Jurnal Biodjati 9 (2): 321-333,November 2024
e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Effects of Wedelia Ethanol Extract on Sperm Quality and Blood Cholesterol Levels in Obese Rats

Yumna Nabilah Iffatuzzahra[1], Diah Kartika Wardani[2], Andika Gundawa Hidayat[3], Ulinuha Farah Makhabati[4], Haris Setiawan[*5]

Received: 17 July 2024
Revise from: 18 July 2024
Accepted: 26 October 2024

DOI: 10.15575/biodjati.v9i2.37673

[1,2,4,5]Program Study of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Jl. Ringroad Selatan, Kragilan, Tamanan, Kec. Banguntapan, Bantul, DIY, Yogyakarta, 55191, (0274) 511830. [3]Program Study of Chemical Engineering, Faculty of Industry Technology, Universitas Ahmad Dahlan, Jl. Ringroad Selatan, Kragilan, Tamanan, Kec. Banguntapan, Bantul, DIY, Yogyakarta, 55191, (0274) 511830

e-mail:
[1]yumnablhra7@gmail.com,
[2]diahkartika767@gmail.com,
[3]andikagundawahidayat@gmail.com,
[4]makhabatiulinuha@gmail.com,
*[*5]haris.setiawan@bio.uad.ac.id*

**Corresponding author*

Abstract. *Obesity is a complex, multifactorial condition contributing to male infertility by increasing oxidative stress, which negatively affects reproductive hormones, sperm quality, and blood cholesterol levels. The Wedelia plant (Sphagneticola trilobata (L.) Pruski) is a natural herbaceous plant containing flavonoids and tannins, known for enhancing sperm quality and inhibiting cholesterol synthesis in the blood. This study aims to determine the potential of Wedelia in improving sperm quality and regulating blood cholesterol levels. The study used 24 male Wistar rats divided into four groups, consisting of C (no HFF, distilled water only), NC (HFF, distilled water), T1 (HFF and 100 mg/Kg BW Wedelia extract), and T2 (HFF and 200 mg/Kg BW Wedelia extract). HFF was administered from weeks 1 to 3, followed with extract treatments from weeks 3 to 5. On day 36, the rats were euthanized for blood collection using hematocrit into EDTA tubes. The rats were dissected for the epididymis, which was then incised in a 10% PBS to release sperm. Sperm motility and count were observed on glass slides, while sperm viability and morphology were analyzed with hematoxylin-eosin using an Optilab microscope (400x magnification). The observation parameters included sperm morphology, count, motility, viability, total cholesterol, LDL, and HDL levels. Data were analyzed using ANOVA with DMRT post hoc testing ($p \leq 0.05$). The results showed that T2 exhibited an increase in sperm count, motility, viability, and morphology in HFF-induced obese rats. T1 and T2 also showed enhanced HDL cholesterol and reduced total and LDL cholesterol levels. In conclucions, a Wedelia leaf extract dose of 200 mg/kg BW optimally improves sperm quality and reduces total and LDL cholesterol levels.*

Keywords: *cholesterol, obesity, sperm, wedelia leaf.*

Citation
Iffatuzzahra, Y. N., Wardani, D. K., Hidayat, A. G., Makhabati, U. F., & Setiawan, H. (2024). Effects of Wedelia Ethanol Extract on Sperm Quality and Blood Cholesterol Levels in Obese Rats. *Jurnal Biodjati*, 9(2), 321-333.

Jurnal Biodjati 9(2):321-333, November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

INTRODUCTION

The 2023 World Obesity Atlas estimates that 38% of the current global population of 3 billion is overweight or obese. Cases of obesity in Indonesia continue to rise annually, with 23.4% or approximately 7 million adults in Indonesia (aged >18 years) classified as obese (Kemenkes, 2023). Obesity is a condition of imbalance between incoming energy intake and energy expenditure resulting in excess energy stored as fat tissue by the body (Rompis et al., 2018). This condition increases the risk of adult male infertility and high cholesterol, making obesity one of Indonesia's most critical health concerns (Wahyuni & Diansabila, 2020).

Male infertility refers to the inability to produce a pregnancy in fertile women (Kumar & Singh, 2015), as men account for approximately 45-50% of infertile couple cases, proving the relationship between male infertility and obesity (Craig et al., 2017). Obese men exhibit low sperm counts due to direct interference with spermatogenesis and impaired Leydig and Sertoli cell functions (Venigalla et al., 2023). Consumption of fatty foods with cholesterol levels exceeding 300 mg daily is linked to obesity and elevated blood cholesterol concentrations (hypercholesterolemia) (Listiyana et al., 2013).

