Ovozoa 13 No. 2, August 2024 p-ISSN 2302-6464 e-ISSN 2722-967X
Available at https://e-journal.unair.ac.id/OVZ/index

Original article

Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke

Fifi Fauziah Ramadhani[1], Yulianna Puspitasari[2*], Hermin Ratnani[3], Budi Utomo[3], Rochmah Kurnijasanti[4], Kadek Rachmawati[4]

[1] Internship Veterinarian in Klinik K5, Kota Kediri, East Java, Indonesia, [2] Division of Veterinary Microbiology, [3] Division of Veterinary Reproduction, [4] Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia

[*] Corresponding author, e-mail: yulianna-puspitasari@fkh.unair.ac.id
Open access under CC BY – SA license, DOI: 10.20473/ovz.v13i2.2024.120-128
Received February 9 2024, Revised September 1 2024, Accepted September 3 2024
Published online September 2024

ABSTRACT

This study investigates the impact of mulberry leaf extract on the viability, plasma membrane integrity, and motility of spermatozoa from male white rats (*Rattus norvegicus*) exposed to e-cigarette smoke. A total of twenty-five male rats were divided into five groups: negative control (NC), positive control (PC), T1, T2, and T3. All groups, except for the NC group, were exposed to e-cigarette smoke. Rats in the T1, T2, and T3 groups received mulberry leaf extract in doses of 100, 200, and 400 mg/kg bw, respectively, while the NC and PC groups were given a placebo of 1% Na-CMC. Both the mulberry leaf extract and the placebo were administered daily, beginning three days prior to the start of e-cigarette smoke exposure, which lasted for 28 days. Results showed that spermatozoa motility, plasma membrane integrity, and viability in the experimental groups were significantly lower than those in the NC group ($p < 0.05$). Conversely, rats in the T1, T2, and T3 groups that received mulberry leaf extract demonstrated significantly greater spermatozoa viability, plasma membrane integrity, and motility compared to the PC group ($p < 0.05$). The T3 group exhibited the most pronounced improvements, with significantly enhanced spermatozoa viability, membrane integrity, and motility ($p < 0.05$) relative to the PC group. These results indicate that mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa viability, plasma membrane integrity, and motility in white rats (*Rattus norvegicus*) subjected to e-cigarette smoke.

Keywords: alkaloids, antioxidant, ethanolic extract, free radical, polyphenols

INTRODUCTION

Smoking is a health problem with a fairly high death rate in the world (Le Foll *et al*., 2022). Cigarettes contained dangerous compounds that could harm health. One burned cigarette produced thousands of types of chemicals. Once identified, the chemical components turned out to be compounds that could be harmful to health, including nicotine, tar, carbon monoxide, nitrosamines, nitrogen oxides, polynuclear aromatic hydrocarbons (Engstrom et al., 2003). Several studies linked chemicals in cigarette smoke to the risk of respiratory disease (Cha *et al*., 2023), immune disorders, cardiovascular disease (Dahdah *et al*., 2022), cancer (Jain *et al*.,

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

2021), diabetes mellitus (Maddatu *et al*. al., 2017), rheumatoid arthritis (Lei *et al*., 2023), and reproductive disorders in men (Osadchuk *et al*., 2023) and women (Dhage *et al*., 2024). It was estimated that billions of people die every year due to cigarette smoke (Le Foll *et al*., 2022).

To reduce the risks posed by tobacco, WHO uses various strategies to encourage people to quit smoking. One example is the nicotine replacement therapy (NRT) method. NRT is a method of administering nicotine without the process of burning tobacco but still provides the sensation of smoking for the user (Wadgave and Nagesh, 2016; Hartmann-Boyce *et al*., 2016). There are several types of NRT, one of which is e-cigarettes. E-cigarette users continued to increase every year because conventional cigarette users were starting to switch to e-cigarettes. Several studies showed that e-cigarettes could help smokers quit traditional cigarettes (Wang *et al*., 2021).

