

Androgen Receptor and Ultrastructural Features of *Nigella sativa* Oil and Nicotine-Treated Male Rat Reproductive Glands

(Ciri-ciri Struktur Ultra dan Reseptor Androgen bagi Kelenjar Pembinaan Tikus Jantan yang Dirawat Nikotin dan Minyak *Nigella sativa*)

LINA, S., ELIZA, H., HASHIDA, N.H.*, IBRAHIM, S.F. & OSMAN, K.

ABSTRACT

Nicotine is claimed to increase free radicals, DNA damage and lipid peroxidation in male reproductive organs. Nigella sativa has been identified to ameliorate these adverse effects due to nicotine intake. This study was conducted to evaluate potential protective effects of N. sativa oil on the adverse effects of nicotine on androgen receptors (AR) and ultrastructural features of rat seminal vesicles and prostate glands. Twenty four Sprague Dawley male rats, 7-9 weeks of age and 200-250 g body weight (BW) were randomly divided into; Group 1 Saline (S), forced fed with 0.1 mL/100 g BW of 0.9% normal saline; Group 2 Nicotine (N), intramuscularly injected with 0.5 mg/100 g BW of nicotine; Group 3 N. sativa (NS), forced fed with 6.0 µL/100 g BW of N. sativa and Group 4 Nicotine-N. sativa (NNS), co-administered with 0.5 mg/100 g BW of nicotine and 6.0 µL/100 g BW of N. sativa. The seminal vesicles and prostate glands were extracted after 100 days of treatment. The seminal vesicle and prostate gland were processed for ultrastructural study and androgen receptor detection. The epithelial cells in prostate gland and seminal vesicle of the N group showed weaker brown staining intensity as compared to that of in the NS and NNS groups. This was consistent with the presence of some ultracellular changes observed in the prostate gland and seminal vesicle tissues of the N group. Findings from this study suggested that administration of N. sativa results in ameliorating effects on both the prostate gland and seminal vesicle structures and functions of the nicotine-treated rats.

Keywords: Androgen receptor; nicotine; Nigella sativa; prostate gland; seminal vesicle

ABSTRAK

Nikotin didakwa meningkatkan radikal bebas, merosakkan DNA dan pengoksidaan lipid dalam organ pembiakan lelaki. Nigella sativa telah dikenal pasti untuk memperbaiki kesan buruk akibat pengambilan nikotin. Kajian ini telah dijalankan untuk menilai potensi kesan perlindungan minyak N. sativa pada kesan buruk nikotin kepada reseptor androgen (AR) dan ciri-ciri struktur ultra vesikel semen dan kelenjar prostat tikus. Dua puluh empat tikus jantan Sprague Dawley, berumur 7-9 minggu dan 200-250 g berat badan (BW) telah dibahagikan secara rawak kepada; Kumpulan 1 Salin (S), diberi makan secara paksaan dengan salin normal 0.9% 0.1 mL/100 g BW; Kumpulan 2 Nikotin (N), disuntik intraotot dengan nikotin 0.5 mg/100 g BW; Kumpulan 3 N. sativa (NS), diberi makan secara paksaan dengan N. sativa 6.0 µL/100 g BW dan Kumpulan 4 Nikotin-N. sativa (NNS), diberi bersama nikotin 0.5 mg/100 g BW dan N. sativa 6.0 µL/100 g BW. Vesikel semen dan kelenjar prostat telah diekstrak selepas 100 hari rawatan. Vesikel semen dan kelenjar prostat telah diproses untuk kajian struktur ultra dan pengesanan reseptor androgen. Sel-sel epitelium dalam kelenjar prostat dan vesikel semen bagi kumpulan N menunjukkan keamatan pewarnaan coklat yang lemah berbanding dengan kumpulan NS dan NNS. Ini adalah konsisten dengan kehadiran beberapa perubahan selular ultra yang diperhatikan dalam kelenjar prostat dan tisu vesikel semen bagi kumpulan N. Penemuan daripada kajian ini mencadangkan bahawa pemberian N. sativa memberi kesan pemulihan kepada struktur dan fungsi kedua-dua kelenjar prostat dan vesikel semen tikus yang dirawat nikotin.

