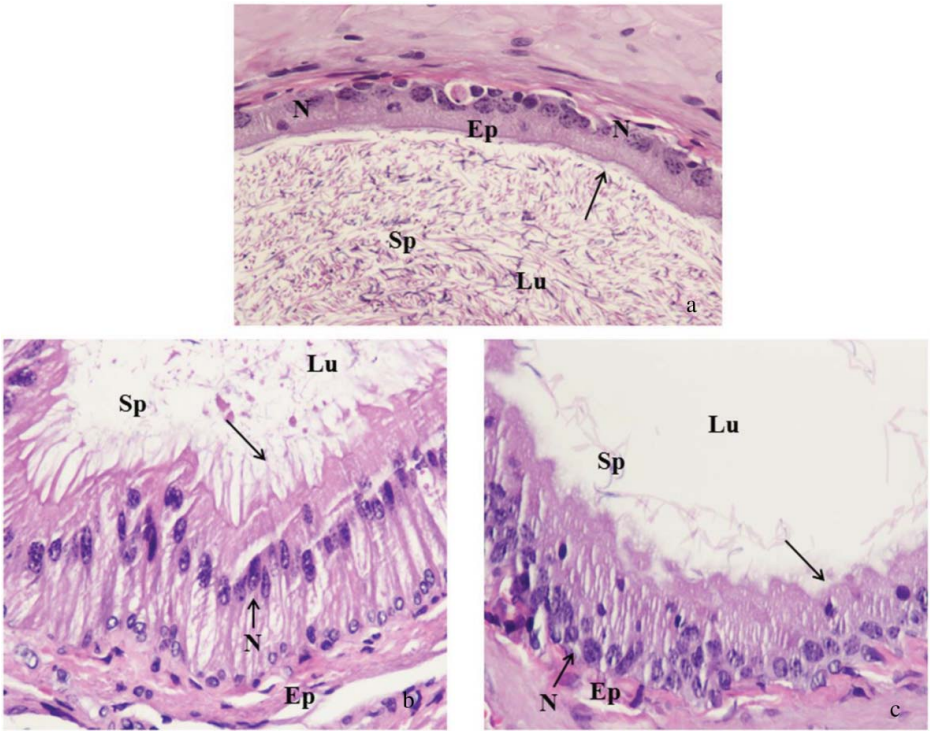


Figure 1. Histopathological changes in rat vas deferens. (a) Vas deferens of control animal showing normal histological features with an outer serous coat, thick muscular coat, and inner mucous layer. The epithelium had ciliated microvilli (arrows). Pseudo-stratified epithelium with distinct principal (P) and basal cells (B) were also seen. The lumen (Lu) was filled with mature spermatozoa (Sp). (b and c) Vas deferens treated with *B. papyrifera* and *B. carterii* showed disturbances in the epithelium (Ep) with loss of ciliated microvilli (arrows) and the inner epithelium lining has become loosened. There were fewer agglomerated spermatozoa (Sp) in the lumen (Lu). The epithelium showed vacuolization as well as exfoliation of principal and basal cell nucleus (N).

Ultrastructural study

The ultrastructural study demonstrated that basal and principal cells were present along the entire length of the vas deferens. These cells have an elliptical nucleus (Figure 2(a)). The basal cells were elongated and nuclei were flattened against the basement membrane (Figure 2(a)). The basal lamina and other endothelial cells are normal, as shown in Figure 2(a) and 2(b). *B. papyrifera*- and *B. carterii*-exposed rats showed reduction in epithelial heights and degeneration of cells (Figures 3(a), 3(b), 4(a), and 4(b)). The nuclei were pycnotic and height of stereocilia was condensed. The lumen was devoid of sperm and filled with lymphocytes and debris of degenerated sperm (Figures 3(b) and 4(b)). The basement membrane was loose and disrupted. Cells displayed vacuolization and cell debris was evident due to cytolysis. Few cells exhibited signs of degeneration (Figures 3(a), 3(b), 4(a), and 4(b)).

Marked changes in principal cells exposed to *B. papyrifera* and *B. carterii* were decreased number of coated micropinocytotic vesicles, invaginations of luminal surface, and disruption in mitochondrial cristae (Figures 3(a), 3(b), 4(a), and 4(b)). Further, the multi-vesicular bodies were increased and contained homogeneous or heterogeneous