E-cigarettes contained propylene glycol, vegetable glycine (glycerol), ethylene glycol, polyethylene glycol mixed with flavorings, and nicotine (Komura *et al*., 2022). Polyethylene glycol could cause oxidative stress by increasing reactive oxygen species (ROS) (Patlolla *et al*., 2019). Exposure to polyethylene glycol severely affected the reproductive system of male Wistar rats (Ajonuma *et al*., 2024).

Mulberry plants (*Morus alba* L.) were recognized for their antioxidant properties, containing beneficial compounds like polyphenols, alkaloids, flavonoids, and terpenoids in their leaves (Chen *et al*., 2021). Flavonoids, with their hydroxyl groups, could donate hydrogen atoms to stabilize ROS compounds. Quercetin, a notable flavonoid found in mulberry leaves, exhibited strong antioxidant activity (Zheng *et al*., 2021). The ethanol extract of mulberry leaves had demonstrated potent antioxidant properties, as confirmed by the DPPH assay (Chen *et al*., 2022). Given the high antioxidant potential of quercetin in mulberry leaves, it is hypothesized that it may mitigate free radical damage induced by e-cigarette smoke.

Therefore, the aim of this study was to determine the effect of exposure to e-cigarette smoke on the viability, plasma membrane integrity and motility of white rat spermatozoa by administering mulberry leaf extract.

MATERIALS AND METHODS

The collected mulberry leaves were washed with running water to remove dirt or particles on the leaves. Next, the leaves were cut into small pieces and air dri. Dried mulberry leaves were blended to be a fine powdered simplicia. Simplicia powder was extracted using the maceration method using 65% ethanol, for 24 hours. After filtering, the filtrate was evaporated using a rotary evaporator to obtain the extract (Indawati *et al*., 2023). The extract was resuspended in 1% Na-CMC and the concentrations were adjusted according to the doses. Regarding antioxidant activity, the effective oral dose of mulberry leaf extract was 200 mg/kg bw (He *et al*., 2018). Therefore, in this study the oral dose of mulberry leaf extract dose used were 100 mg, 200 mg, and 400 mg/kg bw.

The procedures used in this study have been approved by the Universitas Airlangga Animal Research Ethical Commission reference number 1.KEH.073.-3.2022. Twenty-five male white rats were allocated into negative control (NC), positive control (PC), T1, T2 and T3 group. All rats, except those in the NC groups were exposed to e-cigarette smoke. Rats in groups T1, T2 and T3 each received mulberry leaf extract doses of 100, 200, and 400 mg/kg bw, respectively. Rats in the NC and PC groups received 1% Na-CMC, as placebo. Mulberry leaf extract and placebo were administered orally at a volume of 1.5 mL daily, starting 3 days before the start of e-cigarette smoke exposure; exposure to e-cigarette smoke was carried out for 28 days (Table 1).

The exposure to e-cigarette smoke was conducted for 30 minutes (three puffs per 10s interval) with 1 mL of liquid for each exposure and a nicotine content of 18mg/mL/day. On day-

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

39, all the rats were sacrificed to have their testicles and cauda epididymis collected. The cauda epididymis was separated by cutting the proximal end of the corpus epididymis and the distal part of the vas deferens. The isolated cauda epididymis was placed in a petri dish containing 0.2 mL of 0.9% sodium chloride solution. Incisions were made in the cauda epididymis, and the spermatozoa were allowed to swim out. After the cauda epididymis tissue was removed, spermatozoa suspension was mixed homogeneously. Then, spermatozoa viability, plasma membrane integrity and motility of the spermatozoa were evaluated (Alifia *et al*., 2023).

Table 1 Treatment in the experimental groups consisted of exposure to e-cigarette smoke and oral administration of mulberry leaf extract (*Morus alba* L.)

	day-8 to day-10	day-11 to day-38
NC	1% Na-CMC	1% Na-CMC
PC	1% Na-CMC	1% Na-CMC + exposure to e-CS
T1	100 mg/kg bw MLE	100 mg/kg bw MLE + exposure to e-CS
T2	200 mg/kg bw MLE	200 mg/kg bw MLE + exposure to e-CS
T3	400 mg/kg bw MLE	400 mg/kg bw MLE + exposure to e-CS

NC: negative control; PC: positive control; T1, T2, T3: treatment 1, 2, 3; Na-CMC: sodium carboxymethyl cellulose; e-CS: e-cigarette smoke; bw: body weight, MLE: mulberry leaf extract.