Kata kunci: Kelenjar prostat; Nigella sativa; nikotin; reseptor androgen; vesikel semen

INTRODUCTION

A number of epidemiological and experimental studies showed detrimental effects of nicotine administration on male sexual functions in animals and humans (Oyeyipo et al. 2011; Sankako et al. 2013). Nicotine exhibited several marked testicular toxicities including reduced weights of the testes, epididymis and seminal vesicle, impaired semen quality and testicular histopathology. Nicotine

also enhanced oxidative stress and increased production of reactive oxygen species (ROS) and lipid peroxidation, which led to tissue oxidative damage (Aitken et al. 2014). Thus, exposure to cigarette smoking containing nicotine could lead to adverse effects on the reproductive system (Zenzes 2000).

Herbal medicine has been used as alternative treatment for male infertility especially in developing

and underdeveloped countries (Safarinejad & Safarinejad 2012). Plants are widely used in traditional practices for enhancing fertility and may contain aphrodisiac properties (D'Cruz et al. 2010; McDonald 2004). Current evidence demonstrates that some medicinal herbs can scavenge free radicals and prevent the deleterious consequences of oxidative stress (Atanassova et al. 2011; Awa et al. 2012). One of the medicinal plants that has been extensively studied for its pharmacological properties and antioxidant activities is *N. sativa* (Ashraf et al. 2011; Ismail et al. 2010). Numerous earlier studies recorded the positive effects of *N. sativa* oil, not only in spermatogenesis, but also in the overall reproductive parameters in male rats (Al-Sa'aidi et al. 2009; Kolahdooz et al. 2014). Zahra et al. (2016) reported valuable therapeutic effects of *N. sativa* seed powder, oil, extracts (aqueous, ethanolic, and methanolic) and thymoquinone on different disorders with a wide range of safe doses based on extensive and clinical studies.

Thymoquinone (TQ), the main constituent of the volatile oil derived from *N. sativa* gave beneficial effects on the semen quality of heat stress-induced reproductive system (Al-Zahrani et al. 2012). A previous study also demonstrated protective and antioxidant effects of TQ on functions of testicular and accessory sex glands against oxidative stress induced by hydrogen peroxide (Tawfeek et al. 2006).

To date, reports and data on the effects of *N. sativa* oil against detrimental effects of nicotine on male reproductive system are still lacking. Hence, the present study was designed to investigate the possible protective effect of *N. sativa* oil (NSO) on nicotine administered rats through the assessment of androgen receptors and ultrastructural features of the prostate and seminal vesicle glands.

MATERIALS AND METHODS

ANIMAL MAINTENANCE

Twenty four Sprague Dawley rats (7-9 weeks, average weight 200-250 g) were used in this experiment. Water and food in the form of pellets were given *ad libitum* to the rats. The rats were allowed to acclimatise to 12 h of light and 12 h of darkness per day. Wood shavings were used as bedding and changed once every three days to maintain clean environment for the rats.

EXPERIMENTAL DESIGN

Twenty four Sprague Dawley male rats, were randomly divided into 4 groups with 6 rats for each group. Group 1 (S), was forced fed with 0.9% normal saline, 0.1 mL/100 g body weight (BW); Group 2 (N), was intramuscularly (i.m.) injected with nicotine, 0.5 mg/100 g BW (L-Nicotine, 99+%, CAS RN: 54-11-5); Group 3 (NS), was forced fed with *N. sativa*, 6.0 µL/100 g BW (Dogaci, Turkey) and Group 4 (NNS), was co-administered with nicotine, 0.5 mg/100 g BW and *N. sativa*, 6.0 µL/100 g BW. Treatments were carried out once daily at 9.00 a.m for 100 days. The protocols used

were approved by the Institutional Animal Care and Use Committee, University of Malaya (UM IACUC) (reference number of ISB/20/04/2012/DSHA (R)). The seminal vesicle and prostate gland were processed for ultrastructural study and androgen receptor detection.