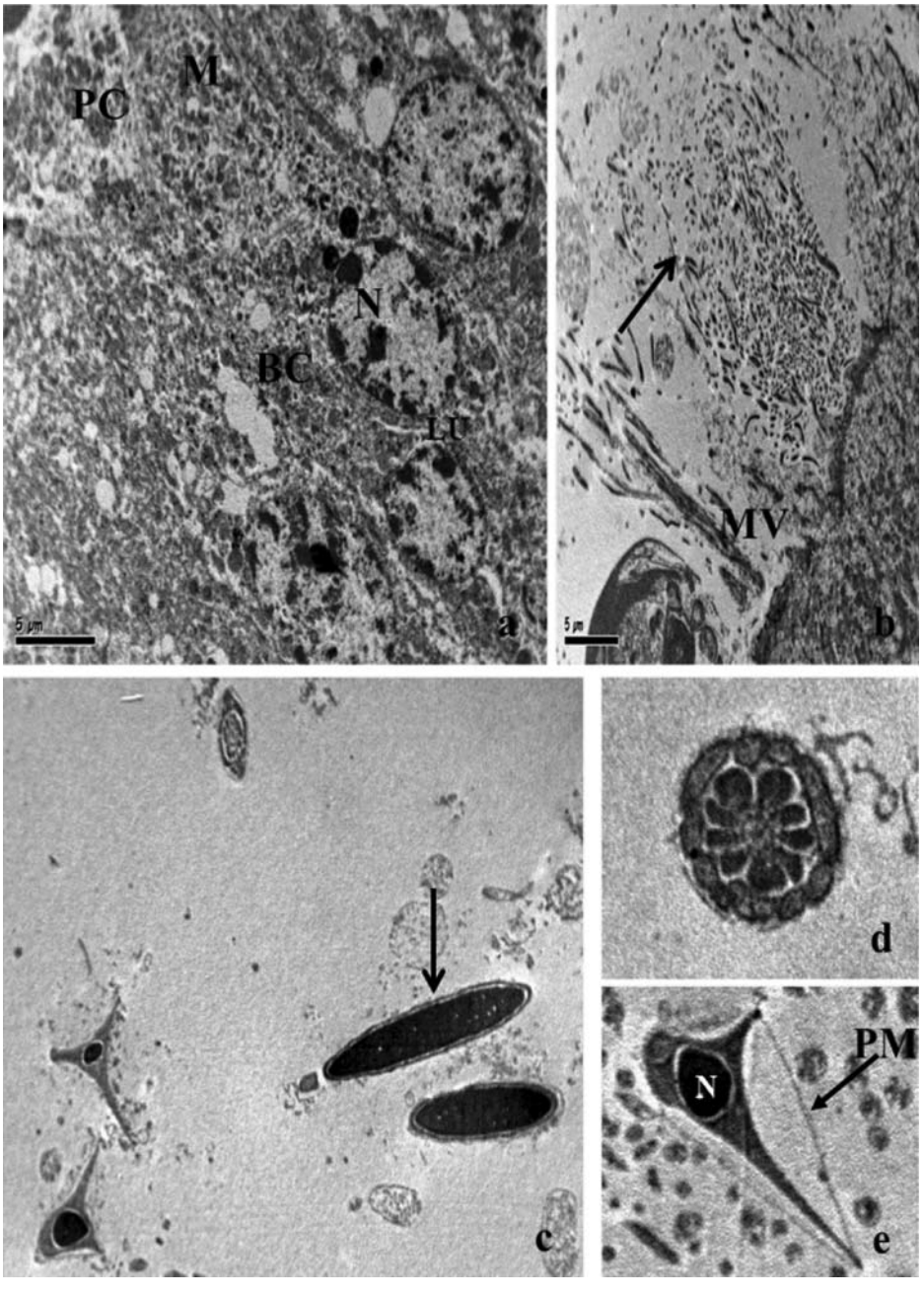


Figure 2. Electron micrographs of control vas deferens. (a) The basal cells (BC) were elongated and nuclei were flattened against the basement membrane The principal cells (PC) were present along the entire length of the vas deferens. These cells have an elliptical nucleus (N). The basal lamina and other endothelial cells are normal (X10.000). (b) In control, rats showed normal spermatozoa (arrows) projected towards the lumen (Lu) (X8000). (c and e) In transverse section, the perforatorium and acrosome were enclosed with the plasma membrane (PM). A distinguish acrosome was covered with acrosomal membrane (arrows). The whole spermatozoon was intact with all the membranes and organelles (X8000). (d) A transverse section of tail region showing 9 + 2 arrangement of microtubules. The outer and inner membranes are normal (X12000).