Spermatozoa motility

A drop of spermatozoa suspension was mixed thoroughly with an equal volume of physiological saline solution (0.9% w/v sodium chloride) on a slide, which was then covered with a coverslip. The assessment of spermatozoa motility was carried out using a microscope (Nikon Eclipse E100) with 400x magnification. The percentage of spermatozoa with progressive motility was calculated on 100 spermatozoa (Sari *et al*., 2023).

Spermatozoa viability

Slides for examining spermatozoa viability were prepared by dropping one drop of spermatozoa suspension onto an object glass and one drop of eosin-nigrosin stain, and mixed thoroughly. Thin smear was made, and fixed afterward over a flame. The slides were observed under a microscope (Nikon Eclipse E100) at 400x magnification on 100 spermatozoa. Live spermatozoa were unstained, and dead spermatozoa were stained with eosin (Sari *et al*., 2023).

Spermatozoa plasma membrane integrity

Spermatozoa suspension (0.1 mL) was diluted 10 times in hypoosmotic solution (1.352 g fructose and 0.735 g sodium citrate dihydrate dissolved in 100 mL distilled water) and incubated at 37°C. After incubation for 30 minute, spermatozoa were examined under a light microscope (Nikon Eclipse E100) at 400x magnification. Spermatozoa whose plasma membranes were intact showed curved or bent spermatozoa tails, while spermatozoa whose plasma membranes were damaged showed straight tails (Susilowati *et al*., 2021).

Data analysis

Data on spermatozoa motility, viability and plasma membrane integrity were analyzed using ANOVA. Duncan's range test was used after analyzing the data to see if there was a significant difference ($p < 0.05$). All tests were carried out using Statistical Product and Service Solution (SPSS) v 26 for Windows.

RESULTS

Exposure to e-cigarettes for 28 days (PC) caused a decrease ($p < 0.05$) in viability, intact plasma membrane, and motility of rat spermatozoa compared to the NC group of rats.

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

Administration of mulberry leaf extract at doses of 100, 200, and 400 mg/kg bw to rats exposed to e-cigarette smoke was followed by an increase ($p < 0.05$) in viability, intact plasma membrane, and motility of rat spermatozoa compared to the PC group of rats. The highest dose in this study (400 mg/kg bw, T3) showed higher viability, intact plasma membrane, and motility of spermatozoa ($p < 0.05$) compared to those in groups PC, T1, and T2, and showed lower viability, intact plasma membrane, and motility of spermatozoa ($p < 0.05$) compared to those in group NC (Table 2).

Table 2 Viability, plasma membrane integrity, and motility of white rats (*Rattus norvegicus*) spermatozoa after exposure to e-cigarettes and administration of mulberry leaf extract

	viability	intact plasma membrane	motility
NC	90.50 ± 4.10 [a]	78.73 ± 5.37 [a]	78.25 ± 4.46 [a]
PC	29.19 ± 7.45 [e]	28.55 ± 8.14 [d]	26.84 ± 4.65 [d]
T1	40.18 ± 3.99 [d]	39.06 ± 4.08 [d]	36.92 ± 3.09 [c]
T2	49.72 ± 3.35 [c]	46.35 ± 2.46 [c]	42.38 ± 5.79 [c]
T3	69.87 ± 12.05 [b]	65.27 ± 14.01 [b]	60.74 ± 5.35 [b]

Different superscripts within the same column demonstrate a statistically significant difference ($p < 0.05$); NC: negative control group, where rats were administered 1% Na-CMC; PC: positive control group, where rats were exposed to e-cigarette smoke and received 1% Na-CMC; T1, T2, and T3: groups of rats exposed to e-cigarette smoke and administered 100, 200, and 400 mg/kg bw of mulberry leaf extract, respectively; both Na-CMC and mulberry leaf extract were given orally at a daily volume of 1.5 mL, starting three days prior to the initiation of e-cigarette smoke exposure; the exposure period lasted for 28 days.