Free radicals from obesity negatively affect both reproductive health and cholesterol levels, this can be mitigated by providing antioxidants. The Wedelia plant (*Sphagneticola trilobata* (L.) Pruski) has demonstrated antioxidant potential, containing high levels of flavonoids, tannins, terpenoids, phenols, and saponins (Mardina et al., 2020). The total flavonoid content in Wedelia plants measures 103.61 mg/g (Afzal & Rajesh, 2021), a significantly higher level compared to that in purslane plants (*Portulaca oleracea*), which contains 3.27 mg/g (Budiawan et al., 2021).

Research by Ismail (2018) demonstrates that diets high in saturated fats can elevate cholesterol levels and promote the production of oxygen radicals and lipid peroxides. This leads to increased Reactive Oxygen Species (ROS) and oxidative stress, damaging sperm cell membranes, protein structures, and DNA, making antioxidant compounds critical in mitigating this oxidative damage (Billah et al., 2022). Despite the recognized benefits, research on the antioxidant potential of Wedelia plants in Indonesia, particularly the flavonoid compounds effective against obesity-induced free radicals, remains limited. These natural antioxidants may help repair cellular damage associated to increased ROS concentration. This study, therefore, focuses on the benefits of Wedelia leaf extract and its content as a natural antioxidant. This study also investigates the effects of Wedelia leaf extract as a natural antioxidant on sperm quality parameters (motility, count, viability, morphology) and blood cholesterol profiles (total cholesterol, LDL, HDL) following 21 days of high-fat feed (HFF) administration.

MATERIALS AND METHODS

Ethical Clearance

This in vivo (preclinical) experimental study used male Wistar rats as test animals to examine the effect of Wedelia plant extract on sperm quality and blood cholesterol levels. The study was conducted at the Laboratory of Animal Structure and Physiology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan Yogyakarta, with ethical clearance granted by the UAD Ethics Committee (No: 012404084). Wedelia plants were collected from rice fields behind Campus 4 of Universitas Ahmad Dahlan,

Bantul, Yogyakarta and then identified at the Laboratory of Plant Physiology Structure, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan Yogyakarta (No: 377/Lab.Bio/B/VII/2024) as *Sphagneticola trilobata* (L) Pruski

.

Preparation of Ethanol Extract of Wedelia Plant

Wedelia leaves (15 kg) were harvested, washed with running water, and dried in a cabinet dryer at 50ºC for approximately 3 days. The dried simplicia (medicinal herbal leaves) was pulverized into fine powder using a blender, yielding 1.3 kg of simplicia powder. The powder was filtered using a Buchner funnel and Whatman No. 1 paper and then macerated in 96% ethanol for around 4 days. The macerate was evaporated using a rotary evaporator at 60ºC for approximately 3 hours, resulting in a thick paste weighing 137 g. The paste was stored in a refrigerator at -4ºC.

Acclimatization of Test Animals

This study used 24 male Wistar rats, aged 3 months and weighing ±150 g, divided into four treatment groups. Rats were acclimatized for 7 days in a cage measuring 150 × 18 cm with a temperature maintained at 22±3ºC with 30-70% room humidity, and 12 hours of light-dark cycles. Chaff was changed every 3 days, and the rats were provided with Rat Bio feed and drinking water ad libitum. Body weight and length were recorded weekly using a digital scale (Oxone).

Preparation and Feeding of High-Fat Feed (HFF)

High-Fat Feed (HFF) formulation consisted of 2 mL of pork oil and 1 mL of quail egg yolk. The test animals were induced with 2 mL HFF in the morning and 1 mL in the afternoon orally using rat oral gavage and a 1 mL syringe for 21 days. High-fat feeding treatments included Negative Control (NC), Treatment 1 (T1), and Treatment 2 (T2). Body weight and length of the test animals were measured until they reached the Lee Obesity index (>0.3), calculated as follows (BPOM, 2020):

$$Lee\ index = \frac{\sqrt[3]{body\ weight\ (g)}}{Nasoanal\ length\ (cm)} \times 1000$$

Administration of Wedelia Extract to Test Animals

Wedelia leaf extract was administered orally via gavage and 1 mL syringe for 14 days. Groups included a Control (C) receiving only distilled water, Treatment 1 (T1) receiving 100 mg/Kg BW/day, and Treatment 2 (T2) receiving 200 mg/Kg BW/day (Azizah et al., 2018). The combined HFF and Wedelia extract treatments over 21 and 14 days, showed significant changes in sperm quality and blood cholesterol levels.