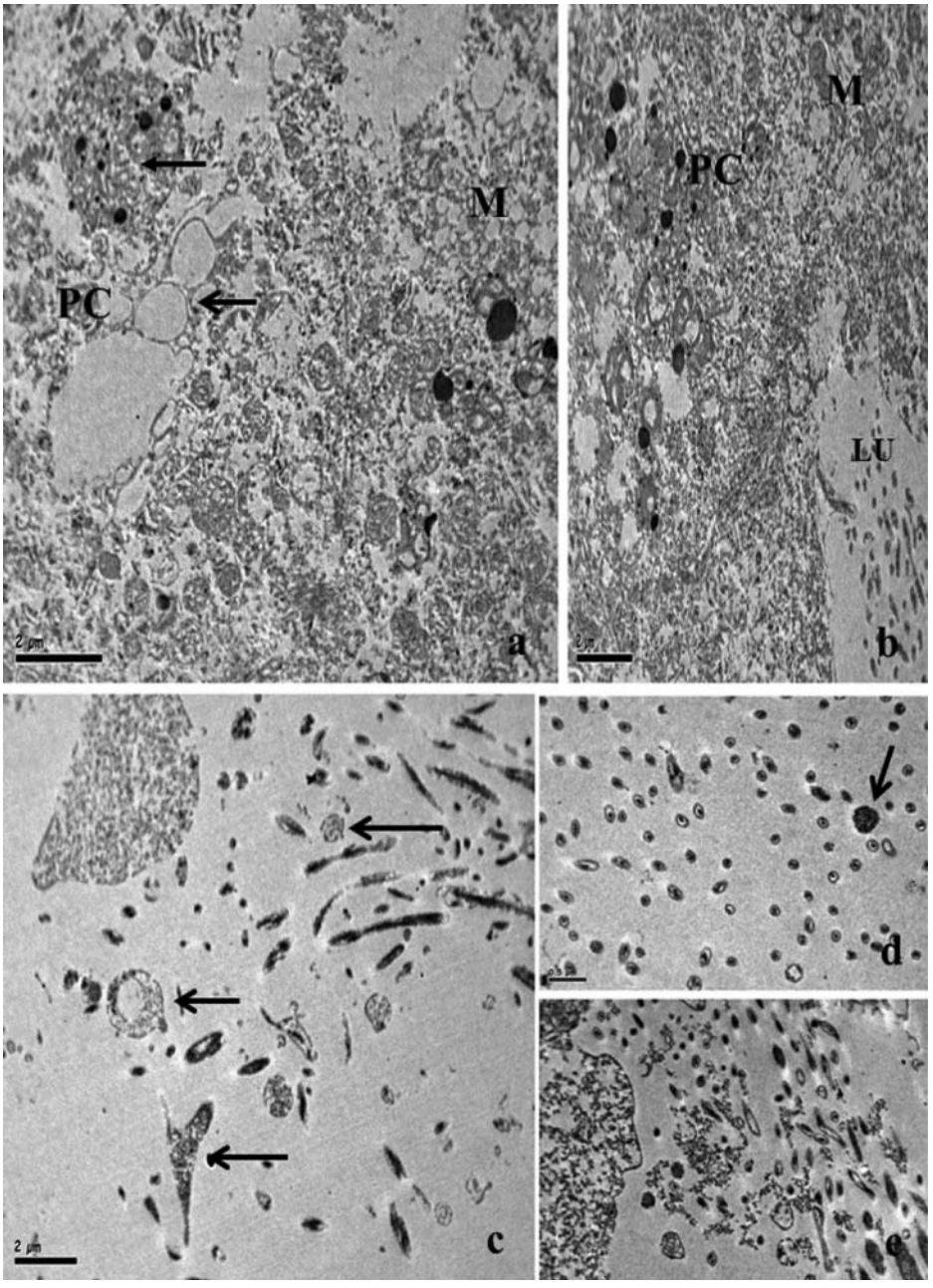
materials. The principal cells reflected alterations in terms of vesicular elements and lysosomal bodies (Figures 3(a) and 4(a)). Decreased lipid droplets were also observed in cells. Lysosomes activity increased either in the basal cytoplasm or in the supranuclear cytoplasm or in both. Further, basal cells showed the absence of scattered spherical electron-dense granules in the cytoplasm. Disruption of mitochondrial cristae and Golgi apparatus were also evident in this region (Figures 3(a), 3(b), 4(a), and 4(b)). This assessment revealed a group of cylindrical epithelial cells with electron-dense cytoplasm. These electron-dense cells were postulated to contain severe degeneration. Electron lucent cells were found to have less vacuole formation compared with other cells. A group of electron lucent cells included autophagic vacuoles.

Ultrastructural changes in spermatozoa

At ultrastructural studies, control rats showed normal spermatozoa (Figure 2(c)–(e)). Animals exposed to *B. papyrifera* and *B. carterii* revealed disruption in plasma membrane and acrosomal membranes. The surface became rough accumulated with debris and elusive materials (Figures 3(c), 3(e), and 4(c)–(e)). Tip of the sperm head showed disruption of plasma membrane as well as acrosome (Figures 3(c), 3(e), and 4(c)–(e)). The perforatorium was condensed and most of its surface was covered by a thin layer of acrosomal sac (Figures 3(c) and 4(c)). Many serrations were observed at the head region of the spermatozoa. The shape and size of the sperm head changed significantly (Figures 3(c)–(e) and 4(c)–(e)). There was severe dorsoventral constriction in the mid-head region of most sperms (Figures 3(c) and 4(c)). The anterior and caudal portion of the sperm heads revealed loss of plasma membrane, acrosome, and the presence of small vesicles on ventral surface of perforatorium (Figures 3(c) and 4(c)). The tail section of sperm exposed to *B. papyrifera* and *B. carterii* showed disruption and complete degeneration of mitochondrial sheath and loss of plasma membrane (Figures 3(d) and 4(d)). The commencement of degeneration of mitochondria was observed in almost all sheaths either on one or both sides and their abnormal pattern of outer dense fibers (Figures 3(d) and 4(d)(arrows)). Different parts of the tail region exhibited discontinuation of plasma membrane and fibrous sheath, respectively (Figure 3(d) and 4(d)). Most of the spermatozoa displayed a splitting of the tail and distinct visibility of balloon-like cytoplasmic droplets in the mid-region of the tail (Figure 3(e) and 4(e) (arrows)).

Discussion

The vas deferens is a muscular cylinder duct that contracts unidirectional during ejaculation to release seminal fluid into ejaculatory duct. This contractile activity is biphasic in rodents and mediated by autonomic nervous system. The first phase is a twitch-like contraction mediated by the synaptic release of ATP and the second phase is a tonic contraction mediated by the synaptic release of NA (Pampal et al. 2010). In addition, distal vas deferens possesses a higher threshold requisite for testosterone for the maintenance of its structural and biochemical integrity than the proximal vas deferens (Chinoy and Chinoy 1983). Recently, Mukhtar et al. (2013) and Ahmed et al. (2013, 2014) demonstrated the toxicity of *B. papyrifera* and *B. carterii* on spermatogenesis, epithelial cells of cauda epididymis, biochemical compositions, sperm kinetics, gonadal hormones, and morphometric variations in different male reproductive organs. In this study, the toxicity of *B. papyrifera* and *B. carterii* on vas deferens was examined especially in epithelial cells, seminal plasma, and in the spermatozoa.