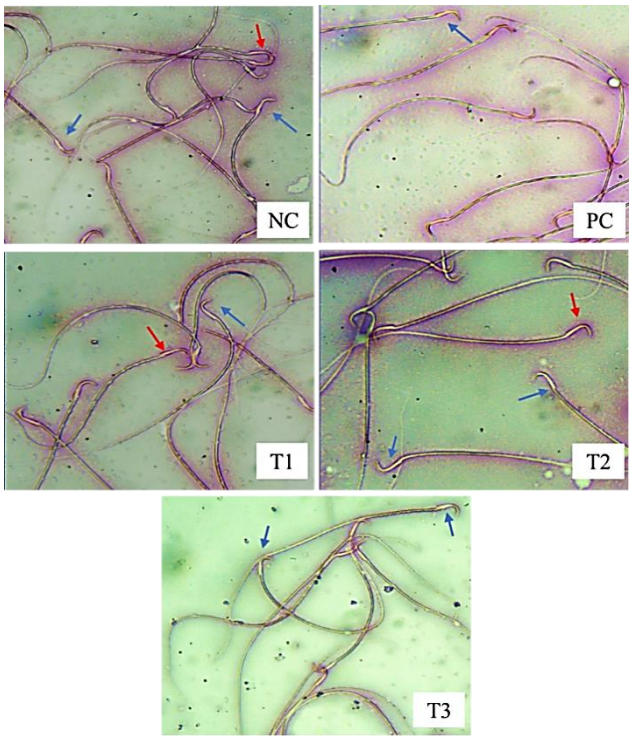

Figure 1 Viability of rats (*Rattus norvegicus*) spermatozoa following exposure to e-cigarettes and treatment with mulberry leaf extract; analysis conducted using a light microscope (Nikon Eclipse E100) at 400x magnification; blue arrow: live spermatozoa (unstained/transparent); red arrow: dead spermatozoa (stained with eosin); NC: negative control group, where rats received 1% Na-CMC; PC: positive control group, where

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

rats were exposed to e-cigarette smoke and received 1% Na-CMC; T1, T2, and T3: groups of rats exposed to e-cigarette smoke and treated with 100, 200, and 400 mg/kg bw of mulberry leaf extract, respectively; both Na-CMC and mulberry leaf extract were administered orally at a daily volume of 1.5 mL, starting three days before e-cigarette smoke exposure; the exposure lasted for 28 days.