Blood and Epididymal Organ Collection

On day 36, rats were anesthetized with chloroform, and approximately 2 mL of blood was drawn from the orbital sinus using a microhematocrit tube, then transfered in EDTA tubes. Blood samples were stored in a cooler for subsequent total cholesterol, LDL, and HDL tests. After blood collection, rats were anesthetized, euthanized by cervical dislocation, and dissected for epididymis extraction using surgical instruments (surgical knife and scissors).

Total, HDL, and LDL Cholesterol Level Tests

On the 36th day, the rats were anaesthetized using chloroform and then blood was drawn through the orbital sinus

Jurnal Biodjati 9(2):321-333, November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

using a microhematocrit and loaded into an EDTA tube. The collected blood was tested for total cholesterol, HDL, and LDL levels. Total cholesterol levels were measured using CHOD PAP reagent kit, HDL using HDL cholesterol Beckman Coulter reagent kit, and LDL using LDL-cholesterol Backman Coulter reagent. All tests were performed using a UV-Vis spectrophotometer.

Preparation of Sperm Smear Preparations

The epididymis was placed in a 6 cm petri dish containing 1 mL of 10% PBS solution at 36ºC to collect the sperm. The organ was incised using surgical scissors until the sperm came out. Sperm suspension was dripped on an object glass and then removed using another object glass and left to dry. The smear preparations were fixed using 70% methanol for approximately 4 minutes, evenly stained with Giemsa dye, and allowed to dry.

Observation of Sperm Quality

A portion of the epididymis organ was cut and placed in a 6 cm petri dish containing 1 mL of 10% PBS solution at 36ºC to collect the sperm. The sperm suspension (10 µL) was drawn using an erythrocyte pipette, placed in a Neubauer Improved hemocytometer chamber, and examined under a light microscope at 400x magnification for sperm count and motility. According to Setiawan & Subagja (2023) the calculation of the number of sperm cells can use the following formula:

Number of Sperm Cells= 5 boxes×50.000×100 (cell/mL)

Sperm motility was classified into four grades: Grade I for immotile sperm, grade II for non-progressive sperm motility with tail movement only (vibrating-like-movement), grade III for slow, non-linear sperm movement (non-linear-motility) that only moves in circles, and grade IV for progressive progressive, straight-line motility (Setiawan & Subagja, 2023).

Sperm morphology and viability were observed through smear preparations. Sperm suspension was dripped on an object glass and then removed using another object glass and allowed to dry. The smear preparations were fixed using 70% methanol for ± 4 minutes, evenly stained with Giemsa dye, and dried. Sperm morphology was classified into either normal or abnormal. Sperm viability was assessed based on membrane permeability: a part of viable (live) sperm appeared unstained (transparent), whereas non-viable (dead) sperm had purple-stained heads. The formulas for calculating the percentage of sperm motility and viability according to Unitly et al. (2022) are as follows:

$$\%\ \text{Motility} = \frac{\text{Progressive spermatozoa count}}{\text{Total number of spermatozoa observed}} \times 100\%$$

$$\%\ \text{Viability} = \frac{\text{Number of unstained spermatozoa heads}}{\text{Total number of spermatozoa observed}} \times 100\%$$

Data Analysis

All research parameters were tested for homogeneity and normality. Normally distributed and homogeneous data were analyzed using One Way ANOVA, followed by with Duncan Multiple Range Test (DMRT) ($p \leq 0{,}05$) to determine differences between treatments. Data analysis was carried out using SPSS software, version 15.

RESULTS AND DISCUSSION

Rat Obesity Index

This study shows increase in the obesity index for the NC, T1, and T2 groups from week 0 to week 3 following administration of High-Fat Feed (HFF) (Figure 1A). This highlights a significant difference between the treatment and the negative control groups prior to Wedelia leaf extract administration

(p≤0,05). The rats fed with High Fat Feed (HFF) exhibited pronounced obesity, with obesity index reaching 0.4, attributable to the high cholesterol content in pork oil and quail egg yolk. Consistent with the research by Dias et al. (2021), HFF consumption can promote obesity by elevating adiposity and leptin levels, thus stimulating the development of hypertension and glucose intolerance.