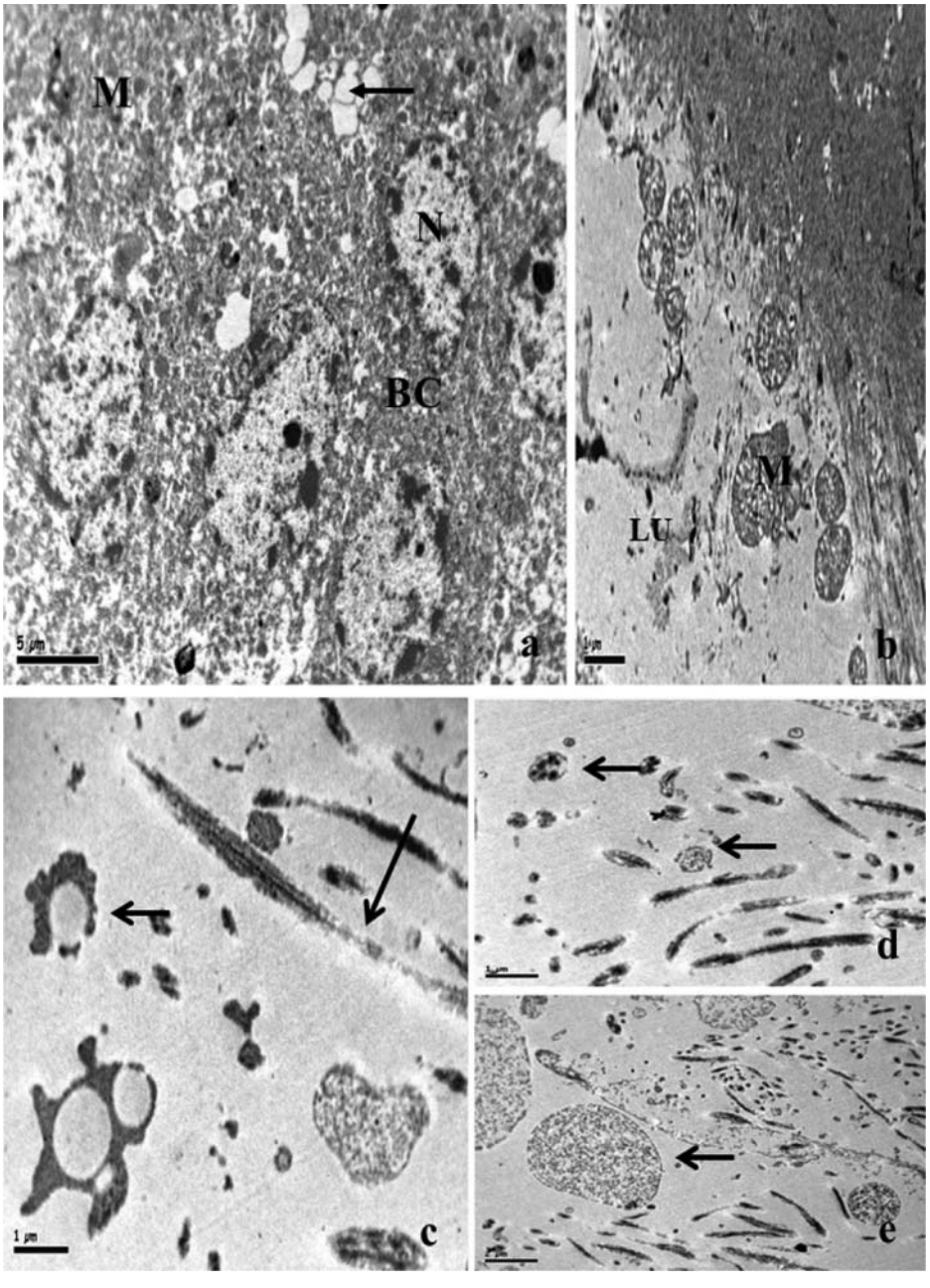
In this study, rats exposed to *B. papyrifera* and *B. carterii* exhibited significant decrease in vas deferens levels of proteins, carbohydrate, sialic acid, and carnitine (Table 1). Carnitine is an important metabolite which helps in sperm maturation, motility, and fertilizing capacity (80%) (de la Luz et al. 2003; Casillas, Villalobos, and Gonzalez 1984). Carnitine was shown to possess an important cellular function, such as transferring long-chain fatty acids across the inner mitochondrial membrane for β-oxidation (Bremer 1977).

Fructose, a vital source of energy, plays an important role in the motility of mature gametes and is considered as an indicator for androgen levels (Mukhtar et al. 2013; Ahmed et al. 2013, 2014; Mann and Lutwak-Mann 1981). Patel, Skandhan, and Mehta (1988) found a positive correlation between levels of fructose and percentage of motile sperm. In this study, the decreased levels of fructose in vas deferens (Table 2) directly affected sperm motility and contractility (Mukhtar et al. 2013; Ahmed et al. 2013, 2014; Chinoy and Chinoy 1983; Chinoy and Ranga Geetha 1984; Busch, Wald, and Borda 2000). In addition, fructose reduced motility of vas deferens and contributed to male infertility (Steers 1994; Göçmez et al. 2010; Chinoy and Chinoy 1981; Mulryan et al. 2000). The above findings support the data in our present study.