IMMUNOHISTOCHEMISTRY STUDY FOR ANDROGEN RECEPTOR IDENTIFICATION

The prostate gland and seminal vesicle were fixed in 10% formalin solution at room temperature for a week. The sectioned tissues of 5 µm thickness were placed on Poly-L-Lysine coated slides. Immunohistochemical staining procedure was conducted according to the protocol in immunoperoxidase secondary detection kit (CHEMICON IHC: DAB 500) with some modifications. The sectioned tissues were deparaffinised using xylene for five minutes with four changes. All slides were incubated overnight with mouse anti-androgen receptor primary (1°) antibody (Santa Cruz Biotechnology: AR 441: sc-7305) at room temperature. The negative control slides were incubated in antibody diluent. On the following day, all treated and negative control slides were incubated in secondary (2°) antibody for 2 h. Treated slides were observed under light microscope (Nikon Eclipse 80i) at 40× magnification using NIS-Element Imaging System Software.

TRANSMISSION ELECTRON MICROSCOPE (TEM)

The harvested prostate gland and seminal vesicle were fixed in 4% glutaraldehyde. The fixed tissues were then washed with three changes of cacodylate buffer before being postfixed in 1% osmium tetroxide (OsO₄) in cacodylate buffer for 2 h at 4°C. Next, the specimens were stored in cacodylate buffer overnight. After 48 h, specimens were washed with three changes of double distilled water prior to incubation in uranyl acetate for 10 min. The specimens were then once again washed with three changes of double distilled water. The specimens were later dehydrated in increasing alcohol concentrations. Then, the specimens were infiltrated with two changes of propylene oxide for 15 min, followed by a mixture of propylene oxide and epon at a ratio of 1:1 and 1:3 for 1 and 2 h, respectively. The specimens were kept in pure epon overnight. Embedded specimen in epon were sectioned, mounted on copper grids and stained with uranyl acetate and lead citrate. Stained sections were examined and photographed under electron microscope (LEOLIBRA 120).

RESULTS

IMMUNOHISTOCHEMISTRY STUDY FOR ANDROGEN RECEPTOR IDENTIFICATION

The nicotine group of rats exhibited a weak brown staining intensity at the epithelial cells of prostate (Figure 1(a)) indicating minimal presence of androgen receptor. However, the nicotine-*N. sativa*, *N. sativa* and saline groups showed stronger brown staining intensity (Figure