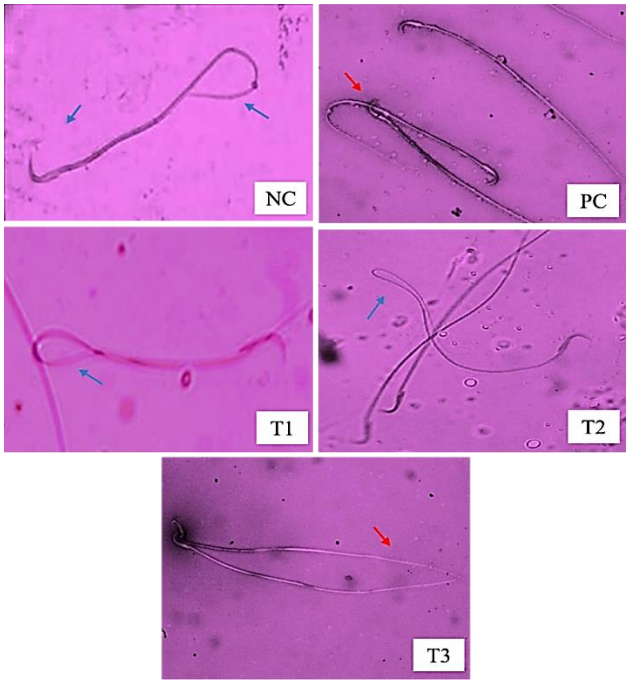

Figure 2 Intact plasma membranes of spermatozoa in rats (*Rattus norvegicus*) following exposure to e-cigarette smoke and treatment with mulberry leaf extract; evaluation performed using a light microscope (Nikon Eclipse E100) at 400x magnification; blue arrow: intact plasma membranes (bent/curved spermatozoa tails); red arrow: damaged plasma membranes (straight spermatozoa tails); NC: negative control group, where rats received 1% Na-CMC; PC: positive control group, where rats were exposed to e-cigarette smoke and received 1% Na-CMC; T1, T2, and T3: groups of rats exposed to e-cigarette smoke and treated with 100, 200, and 400 mg/kg bw of mulberry leaf extract, respectively; Na-CMC and mulberry leaf extract were administered orally at a daily volume of 1.5 mL, beginning three days prior to e-cigarette smoke exposure; the exposure duration was 28 days.

DISCUSSION

Viability refered to the ability of spermatozoa to survive during fertilization. The integrity of the spermatozoa plasma membrane indicated the maintenance of its physiological functions, crucial for effective transport mechanisms. Motility described the capacity of spermatozoa to swim progressively through the female reproductive tract to achieve fertilization (Mahé *et al*., 2021). In this study, exposure to e-cigarettes containing 18 mg/mL nicotine for 28 days resulted in diminished plasma membrane integrity, viability, and motility of spermatozoa. This decline could be attributed to the toxic compounds present in cigarette smoke. E-cigarette smoke exposure introduces metals like cadmium and nickel, which contributed to lipid peroxidation in spermatozoa cell membranes (Montjean *et al*., 2023).

E-cigarette smoke is known to contain free radicals in the form of reactive oxygen species (ROS) (Menicagli *et al*., 2020). An excess of ROS could lead to oxidative stress. Prolonged oxidative stress (over 20 days) could cause cellular damage (Coluzzi *et al*., 2017). Three primary mechanisms contribute to this damage:

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

lipid peroxidation, which compromised cell membranes (Wong-Ekkabut *et al*., 2007), DNA damage leading to mutations and cell death (Martins *et al*., 2021), and modifications of oxidized proteins (Viedma-Poyatos *et al*., 2021). Free radicals could covalently bind to membrane enzymes or receptors, altering cellular activity. Lipid peroxidation destabilized and damaged the plasma membrane (Petersen, 2017).

Spermatozoa plasma membranes are rich in polyunsaturated fatty acids (PUFAs), making them particularly vulnerable to ROS. Lipid peroxidation initiated by extracting hydrogen atoms from the methylene group (-CH2) in PUFAs, leading to compromised membrane integrity and reduced spermatozoa motility (Rodak and Kratz, 2023). The observed decrease in spermatozoa viability correlated with plasma membrane damage. Furthermore, ROS could impair intracellular enzymes, lipoproteins, ATP levels, and potassium ions, disrupting membrane permeability (Dutta *et al*., 2019). Membrane permeability was vital for nutrient transport, crucial for cellular metabolism. Disruption of spermatozoa membrane permeability could hinder nutritional supply and resulted in cell death. The membrane served to protect cellular components and regulates intracellular and extracellular exchanges (Stewart *et al*., 2018).

Free radicals also contributed to reduced mitochondrial ATP production, as they could diminish the frequency of spermatozoa tail movements (Gallo *et al*., 2021). Mitochondria were responsible for energy production, which was essential for spermatozoa motility. ATP was crucial for the motility of spermatozoa (Costa *et al*., 2023). Normal spermatozoa physiology relies on a balance between oxidants and antioxidants. However, when ROS levels exceed the capacity of endogenous antioxidants (Kowalczyk, 2022), exogenous antioxidants became necessary (Chaudhary *et al*., 2023).