Upon Wedelia leaf extract administration from week 3 to week 5, there was a significant difference between the NC group and the treatment groups (p≤0,05), where a decrease in the index value in 100 mg/Kg BW and 200 mg/Kg BW was observed in treatment groups, while no change was observed in the NC group (Figure 1A). These results indicate that Wedelia leaf extract effectively reduced obesity index values post-HFF administration, likely due to its antioxidant properties. Roy et al. (2021) states that antioxidants can accelerate the metabolic process associated to reducing body fat which results in decreased body weight.

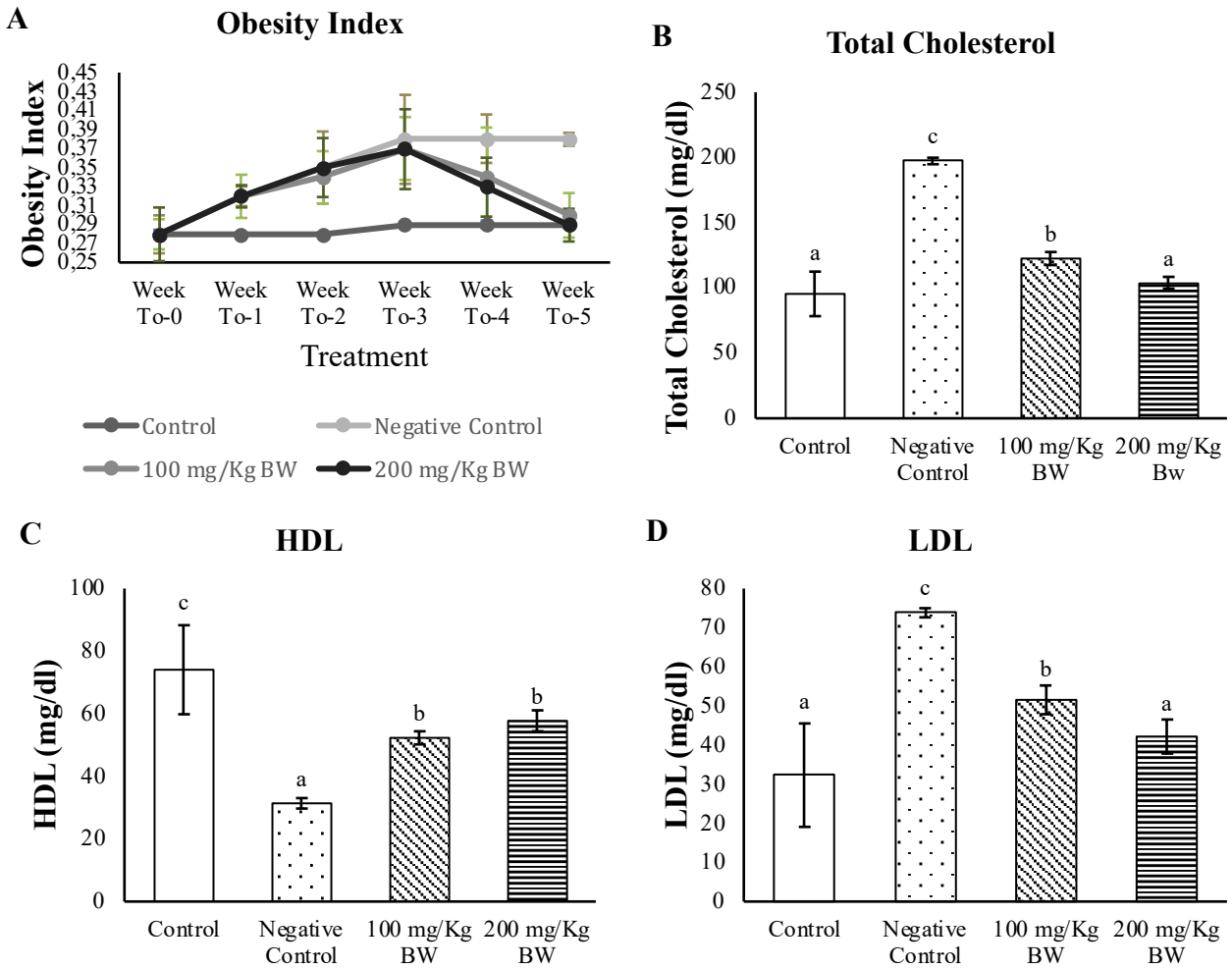

Figure 1. Obesity index graph (A). Week 0-3 (W0-W3) of HFF administration in treatments Negative Control, 100 mg/Kg BW, and 200 mg/Kg BW. Weeks 3-5 (W3-W5) of Wedelia leaf extract administration in 100 mg/Kg BW and 200 mg/Kg BW. Histogram of total cholesterol (B); HDL cholesterol (C); and LDL cholesterol (D). Control (0 mg/Kg BW extract and no HFF), Negative Control (HFF), Treatment 1 (HFF and 100 mg/Kg BW extract), Treatment 2 (HFF and 200 mg/Kg BW extract). Mean ± SD, superscript a-c indicates significant differences between treatments (p≤0,05).

Jurnal Biodjati 9(2):321-333, November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Total, HDL, and LDL Cholesterol Level

The results of cholesterol level analysis revealed that Wedelia leaf ethanol extract, particularly at a dose of 200 mg/Kg BW, significantly decreased total cholesterol (Figure 1B) and LDL levels (Figure 1D) while increasing HDL levels (Figure 1C), as indicated by a significant difference between NC and both T1 and T2 groups ($p \leq 0.05$). Excessive fat consumption raises blood cholesterol levels, facilitating free radical formation (Nashriana et al., 2015). These free radicals from cholesterol oxidation can damage endothelial cells, leading to atherosclerosis (Yuliani & Deinina, 2015).

Wedelia leaf ethanol extract's phytochemical compounds, such as flavonoids and tannins, potential to reduce blood cholesterol levels (Witosari & Widyastuti, 2014). Flavonoid and tannin compounds are antioxidant compounds that prevent LDL oxidation (Rumianti, 2011), reduce the activity of the acyl-CoA cholesterol acyltransferase (ACAT) enzyme, and inhibit cholesterol absorption in the digestive tract (Mutia et al., 2018).