The ultrastructural findings of our study demonstrated morphological changes of vas deferens and these morphological changes might be the basis for diminshed motility of vasa differentia (Brown et al. 1983; Ghodesawar, Ahamed, and Aladakatti 2004). In this study, the principal cells lost most of their microvilli and formed apical blebs; which appeared to produce dense secretory material that was found in the lumen (Ghodesawar, Ahamed, and Aladakatti 2004; Swan et al. 1990). The epithelium and principal cells were markedly altered in our investigation which supports data in other studies (Swan et al. 1990). *Azadirachta indica* leaves affect the height of the epithelium and diameter of the nuclei. In addition, sperm were packed at the center of the lumen (Swan et al. 1990; Nistal, Santamaría, and Paniagua 1992; Hermo and de Melo 1987). Further, various extracts also affect the internal milieu of the vas deferens producing change in spermatozoa in treated albino rats (Chinoy and Ranga Geetha 1984). These alterations were frequent in our observations.

The vas deferens plays an important role in the transport of sperm (Balasubramanian, Pereira, and Govindarajulu 1981). In addition to this, it is absorptive in nature as manifested by signs of pinocytosis in the lumen and the presence of multi-vesicular bodies and abundant lysosomes (Balasubramanian, Pereira, and Govindarajulu 1981; Murakami,

Figure 3. Electron micrographs of vas deferens exposed with *B. papyrifera*. (a) It showed great reduction in epithelial heights and in the nuclear diameters. The nuclei existed pycnotic and the height of stereocilia was condensed (arrows). The principal cells showed vacuolization (arrows) (X8000). (b) The lumen was devoid of sperm and filled with lymphocytes and debris of degenerated sperm (Lu). The basement membrane was loosened and disrupted. The cell showed vacuolization and the cell debris was evident due to cytolysis. Many cells exhibited signs of degeneration (arrows). Multi-vesicular bodies were increased and contained a homogeneous or heterogeneous material. The principal cells reflected the change in terms of vesicular elements and lysosomal bodies. Decreased lipid droplets were also observed in cells. The basal cells showed the absence of the scattered spherical electron-dense granules in the cytoplasm (X8000). (c and e) Animals exposed to *B. papyrifera* reveal the disruption in plasma membrane and acrosomal membranes. The surface became rough accumulated with debris and elusive materials (arrows). Tip of the sperm head showed disruption of the plasma membrane as well as acrosome (arrows). The perforatorium was condensed and most of its surface was covered by a thin layer of acrosomal sac. Many serrations were observed at head region of the spermatozoa. The shape and size of the sperm head has changed significantly (arrows). There was severe dorsoventrally constriction in the mid-head region of most sperms (X6000). (d) The tail section of sperm exposed to *B. papyrifera* showed disruption and complete degeneration of mitochondrial sheath and a loss of plasma membrane (arrows). The commencements of degeneration of mitochondria were observed in almost all sheaths either on one or both sides and there was abnormal pattern of outer dense fibers. Different parts of the tail region exhibits discontinuation of plasma membrane and fibrous sheath, respectively (arrows). Most of the spermatozoa showed a splitting of the tail and distinct visibility of balloon-like cytoplasmic droplets in the mid-region of the tail (e) (X8000).



Sugita, and Hamasaki 1982, 1986). In the present study microfilaments in the lumen were lacking; nuclei of cells were irregularly outlined and displayed deep indentation. Similar observations were noted in human and other animals produced by different treatments (Swan et al. 1990; Nistal, Santamaría, and Paniagua 1992; Hermo and de Melo 1987). Existing cytological evidence does not support that basal cells are involved either in

secretion or in absorption (Dutt 1999). However, Veri, Hermo, and Robaire (1993) and Regadera et al. (1993) proposed that basal cells were involved in a scavenging role and phagocytized the degenerated sperm. Further, these cells play an important role in the renewal of the epithelium or in the secretion of recognition molecules of sperm membrane (Dutt 1999). Ultrastructural changes indicate that the principal cells and basal cells are affected thus, altering the composition of the vas fluid. This postulation was supported by earlier studies (Busch, Wald, and Borda 2000; Swan et al. 1990; Hermo and de Melo 1987).

Further, this study was also undertaken to evaluate the effects of *B. papyrifera* and *B. carterii* on ultrastructure of rat sperm present in the lumen of vas deferens. Data showed distinct ultrastructural changes such as major damage in all sperm flagellae including defect in mid-piece mitochondria. The sperms were immotile in agreement with previous studies (Ahmed et al. 2013, 2014; Hoffer 1982). Swami, Ramanathan, and Charan Jeganath (2007) noted agglutinated dead sperms suggesting the possibility of infertility when rats were chronically exposed to noise stress. Further changes were also reported in spermatozoa such as spiral mitochondria, damaged outer dense fibers, and axoneme. Based on the present findings, data suggest that these plants severely damage spermatozoa in the lumen of vas deferens. Similar changes were noted by treatment with lead acetate and gossypol (Ahmed et al. 2014; Hoffer 1982; Piasecka et al. 1996; Swan et al. 1990). Hikim et al. (2000) found that oral administration of triptolide affected all lumen sperm and exhibited complete absence of plasma membrane over the entire middle and principal piece.