This study demonstrated that mulberry leaf extract (*Morus alba* L.) at a dose of 400 mg/kg bw/day was the most effective for preserving motility, viability, and plasma membrane integrity of spermatozoa exposed to e-cigarette smoke. Mulberry leaf extract contained antioxidant compounds such as alkaloids, flavonoids, phenolics, terpenoids, tannins, and saponins (Batiha *et al*., 2023). Flavonoids acted as reducing agents, hydrogen donors, and metal chelators, exhibiting biological activities that supported metabolic function. They played a critical role in reducing ROS levels by inhibiting free radical formation, capturing free radicals through hydrogen atom donation, and interrupting chain reactions, thereby allowing testicular germ cells to withstand oxidative stress. Quercetin, a flavonoid derivative found abundantly in mulberry leaves, has significant antioxidant properties (Hassanpour and Doroudi, 2023).

Quercetin helped protect the testes from ROS and supported male germ cell function by reducing ROS and hydroxyl radical production under hypoxic conditions. It also aided in restoring endogenous homeostasis by enhancing glutathione (GSH) levels and facilitating free radical enzyme release, thereby safeguarding cells from oxidative damage by bolstering endogenous antioxidant defenses (Xu *et al*., 2019).

The administration of mulberry (*Morus alba* L.) leaf extract at 400 mg/kg bw/day, alongside exposure to e-cigarettes with 18 mg/mL nicotine, resulted in superior motility, viability, and plasma membrane integrity compared to lower doses of 100 and 200 mg/kg bw/day. However, even the highest dose (400 mg/kg bw) was insufficient to fully restore spermatozoa viability, plasma membrane integrity, and motility to levels observed in normal rats. Further research is warranted to identify the specific active compounds in mulberry leaves that most effectively preserve spermatozoa motility, viability, and plasma membrane integrity to revert them to normal conditions.

CONCLUSION

In conclusion, mulberry leaf extract (*Morus alba* L.) enhanced the motility, viability, and plasma membrane integrity of spermatozoa in

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

white rats (*Rattus norvegicus*) exposed to e-cigarette smoke.

ACKNOWLEDGEMENT

I would like to thank Dr. Ira Sari Yudaniayanti, drh., M.P. for expertise support, and to Talitha Putri Salsabila and Fikri Maulana Putra for technical assistance.

AUTHOR'S CONTRIBUTIONS

Fifi Fauziah Ramadhani (FFR), Yulianna Puspitasari (YP), Hermin Ratnani (HR), Rochmah Kurnijasanti (RK), Budi Utomo (BU), Kadek Rachmawati (KR)

FFR: under the supervision of YP and HR, drafted the concept, gathered, analyzed, and interpreted data, and drafted the manuscript. RK, BU, and KR: read the manuscript carefully and revised it for intellectual content material. The final manuscript was approved by all authors.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

FUNDING INFORMATION

The authors provided funding for this study

REFERENCES

Ajonuma L, Bamiro S, Adeyinka R, Fapohunda D, Makanjuola S. 2024. P-100 prolonged exposure to polyethylene glycol (peg) 6000 severely affects the reproductive system of male Wistar rats. Hum Reprod. 39: deae108.474.

Alifia AS, Wurlina W, Soeharsono S, Hernawati T, Sudjarwo SA, Utomo B, Mulyati S, Purnama MTE. 2023. Effect of white guava (*Psidium guajava* L.) fruit juice on the quality of lead acetate induced rats (*Rattus norvegicus*) spermatozoa. Ovozoa : J Anim Reprod. 12: 131-40.

Batiha GE, Al-Snafi AE, Thuwaini MM, Teibo JO, Shaheen HM, Akomolafe AP, Teibo TKA, Al-Kuraishy HM, Al-Garbeeb AI, Alexiou A, Papadakis M. 2023. *Morus alba*: A comprehensive phytochemical and pharmacological review. Naunyn Schmiedebergs Arch Pharmacol. 396: 1399-413.

Cha SR, Jang J, Park SM, Ryu SM, Cho SJ, Yang SR. 2023. Cigarette smoke-induced respiratory response: Insights into cellular processes and biomarkers. Antioxidants 12: 1210.

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi-Rad J. 2023. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front Chem. 11: 1158198.

Chen C, Mohamad Razali UH, Saikim FH, Mahyudin A, Mohd Noor NQI. 2021. *Morus alba* L. Plant: Bioactive compounds and potential as a functional food ingredient. Foods 10: 689.