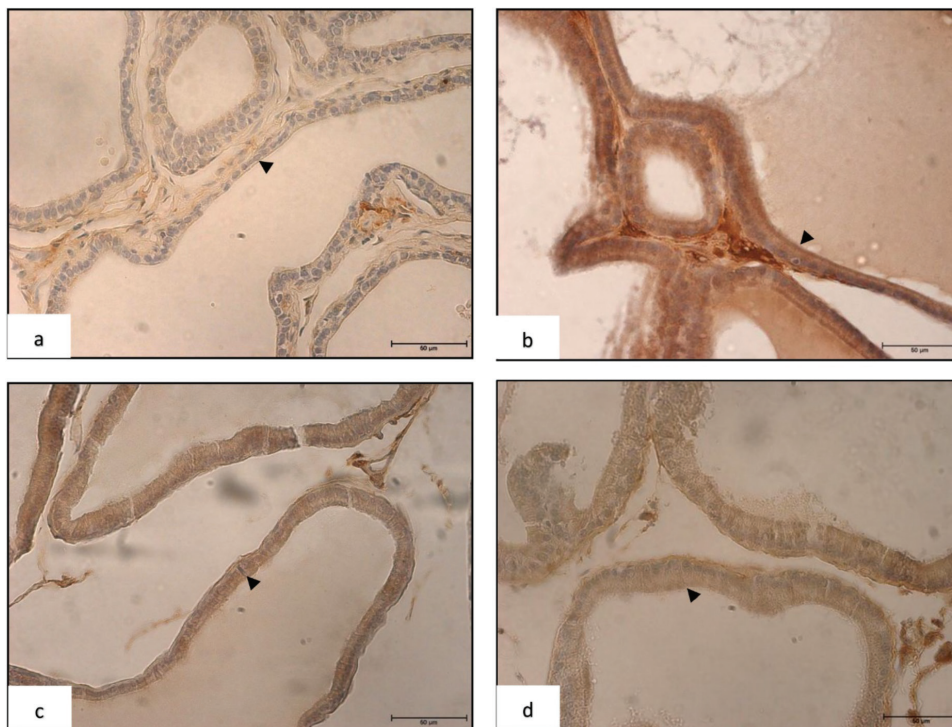


FIGURE 1. Photomicrograph of the prostate gland for; (a) N (b) NNS (c) NS and (d) S groups stained with anti-androgen antibody (40 \times). Lower intensity of brown staining (arrowhead) of the glandular epithelium in the N group compared to the NNS, NS and S groups. Bar = 50 μ m

1(b), 1(c) and 1(d)). Similarly, the epithelial cells of the seminal vesicle of the nicotine group (Figure 2(a)) also exhibited weak brown staining intensity while the nicotine-*N. sativa*, *N. sativa* and saline groups showed stronger brown staining (Figure 2(b), 2(c) and 2(d)).

TRANSMISSION ELECTRON MICROSCOPE (TEM)

PROSTATE GLAND

In the nicotine group of rats, the nuclei of the epithelial cells were noted to have irregular shapes with prominent invagination of membrane. Furthermore, dilated cisternae of the endoplasmic reticulum in the cytoplasm and notable spacings among adjacent cells were observed. A decreased in number and discontinuity of microvilli were evident at the apical region of the epithelial cells in the nicotine group (Figure 3(a)). The nuclei observed in the nicotine-*N. sativa* group were somehow similar as that in the nicotine group. However, the invagination of the nuclei membrane were not as prominent as shown in the nicotine group. A mild dilation of the endoplasmic reticulum cisterns was also observed in the nicotine-*N. sativa* group (Figure 3(b)). The nuclei in the *N. sativa* group were normal in shape with evident nucleoli and enclosed by well-defined nuclei membrane. In addition, the cytoplasm of the epithelial cells also contained distinct endoplasmic reticulum. Microvilli were also noted to be more in the prostate gland of *N. sativa*, nicotine-*N. sativa* and saline groups (Figure 3(b), 3(c) and 3(d)) as compared to the nicotine group.

SEMINAL VESICLE

The ultrastructural features of the seminal vesicle in the nicotine group of rats showed the microvilli were sparsely present at the apical part of the epithelial cells (Figure 4(a)) as compared to the other groups of treated rats (Figure 4(c) and 4(d)). In addition, the nucleoli were noted to be enclosed in a well-defined nuclear membrane in all groups (Figure 4(a), 4(b) and 4(c) (inset)).

DISCUSSION

In the present study, the positive effects observed in the immunohistochemistry (IHC) and transmission electron microscope (TEM) studies of the prostate gland and seminal vesicle of the nicotine-*N. sativa* group could be attributed to the antioxidant properties of *N. sativa* oil that inhibited excessive free radical generation due to nicotine administration. *N. sativa* is known to exhibit antioxidant activities with excellent ability to scavenge free radicals (Burits & Bucar 2000). To date, antioxidants are known to have the ability to reduce oxidative stress by breaking the oxidative chain reaction (Chen et al. 2006; Elbetieha et al. 2011). Compounds with antioxidant properties can transfer electrons to oxidizing agents and inhibit free radical production (Adedara et al. 2014).