The mitochondrial sheath is believed to be the source of energy for sperm motility and outer dense fibers (Fawcett 1975). These outer dense fibers provide additional strength to protect sperm from damage by shear forces encountered during ejaculation (Baltz, Williams, and Cone 1990). The spermatozoa in this study revealed several abnormalities, mainly abnormal patterns of the outer dense fibers and components of axoneme displaced on either side (Fawcett 1975; Baltz, Williams, and Cone 1990). Ultrastructural observations of exposed animals showed cytoplasmic droplets at tail region which look like balloons. Spermatozoa ejaculated with cytoplasmic droplets may

Figure 4. Electron micrographs of vas deferens exposed with *B. carterii*. (a) It showed great disturbance in basal cells and the epithelial heights were reduced. The mitochondria become pycnotic and the height of stereocilia was shortened (arrows). The principal cells showed the change in terms of vesicular elements and lysosomal bodies. Decreased lipid droplets were also observed in cells. The principal cells showed vacuolization (arrows) (X10000). (b) The lumen was lacking of sperm and filled with lymphocytes and debris of degenerated sperm (Lu). The mitochondria (M) showed vacuolization and the cell debris was evident due to cytolysis. Many cells exhibited signs of degeneration (X6000). (c and d) Animals exposed to *B. papyrifera* reveal the disruption in plasma membrane and acrosomal membranes. Sperm head showed disruption in the plasma membrane as well as in acrosomal membrane (arrows). The perforatorium was destroyed completely and there is no acrosomal layer. Many serrations were observed at head region of the spermatozoa. The shape and size of the sperm head has changed drastically (arrows). There was severe dorsoventrally constriction in the mid-head region of most sperms (d) (X8000). (d and e) The tail section of sperm showed disruption and degeneration of mitochondrial sheath and a loss of plasma membrane (arrows). The originations of degeneration of mitochondria were observed in almost all sheaths. There was abnormal pattern of outer dense fibers. Different parts of the tail region exhibits discontinuation of plasma membrane and fibrous sheath, respectively (arrows). Most of the spermatozoa showed a splitting of the tail and distinct visibility of balloon-like cytoplasmic droplets in the mid-region of the tail as shown in (e) (X6000).

be correlated with altered vas milieu and reduced potency (Cummins 1973; Bedford 1976). The presence of cytoplasmic droplets in *B. papyrifera* and *B. carterii* exposed rats may alter the lumen. Similar observations were noted in studies including *Carica papaya*; vincristine, aflatoxin B1, and in the same plants at epididymal spermatozoa (Ahmed et al. 2014; Chinoy, D'Souza, and Padman 1995; Akbarsha and Averal 1996; Agnes and Akbarsha 2003).

Acknowledgments

The authors wish to thank Transmission Electron Microscope Unit College of Science for electron microphotographs.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research project No RGP-VPP-300.

References

Agnes, V.F., and M.A. Akbarsha. 2003. "Spermatotoxic Effect of Aflatoxin B (1) in the Albino Mouse." *Food and Chemical Toxicology* 41: 119–130.

Ahmed, M., N. Al-Daghri, A.H. Harrath, M.S. Alokail, R.H. Aladakatti, M.A. Ghodesawar, and S. Alwasel. 2013. "Potential Ultrastructural Changes in Rat Epididymal Cell Types Induced by Boswellia Papyrifera and Boswellia Carterii Incense." *Comptes Rendus Biologies* 336: 392–399.

Ahmed, M., D. Ali, A.H. Harrath, H. Tajamul, N.M. Al-Daghri, S.A. Majed, R.H. Aladakatti, and M.A. Ghodesawar. 2014. "Ultrastructural and Hormonal Changes in Rat Cauda Epididymal Spermatozoa Induced with *Boswellia Papyrifera* and *Boswellia Carterii*." *Comptes Rendus Biologies* 4: 250–257.

Akbarsha, M.A., and H.I. Averal. 1996. "Epididymis as a Target Organ for the Toxic Effect of Vincristine: Light Microscopic Changes in the Epididymis Epithelial Cell Types." *Biomed Letters* 54: 133–146.

Al-Arafi, S.A., M. Mubarak, and M.S. Alokail. 2004. "Ultrastructure of the Pulmonary Alveolar Cells of Rats Exposed to Arabian Mix Incense (Ma' Amoul)." *Journal of Biological Sciences*. 4: 694–699.

Alokail, M.S., and S.A. Alarifi. 2004. "Histological Changes in the Lung of Wistar Albino Rats (*Rattus Norvegicus*) After Exposure to Arabian Incense (Genus Boswellia)." *Annals of Saudi Medicine* 24: 293–295.

Alokail, M.S., N.M. Al-Daghri, S.A. Al-Arafi, H.M. Draz, H. Tajamul, and S.M. Yakout. 2011b. "Long-Term Exposure to Incense Smoke Alters Metabolism in Wistar Albino Rats." *Cell Biochemistry and Function* 28: 1–6.

Alokail, M.S., A.I. Mohammad, and S.A. Al Arafi. 2011a. "Antioxidant Enzyme Activity and Lipid Peroxidation in Liver of Wistar Rats Exposed to Arabian Incense." *Animal Biology Journal* 2: 1–9.

Balasubramanian, K., B.M. Pereira, and P. Govindarajulu. 1981. "Influence of Castration, Testosterone Propionate, Androstenedione and Estradiol-17 Beta on LDH and its Isoenzymes of Vas Deferens." *Indian Journal of Experimental Biology* 19: 419–421.

Baltz, J.M., P.O. Williams, and R.A. Cone. 1990. "Dense Fibres Protect Mammalian Sperm Against Damage." *Biology of Reproduction* 43: 485–491.

Bauer, J.D., P.G. Ackermen, and G. Toro. 1974. *Clinical Laboratory Methods*. St. Louis, MO: C.V. Mosty.