Chen C, Mokhtar RAM, Sani MSA, Noor NQIM. 2022. The effect of maturity and extraction solvents on bioactive compounds and antioxidant activity of mulberry (*Morus alba*) fruits and leaves. Molecules 27: 2406.

Coluzzi E, Buonsante R, Leone S, Asmar AJ, Miller KL, Cimini D, Sgura A. 2017. Transient ALT activation protects human primary cells from chromosome instability induced by low chronic oxidative stress. Sci Rep. 7: 43309.

Costa J, Braga PC, Rebelo I, Oliveira PF, Alves MG. 2023. Mitochondria quality control and male fertility. Biology 12: 827.

Dahdah A, Jaggers RM, Sreejit G, Johnson J, Kanuri B, Murphy AJ, Nagareddy PR. 2022. Immunological insights into cigarette smoking-induced cardiovascular disease risk. Cells 11: 3190.

Dhage VD, Nagtode N, Kumar D, Bhagat AK. 2024. A narrative review on the impact of smoking on female fertility. Cureus 16: e58389.

Dutta S, Majzoub A, Agarwal A. 2019. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab J Urol. 17: 87-97.

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

Engstrom PF, Clapper ML, Schnoll RA. 2003. Physiochemical composition of tobacco smoke. In: Kufe DW, Pollock RE, Weichselbaum RR, *et al*. (Eds). Holland-Frei Cancer Medicine. 6th Ed. Hamilton (ON): BC Decker.

Gallo A, Esposito MC, Tosti E, Boni R. 2021. Sperm motility, oxidative status, and mitochondrial activity: Exploring correlation in different species. Antioxidants 10: 1131.

Hartmann-Boyce J, Chepkin SC, Ye W, Bullen C, Lancaster T. 2018. Nicotine replacement therapy versus control for smoking cessation. Cochrane Database Syst Rev. 5: CD000146.

Hassanpour SH, Doroudi A. 2023. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 13: 354-76.

He X, Fang J, Ruan Y, Wang X, Sun Y, Wu N, Zhao Z, Chang Y, Ning N, Guo H, Huang L. 2018. Structures, bioactivities and future prospective of polysaccharides from *Morus alba* (white mulberry): A review. Food Chem. 245: 899-910.

Indawati I, Kunaedi A, Karlina N, Fadhillah Z, Cantika CD, Khulfiah AA. 2023. Study on the optimization of mulberry leaf extract by macerating ethanol and microwave assisted extraction method (MAE) with natural deep eutectic solvents (NADES). J Farmasi Sains dan Praktis 9: 11-9.

Jain D, Chaudhary P, Varshney N, Bin Razzak KS, Verma D, Khan Zahra TR, Janmeda P, Sharifi-Rad J, Daştan SD, Mahmud S, Docea AO, Calina D. 2021. Tobacco smoking and liver cancer risk: Potential avenues for carcinogenesis. J Oncol. 2021: 5905357.

Komura M, Sato T, Yoshikawa H, Nitta NA, Suzuki Y, Koike K, Kodama Y, Seyama K, Takahashi K. 2022. Propylene glycol, a component of electronic cigarette liquid, damages epithelial cells in human small airways. Respir Res. 23: 216..

Kowalczyk A. 2022. The role of the natural antioxidant mechanism in sperm cells. Reprod Sci. 29: 1387-94.

Le Foll B, Piper ME, Fowler CD, Tonstad S, Bierut L, Lu L, Jha P, Hall WD. 2022. Tobacco and nicotine use. Nat Rev Dis Primers 8: 19.

Lei T, Qian H, Yang J, Hu Y. 2023. The exposure to volatile organic chemicals associates positively with rheumatoid arthritis: a cross-sectional study from the NHANES program. Front Immunol. 14: 1098683.

Maddatu J, Anderson-Baucum E, Evans-Molina C. 2017. Smoking and the risk of type 2 diabetes. Transl Res. 184: 101-7.