Findings from a previous study showed that generation of reactive oxygen species (ROS) as a result of oxidative stress was depressed by antioxidant effect of *N. sativa* seeds (Parveen & Shadabig 2011). In the present study,

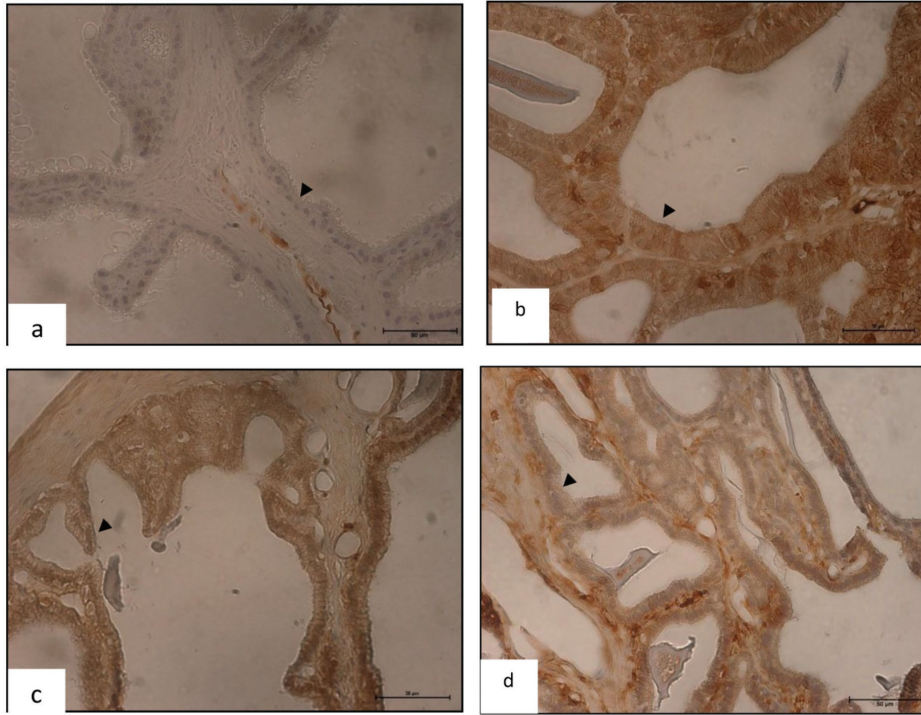


FIGURE 2. Photomicrograph of the seminal vesicle for; (a) N (b) NNS (c) NS and (d) S groups stained with anti-androgen antibody (40X). Higher intensity of brown staining (arrowhead) of the glandular epithelium in the NNS, NS and S groups compared to the N group. Bar = 50 µm

