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ORIGINAL LITERARY WORK DECLARATION

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Name of Degree : MASTER OF PHILOSOPHY

Title of Project Paper/Research Report/Dissertation/Thesis ("This Work"):

EFFECTS OF *HABBATUS SAUDA* (*NIGELLA SATIVA*) OIL ON SPERM AND TESTIS PARAMETERS OF NICOTINE TREATED RATS

Field of study : LIVE SCIENCES (BIOLOGY)

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ABSTRACT

Nicotine is considered as one of the most toxic substances that can be found in tobacco smoke. Besides being used as a natural insecticide, nicotine is pharmacologically active and has a negative impact on the reproductive system and fertility of males. In contrast, *Nigella sativa* seeds and oil have a lot of positive properties, such as antipyretic, analgesic, antihypertensive and antineoplastic. This study was carried out to investigate the potential protective effect of *Nigella sativa* (*habbatus sauda*) oil on nicotine treated male rat reproductive system. Forty-five *Sprague-Dawley* male rats (7-9 weeks old, 200-250g) were randomly divided into 5 groups; Nicotine (N) (0.5mg/100g), Nicotine Control (NC) (saline, 0.1ml/100g), *habbatus sauda* oil (HS) (6.0μl/100g), *habbatus sauda* Control (HSC) (corn oil, 0.1ml/100g) and nicotine-*habbatus sauda* (NHS) (0.5mg/100g BW nicotine and 6.0μl/100g *habbatus sauda*) groups and treated for 100 days. Sperm parameters, seminiferous tubules measurements, blood hormonal level and body weight increment were evaluated**.** Rats in NHS group (1.29±0.04 x 10^6 sperm/ml) showed significantly higher sperm motility compared to N group (1.04±0.04 x 10^6 sperm/ml). The HS group (90.69±0.01 %) had the highest percentage of normal sperm followed by NHS (88.52±0.02 %) and N (82.05±0.02 %) groups ($p<0.05$). Percentage of live sperm for NHS group (95.96±0.01 %) was significantly higher than N group (93.45±0.01 %) but significantly lower than HS group (96.80±0.00 %) ($p<0.05$). The NHS group showed significantly smaller diameter of seminiferous tubules (245.42±1.34µm) compared to N group (247.12±1.59µm) and HS group (249.95±1.37µm). Comparison on lumen diameter showed that N group had significantly wider lumen (100.74±1.75µm) compared to NHS (67.41±1.92µm) and HS (66.65±1.57µm) groups. Width of spermatogonia layer of NHS group (16.12±0.24µm) was significantly lower compared to HS (17.59±0.25 µm) and N (19.51±0.29µm)

groups. The NHS group (35.94±0.39µm) had significantly widest spermatocytes layer followed by HS (34.56±0.40µm) and N (32.56±0.41µm) groups ($p<0.05$). Both HS (38.05±0.61µm) and NHS (37.84±1.22µm) groups had significantly wider spermatid and sperm layer compared to N group (21.51±0.51µm). Treatments did not significantly affect blood hormonal level (testosterone, LH and FSH) and body weight increment pre and post treatment ($p\geq0.05$). This study suggests that *Nigella sativa* (*habbatus sauda*) oil possessed protective effects against detrimental nicotinic effects on sperm quality and testis parameters of male rats.

ABSTRAK

Nikotin dianggap sebagai salah satu bahan paling toksik yang boleh ditemui dalam asap tembakau. Selain digunakan sebagai racun serangga semulajadi, nikotin adalah aktif secara farmakologi dan mempunyai kesan negatif terhadap sistem pembiakan serta kesuburan lelaki. Sebaliknya, biji dan minyak *Nigella sativa* mempunyai banyak ciri-ciri positif seperti antipiretik, analgesik, anti-hipertensi dan antineoplastik. Kajian ini dijalankan bagi mengkaji potensi kesan perlindungan minyak *Nigella sativa* (*habbatus sauda*) terhadap sistem pembiakan tikus jantan yang diberikan nikotin. Empat puluh lima ekor tikus jantan *Sprague-Dawley* (berumur 7-9 minggu, 200-250g) dibahagikan secara rawak kepada kumpulan: nikotin (N) (0.5mg/100g), kawalan nikotin (NC) (salina, 0.1ml/100g), *habbatus sauda* (HS) (6.0µl/100g), kawalan *habbatus sauda* (HSC) (minyak jagung, 0.1ml/100g) dan nikotin-*habbatus sauda* (NHS) (0.5mg/100g nikotin dan 6.0µl/100g *habbatus sauda*) dan dirawat selama 100 hari. Parameter sperma, ukuran tubul seminiferus, tahap hormon darah dan penambahan berat badan tikus telah dinilai. Tikus dalam kumpulan NHS ($1.29\pm0.04 \times 10^6$ sperma/ml) menunjukkan motiliti sperma bersignifikan lebih tinggi berbanding dengan kumpulan N ($1.04\pm0.04 \times 10^6$ sperma/ml). Kumpulan HS (90.69±0.01 %) mempunyai peratusan tertinggi morfologi sperma normal diikuti oleh kumpulan-kumpulan HS (88.52±0.02 %) dan N (82.05±0.02 %) ($p<0.05$). Peratusan sperma hidup bagi kumpulan NHS (95.96±0.01 %) adalah bersignifikasi lebih tinggi daripada kumpulan N (93.45±0.01 %) tetapi bersignifikasi lebih rendah daripada kumpulan HS (96.80±0.00 %) ($p<0.05$). Kumpulan NHS (245.42±1.34µm) menunjukkan diameter yang lebih kecil secara signifikan berbanding kumpulan N (247.12±1.59µm) dan kumpulan HS (249.95±1.37µm). Perbandingan diameter lumen menunjukkan bahawa kumpulan N (100.74±1.75µm) mempunyai lumen yang lebih lebar secara signifikan berbanding dengan kumpulan