Mahé C, Zlotkowska AM, Reynaud K, Tsikis G, Mermillod P, Druart X, Schoen J, Saint-Dizier M. 2021. Sperm migration, selection, survival, and fertilizing ability in the mammalian oviduct†. Biol Reprod. 105: 317-31.

Martins SG, Zilhão R, Thorsteinsdóttir S, Carlos AR. 2021. Linking oxidative stress and DNA damage to changes in the expression of extracellular matrix components. Front Genet. 12: 673002.

Menicagli R, Marotta O, Serra R. 2020. Free radical production in the smoking of e-cigarettes and their possible effects in human health. Int J Prev Med. 11: 53.

Montjean D, Godin Pagé MH, Bélanger MC, Benkhalifa M, Miron P. 2023. An overview of e-cigarette impact on reproductive health. Life 13: 827.

Osadchuk L, Kleshchev M, Osadchuk A. 2023. Effects of cigarette smoking on semen quality, reproductive hormone levels, metabolic profile, zinc and sperm DNA fragmentation in men: Results from a population-based study. Front Endocrinol. 14: 1255304.

Patlolla AK, Kumari SA, Tchounwou PB. 2019. A comparison of poly-ethylene-glycol-coated and uncoated gold nanoparticle-mediated hepatotoxicity and oxidative stress

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.

in Sprague Dawley rats. Int J Nanomedicine 14: 639-47.

Petersen RC. 2017. Free-radicals and advanced chemistries involved in cell membrane organization influence oxygen diffusion and pathology treatment. AIMS Biophys. 4: 240-83.

Rodak K, Kratz EM. 2023. PUFAs and their derivatives as emerging players in diagnostics and treatment of male fertility disorders. Pharmaceuticals 16: 723.

Sari NM, Yuliani GA, Yimer N, Hernawati T, Herupradoto EBA, Hidayatik N. 2023. Reddragon (*Hylocereus polyrhizus*) fruit peel extract increased the motility and viability of spermatozoa of hypercholesterolemic rats (*Rattus norvegicus*). Ovozoa : J Anim Reprod. 12: 33-41.

Stewart MP, Langer R, Jensen KF. 2018. Intracellular delivery by membrane disruption: Mechanisms, strategies, and concepts. Chem Rev. 118: 7409-531.

Susilowati S, Mustofa I, Wurlina W, Triana IN, Utama S, Rimayanti R. 2021. Effect of insulin-like growth factor-1 complex of Simmental bull seminal plasma on post-thawed Kacang buck semen fertility. Vet World 14: 2073-2084.

Viedma-Poyatos Á, González-Jiménez P, Langlois O, Company-Marín I, Spickett CM, Pérez-Sala D. 2021. Protein lipoxidation: Basic concepts and emerging roles. Antioxidants 10: 295.

Wadgave U, Nagesh L. 2016. Nicotine replacement therapy: An overview. Int J Health Sci. 10: 425-35.

Wang RJ, Bhadriraju S, Glantz SA. 2021. E-cigarette use and adult cigarette smoking cessation: A meta-analysis. Am J Public Health 111: 230-46.

Wong-Ekkabut J, Xu Z, Triampo W, Tang IM, Tieleman DP, Monticelli L. 2007. Effect of lipid peroxidation on the properties of lipid bilayers: A molecular dynamics study. Biophys J. 93: 4225-36.

Xu D, Hu MJ, Wang YQ, Cui YL. 2019. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 24: 1123.

Zheng Q, Tan W, Feng X, Feng K, Zhong W, Liao C, Liu Y, Li S, Hu W. 2022. Protective effect of flavonoids from mulberry leaf on AAPH-induced oxidative damage in sheep erythrocytes. Molecules 27: 7625.

How to cite this article: Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K. 2024. Mulberry (*Morus alba* L.) leaf extract enhanced spermatozoa motility, viability, and plasma membrane integrity of rats (*Rattus norvegicus*) exposed to e-cigarette smoke. Ovozoa: Journal of Animal Reproduction. 13: 120-128.

Copyright ©2024 Ramadhani FF, Puspitasari Y, Ratnani H, Utomo B, Kurnijasanti R, Rachmawati K.