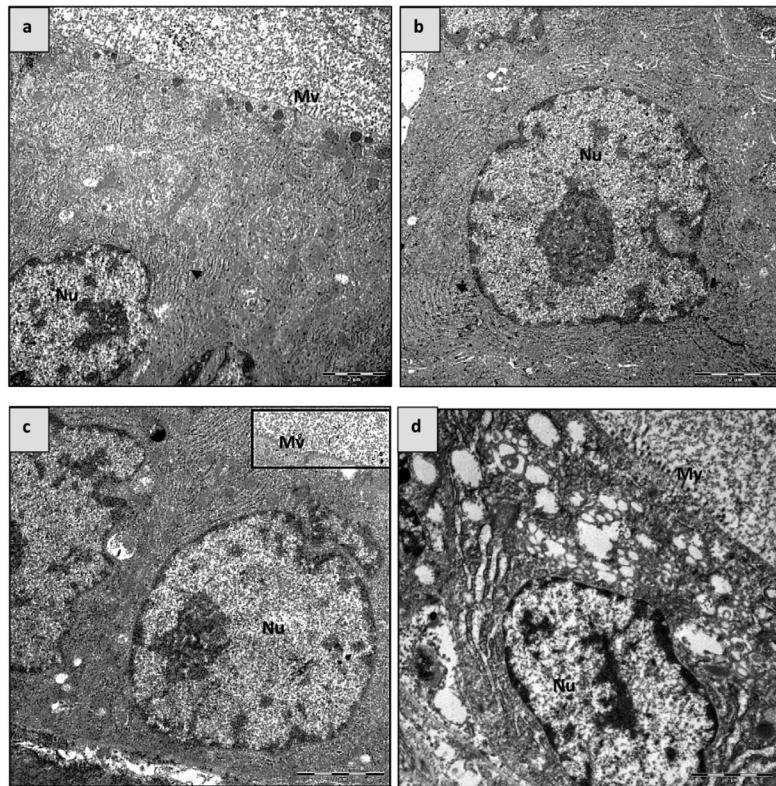


FIGURE 3. Electronmicrograph of the prostate gland for; (a) N group showed minimal number and discontinuity of the microvilli (Mv) and dilated cisternae of endoplasmic reticulum (arrowhead) (b) NNS group showed an irregular shape of nucleus (Nu) with minimal invagination of the membrane and mild dilated cisternae of endoplasmic reticulum (arrowhead), (c) NS group showed nucleus (Nu) with nucleolus enclosed in a well-defined nuclear membrane with abundant microvilli (inset) (Mv) and (d) S group showed abundant microvilli on the apical surface of the cell. Bar: 2µm

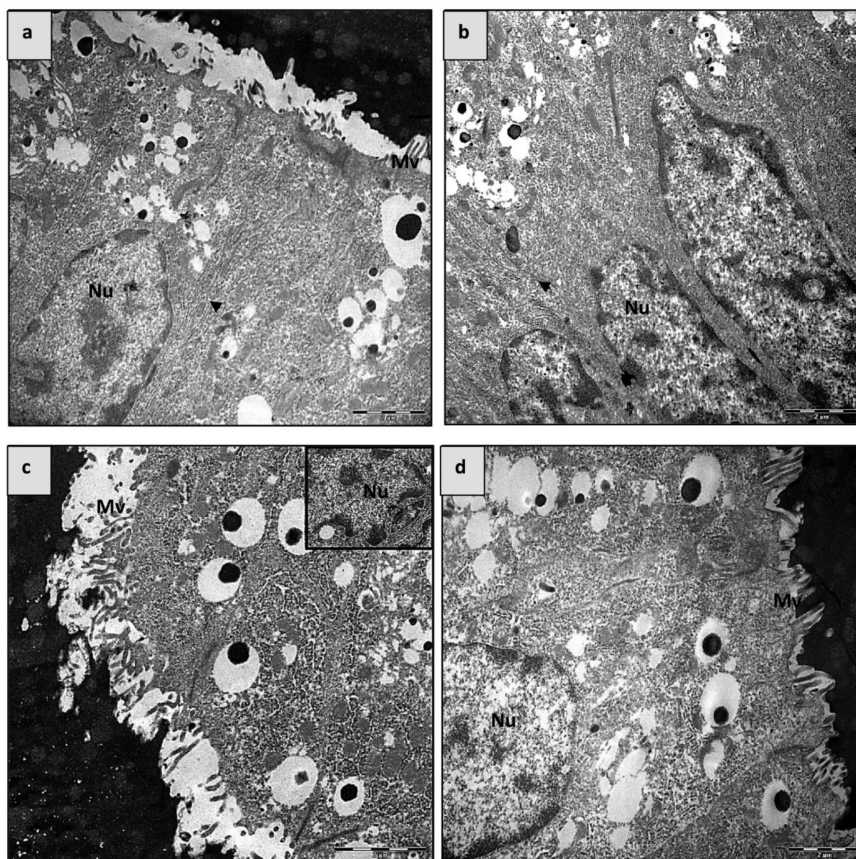


FIGURE 4. Electronmicrograph of seminal vesicle for; (a) N group with sparse microvilli (Mv), (b) NNS group showed an irregular shape of the nucleus (Nu) with minimal invagination of the membrane and mild dilated cisternae of endoplasmic reticulum (arrowhead). Both (c) NS and (d) S groups contained nucleus (Nu) enclosed within a well-defined nuclear membrane and abundant microvilli (Mv) on the apical surface of the cell. Bar: 2 μ m

improvement of both the prostate gland and seminal vesicle histological features is possibly due to the antioxidant properties of *N. sativa* that could neutralize ROS by enhancing the scavenging system.

Result of the present study was in agreement to that reported by Reza et al. (2015). The authors observed positive influence of *N. sativa* on sperm parameters, semen, Leydig cells, reproductive organs and sexual hormones. According to the authors, the main potential mechanism is through the antioxidant properties of *N. sativa*. The main antioxidant component of *N. sativa* is thymoquinone (TQ) that can improve male fertility parameters through promoting antioxidant defence (Reza et al. 2015).