Bedford, J.M. 1976. "Adaptations of Male Reproductive Tract and the Fate of Spermatozoa Following Vasectomy in the Rabbit, Rhesus Monkey, Hamster and Rat." *Biology of Reproduction* 14: 118–142.

Boselli, C., and S. Govoni. 2000. "Alcohol Differentially Affects Noradrenergic and Purinergic Responses in the Bisected Rat Vas Deferens." *Alcohol* 22: 91–96.

Bremer, J. 1977. "Carnitine and its Role in Fatty Acid Metabolism." *Trends in Biochemical Sciences* 2: 207–209.

Brown, D.A., J.R. Docherty, A.M. French, A. MacDonald, J.C. McGrath, and N.C. Scott. 1983. "Separation of Adrenergic and Non-adrenergic Contractions to Field Stimulation in the Rat Vas Deferens." *British Journal of Pharmacology* 79: 379–393.

Busch, L., M. Wald, and E. Borda. 2000. "Influence of Castration on the Response of the Rat Vas Deferens to Fluoxetine." *Pharmacological Research* 42: 305–311.

Camarda, L., T. Dayton, V. Di Stefano, R. Pitonzo, and D. Schillaci. 2007. "Chemical Composition and Antimicrobial Activity of Some Oleogum Resin Essential Oils from Boswellia Spp (Burseraceae)." *Annali Di Chimica* 97: 837–844.

Casillas, E.R., P. Villalobos, and R. Gonzalez. 1984. "Distribution of Carnitine and Acylcarnitine in the Epididymis and Epididymal Spermatozoa During Maturation." *Journal of Reproduction and Fertility*. 72: 197–201.

Cavazos, L.F., R.O. Greep, E.B. Astwood, D.W. Hamilton, and S.R. Geiger, eds. 1975. *Handbook of Physiology*. Washington, DC: American Physiological Society.

Chinoy, N.J., and M.R. Chinoy. 1981. "Infertility Induced by Prostaglandins in Albino Rats by Adrenergic Block in the Vas Deferens." *International Journal of Fertility* 26: 1–7.

Chinoy, M.R., and N.J. Chinoy. 1983. "Differential Androgen Requirements of Proximal and Distal Vas Deferens of Albino Rats and Possible Potentiation of Androgen Action by Ascorbic Acid." *Indian Journal of Experimental Biology* 21: 335–338.

Chinoy, N.J., J.M. D'Souza, and P. Padman. 1995. "Contraceptive Efficacy of *Carica Papya* Seed Extract in Male Mice (*Mus musculus*)." *Phytotherapy Research* 9: 30–36.

Chinoy, N.J., and M. Ranga Geetha. 1984. "Effects of *Carica Papaya* Seed Extracts on the Physiology of the Vas Deferens of Albino Rats." *Acta Europaea Fertilitatis* 15: 59–65.

Cummins, J.M. 1973. "The Effect of Artificial Cryptorchidism in the Rabbit on the Transport and Survival of Spermatozoa in the Female Reproductive Tract." *Journal of Reproduction Fertility* 33: 469–479.

de la Luz Miranda-Beltrán M., A.M. Puebla-Pérez, A. Guzmán-Sánchez, and L. Huacuja Ruiz. 2003. "Male Rat Infertility Induction/Spermatozoa and Epididymal Plasma Abnormalities after Oral Administration of *Kalanchoe Gastonis Bonnieri* Natural Juice." *Phytotherapy Research* 17: 315–319.

Dutt, N.H. 1999. "Structure of Mammalian Epididymis." *Comparative Endocrinology and Reproduction* 201–227.

Fawcett, D.W. 1975. "The Mammalian Spermatozoon." *Developmental Biology* 44: 394–436.

Ghodesawar, M.G., R.N. Ahamed, and R.H. Aladakatti. 2004. "Ultrastructural Changes in Prostate Gland and Vas Deferens Induced by *Azadirachta indica* Leaves in Albino Rats." *Journal of Natural Remedies* 4: 160–167.

Göçmez, S.S., T. Utkan, G. Ulak, N. Gacar, and F. Erden. 2010. "Effects of Long-Term Treatment with Fluoxetine and Venlafaxine on Rat Isolated Vas Deferens." *Autonomic and Autacoid Pharmacology* 30: 197–202.

Hermo, L., and V. de Melo. 1987. "Endocytic Apparatus and Transcytosis in Epithelial Cells of the Vas Deferens in the Rat." *Anatomical Record* 17: 153–163.

Hikim, A.P., Y.H. Lue, C. Wang, V. Reutrakul, R. Sangsuwan, and R.S. Swerdloff. 2000. "Posttesticular Antifertility Action of Triptolide in the Male Rat: Evidence for Severe Impairment of Cauda Epididymal Sperm Ultrastructure." *Journal of Andrology* 1: 431–437.

Hoffer, A.P. 1982. "Ultrastructural Studies of Spermatozoa and the Epithelial Lining of the Epididymis and Vas Deferens in Rats Treated with Gossypol." *Archives of Andrology* 8: 233–246.

Huacuja, L., A.M. Puebla, and A. Carranco. 1997. "Contraceptive Effect on the Male Wistar Rat Oral Administration of *Kalanchoe Blossfeldiana* Crassulaceae Plant Aqueous Crude Extract." *Advanced Contraceptive Delivery Systems* 13: 13–21.

Huang, M.T., V. Badmaev, Y. Ding, Y. Liu, J.G. Xie, and C.T. Ho. 2000. "Anti-tumor and Anti-carcinogenic Effects of Triterpenoid, β-Boswellic Acid." *Biofactors* 13: 225−230.