NHS (67.41±1.92μm) dan HS (66.65±1.57μm). Kelebaran lapisan spermatogonia bagi kumpulan NHS (16.12±0.24μm) adalah secara signifikannya lebih rendah berbanding dengan kumpulan-kumpulan HS (17.59±0.25μm) dan N (19.51±0.29μm). Kumpulan NHS (35.94±0.39μm) mempunyai kelebaran lapisan spermatid yang signifikan diikuti oleh kumpulan-kumpulan HS (34.56±0.40μm) dan N (32.56±0.41μm) ($p<0.05$). Kedua-dua kumpulan HS (38.05±0.61μm) dan NHS (37.84±1.22μm) mempunyai kelebaran lapisan spermatid dan sperma yang signifikan berbanding dengan kumpulan N (21.51±0.51μm). Rawatan-rawatan tidak mempengaruhi secara signifikan aras hormon darah (testosteron, LH dan FSH) dan peningkatan berat badan sebelum dan selepas rawatan ($p\geq0.05$). Kajian ini mencadangkan bahawa minyak *Nigella sativa* (*habbatus sauda*) mempunyai kesan perlindungan terhadap kesan kerosakan nikotin kepada kualiti sperma dan parameter testis tikus jantan.

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Sincerely,

Cho Ping

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ABP	Androgen binding protein
ACTH	Adrenocorticotrophin
AI	artificial insemination
ANOVA	analysis of variance
ATP	Adenosine triphosphate
ART	Assisted reproductive technologies
ASRM	American Society for Reproductive Medicine
AVP	Arginine vasopressin
BSA	Bovine serum albumin
CASA	Computer assisted semen analysis
CDC	Centers for Disease Control and Prevention
cm	centimeter
cm^3	cubic centimeter
CO_2	carbon dioxide
d.f	degrees of freedom
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
Duncan-MRT	Duncan Multiple Range Test
ELISA	enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
g	gram
GH	Growth hormone
GI	Glycemic index
GRH	Gonadothropin releasing hormone
HCl	hydrochloric acid
H_2O_2	hydrogen peroxide

HS	*habbatus sauda*
HSC	*habbatus sauda* control
H&E	Haematoxylin and eosin
ICSI	Intracytoplasmic sperm injection
i.m	Intramuscular
IVF	*In vitro* fertilization
kg	kilogram
L	Lumen
LC	Leydig cell
LH	Luteinising hormone
LN_2	liquid nitrogen
LSD	Least significant difference
LPO	Lipid peroxidation
mg	milligram
mIU	milli-international units
ml	milliliter
mm	millimeter
N	Nicotine
NaCl	sodium chloride
NC	Nicotine control
ng	nanogram
NHS	Nicotine-*habbatus sauda*
NPY	Neuropeptide Y
OAT	Oligoasthenoteratozoospermia
OD	Absorbance
POMC	Pro-opiomelanocortin
PUFA	Polyunsaturated fatty acids
RIA	Radioimmunoassay

ROS	Reactive oxygen species
rpm	revolution per minute
SC	Spermatogenic cells
SE	standard error
SHBG	sex-hormone-binding globulin
SPSS	Statistical Package for the Social Sciences
ST	Seminiferous tubule
TMB	3,3′,5,5′-tetramethylbenzidine
TSH	Thyroid stimulating hormone
TQ	Thymoquinone
TYH	Toyoda Yokoyama Hosi
UM IACUC	Institutional Animal Care and Use Committee, University of Malaya
WHO	World health organisation
µl	microliter
µm	micrometer