Nicotine was reported to adversely affect the male reproductive system and fertility, where its administration in experimental animals caused alterations in the testicular morphometric parameters and structure, sperm functional parameters, fertility indices and hormone (Gamal et al. 2016; Oyeyipo et al. 2015, 2010). A study by Lina et al. (2014) showed that the columnar cells of nicotine treated seminal vesicles were flattened as compared to normal columnar cells. The exposure to nicotine also resulted in high levels of malondialdehyde (MDA), a marker for oxidative stress (Al-Malki & Moselhy 2013; Kiziler et al. 2007). In addition, findings from other studies showed

negative immunoreactivity in the epithelial and stromal cells of the prostate gland due to oxidative stress (Banerjee et al. 2001; Sayed Gabry et al. 2014). Results from the present study supported previous findings that nicotine can damage both accessory sex glands, the prostate gland and seminal vesicle.

In the present study, weak androgen receptor staining observed in both accessory sex glands of nicotine treated group may be attributed to low testosterone level. Previous studies showed that tobacco intake enhanced aromatization of testosterone resulting in weak androgen receptor expression in the prostate gland (Sunanda et al. 2014). Yeh et al. (1989) reported that nicotine and its metabolite, cotinine produced a dose dependent decrease in androstenedione and testosterone concentrations in rats by competitive inhibition of multiple steps in testosterone biosynthesis. According to Meikle et al. (1988), nicotine and its metabolite, cotinine, are inhibitors of 3α -hydroxysteroid dehydrogenase, an important enzyme in the testosterone and dihydrotestosterone metabolism, which can alter the androgenic action on the prostate.

Reddy et al. (1998) also showed that chronic nicotine use on mice provoked reduction in testicular weight and atrophied male accessory sex glands, as a consequence of

the androgenic depletion. The deprivation of testosterone caused by nicotine not only resulted in negative effects on the androgen receptor, but also on the ultrastructural features. This was evidenced by the dilated cisternae of the endoplasmic reticulum found in the prostate gland of nicotine group. A previous study suggested that degradation of biological membranes was characterized by the present of dilation in the cisternae of both Golgi and endoplasmic reticulum as well as formation of autophagic vacuoles (Aumuller et al. 1981).

The recovery of the endoplasmic reticulum cisternae noted in the accessory sex glands of the nicotine-*N. sativa* group may be due to an increase in the testosterone level. This was based on the observation by Thompson and Heidger (1978) that restoration of the cisternae in the granular endoplasmic reticulum, Golgi complex and secretory granules in secretory epithelial cells of the prostate gland were noted after administration of 5 and 2 mg of testosterone for 3 and 7 days, respectively, on castrated rats. Similar findings were also reported by Wright et al. (1996) with different doses of testosterone for 21 days. Madeha et al. (2018) observed improvement of all biochemical parameters and restoration of the tissues of kidney, stomach, testes and liver to normal after 8 weeks of tartrazine and *N. sativa* co-administration in albino rats.

CONCLUSION

Based on the positive effects of *N. sativa* found in the current study and cited by previous researchers albeit the differences in dose and type of extract used; and the differences in experimental approach, more studies and clinical trials are recommended to demonstrate the underlying mechanism involved in contributing to the efficacy of *N. sativa* for the treatment of male infertility. In conclusion, findings from the present study provide additional information on the effects of *N. sativa* in terms of the immunohistochemistry and ultrastructure of the accessory sex glands of nicotine-treated rats.

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Lina, S.
Institute of Graduate Studies
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Eliza, H.
Department of Anatomy, Faculty of Medicine
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Hashida, N.H.*
Center for Foundation Studies in Science
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

Ibrahim, S.F.
Department of Physiology
Faculty of Medicine
Universiti Kebangsaan Malaysia Medical Centre
Jalan Yaacob Latif, Bandar Tun Razak
56000 Cheras, Kuala Lumpur, Federal Territory
Malaysia

Osman, K.
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: nhhpasum@um.edu.my

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