Hussein, G., H. Miyashiro, N. Nakamura, M. Hattori, N. Kakiuchi, and K. Shimotohno. 2000. "Inhibitory Effects of Sudanese Medicinal Plant Extracts on Hepatitis C Virus (HCV) Protease." *Phytotherapy Research* 75: 10−516.

Mann, T. 1964. *The Biochemistry of Semen and of the Male Reproductive Tract*. New York: Wiley.

Mann, T., and C. Lutwak-Mann. 1981. "Secretory Function of the Prostate, Seminal Vesicle, Cowper's Gland and Other Accessory Organs of Reproduction." In *Male Reproductive Function and Semen*, 171−193. London: Springer.

Mukhtar, A., N.M. Al-Daghri, S.A. Majed, and H. Tajamul. 2013. "Potential Changes in Rat Spermatogenesis and Sperm Parameters after Inhalation of *B. Papyrifera* and *B. Carterii* Incense." *International Journal of Environmental Research and Public Health* 10: 830−844.

Mulryan, K., D.P. Gitterman, C.J. Lewis, C. Vial, B.J. Leckie, A.L. Cobb, J.E. Brown et al. 2000. "Reduced Vas Deferens Contraction and Male Infertility in Mice Lacking P2×1 Receptors." *Nature* 6: 86−89.

Murakami, M., T. Nishida, M. Shiromoto, and T. Inokuchi. 1986. "Scanning and Transmission Electron Microscopic Study of the Ampullary Region of the Dog Vas Deferens, with Special Reference to Epithelial Phagocytosis of Spermatozoa and Latex Beads." *Annals of Anatomy* 162: 289−296.

Murakami, M., A. Sugita, and M. Hamasaki. 1982. "Scanning Electron Microscopic Observations of the Vas Deferens in Man and Monkey with Special Reference to Spermiophagy in its Ampullary Region." *Scanning Electron Microscope* 3: 1333−1339.

Nistal, M., L. Santamaría, and R. Paniagua. 1992. "The Ampulla of the Ductus Deferens in Man: Morphological and Ultrastructural Aspects." *Journla of Anatomy* 180: 97−104.

Pampal, A., I.O. Ozen, G. Ekingen, B. Demirogullari, F. Helvacioglu, and G. Take. 2010. "The Morphological Evaluation of Ipsilateral and Contralateral Vasa Deferentia in a Rat Model of Unilateral Spermatic Cord Torsion." *Pediatric Surgery International* 26: 287−292.

Patel, S.M., K.P. Skandhan, and Y.B. Mehta. 1988. "Seminal Plasma Fructose and Glucose in Normal and Pathological Conditions." *Acta Eurpaea Fertilitatis* 19: 329−332.

Piasecka, M., B. Barcew-Wiszniewska, M. Marchlewicz, and L. Wenda-Rózewicka. 1996. "Ultrastructure of Spermatozoa from the Cauda Epididymis in Rat Chronically Treated with Lead Acetate [Pb(II)]." *Polish Journal of Pathology* 47: 65−71.

Regadera, J., P. Cobo, R. Paniagua, F. Martizoz-Garua, J. Placios, and M. Nistal. 1993. "Immunohistochemical and Semi-quantitative Study of the Apical Mitochondria Rich Cells of the Human Pre-Pubertal and Adult Epididymis." *Journal of Anatomy* 183: 507−514.

Reynolds, E.S. 1963. "The Use of Lead Citrate at High pH as an Electron Opaque Stain in Electron Microscopy." *Journal of Cell Biology* 17: 208−212.

Safayhi, H., S.E. Boden, S. Schweizer, and H.P. Ammon. 2000. "Concentration-Dependent Potentiating and Inhibitory Effects of Boswellia Extracts on 5-Lipoxygenase Product Formation in Stimulated PMNL." *Planta Medica* 2: 110−113.

Schweizer, S., A.F. Von Brocke, S.E. Boden, E. Bayer, H.P. Ammon, and H. Safayhi. 2000. "Workup-Dependent Formation of 5-Lipoxygenase Inhibitory Boswellic Acid Analogues." *Journal of Natural Products* 8: 58−61.

Steers, W.D. 1994. "Physiology of the Vas Deferens." *World Journal of Urology* 12: 281−285.

Swami, C.G., J. Ramanathan, and C. Charan Jeganath. 2007. "Noise Exposure Effect on Testicular Histology, Morphology and on Male Steroidogenic Hormone." *Malaysian Journal of Medical Sciences* 14: 28−35.

Swan, M.A., R. Vishwanath, I.G. White, and P.D. Brown-Woodman. 1990. "Electron Microscopic Observations on the Effect of Gossypol on Rat Cauda Epididymis." *Zeitschrift fur Mikroskopisch-Anatomische Forschung* 104: 273−286.

Veri, J.P., L. Hermo, and B Robaire. 1993. "Immunocytochemical Localization of the Yf Subunit of Glutathione S-Transferase. P Shows Regional Variation in the Staining of Epithelial Cells of the Testis, Efferent Ducts and Epididymis of the Male Rat." *Journal of Andrology* 14: 23−44.

Wang, X.D., C. Liu, R.T. Bronson, D.E. Smith, N.I. Krinsky, and M.L. Russell. 1999. "Retinoid Signaling and Activator Protein-1 Expression in Ferrets Given Beta-Carotene Supplements and Exposed to Tobacco Smoke." *Journal of the National Cancer Institute* 91: 60−66.