According to Tamon et al. (2021), flavonoid-rich antioxidants lower total cholesterol and LDL while raise HDL as they improve blood lipid profiles and provide vasoprotective effects. Datu et al. (2021) found that the mechanism of phenolic compounds inhibit HMG-CoA enzyme, improving lipid profile stability, and decreasing cholesterol and fat absorption. Consequently, a reduction in total cholesterol and LDL levels occurs, decreasing the lipid transport in blood (Romadhoni et al., 2014).

Motility of Rat Sperm

The results showed that sperm motility was divided into 4 categories, namely at Grade IV and I showed significant differences between NC and T1 and T2 ($p \leq 0{,}05$) while at Grade III and II showed no significant differences between all treatments ($p > 0{,}05$). In Grade IV there was a high number of motile sperm in the T1 and T2 compared to NC (Figure 2A), while in Grade I there was a low number of immotile sperm in these treatments (Figure 2D).

Administration of toxic substances, as in the obese rat model, negatively impacts the male reproductive system, causing spermatogenesis dysfunction (Campbell & Reece, 2010). Spermatogenesis dysfunction disrupts the process of division or development from germinal epithelial cells to spermatozoa. Good sperm quality requires hormones to regulate the process of spermatogenesis from Leydig cells, such as testosterone and FSH (Arief, 2011). Leydig cells produce testosterone hormone crucial for spermatogenesis (Setiawan et al., 2020). Thus, obesity-induced disruptions in Leydig cells hinder testosterone production, affecting spermatogenesis and decreasing sperm motility (Mu et al., 2021).

Based on the results of the study, it shows that the ethanol extract of Wedelia leaves contains phytochemical compounds that have the ability to increase sperm motility. According to research by Martin & Touaibia (2020), Wedelia leaf extract's flavonoid compounds enhance sperm motility by increasing testosterone levels, which supports normal spermatogenesis. Optimal mitochondrial ATP production enables effective tail movement for motile sperm (Susilo et al., 2018). Flavonoid and tannin compounds are secreted from seminal vesicles during ejaculation as sperm protectors, mitigating oxidative stress from endogenous oxidative DNA damage by neutralizing hydroxyl, superoxide, and hydrogen peroxide radicals and preventing spermatozoa agglutinas (Al-Sultani et al., 2013). Flavonoid and tannin compounds enter the mitochondria through the facilitated glucose transporter (glut 1), protecting the sperm from oxidative injury (Unitly et al., 2022).

Jurnal Biodjati 9(2):321-333, November 2024

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

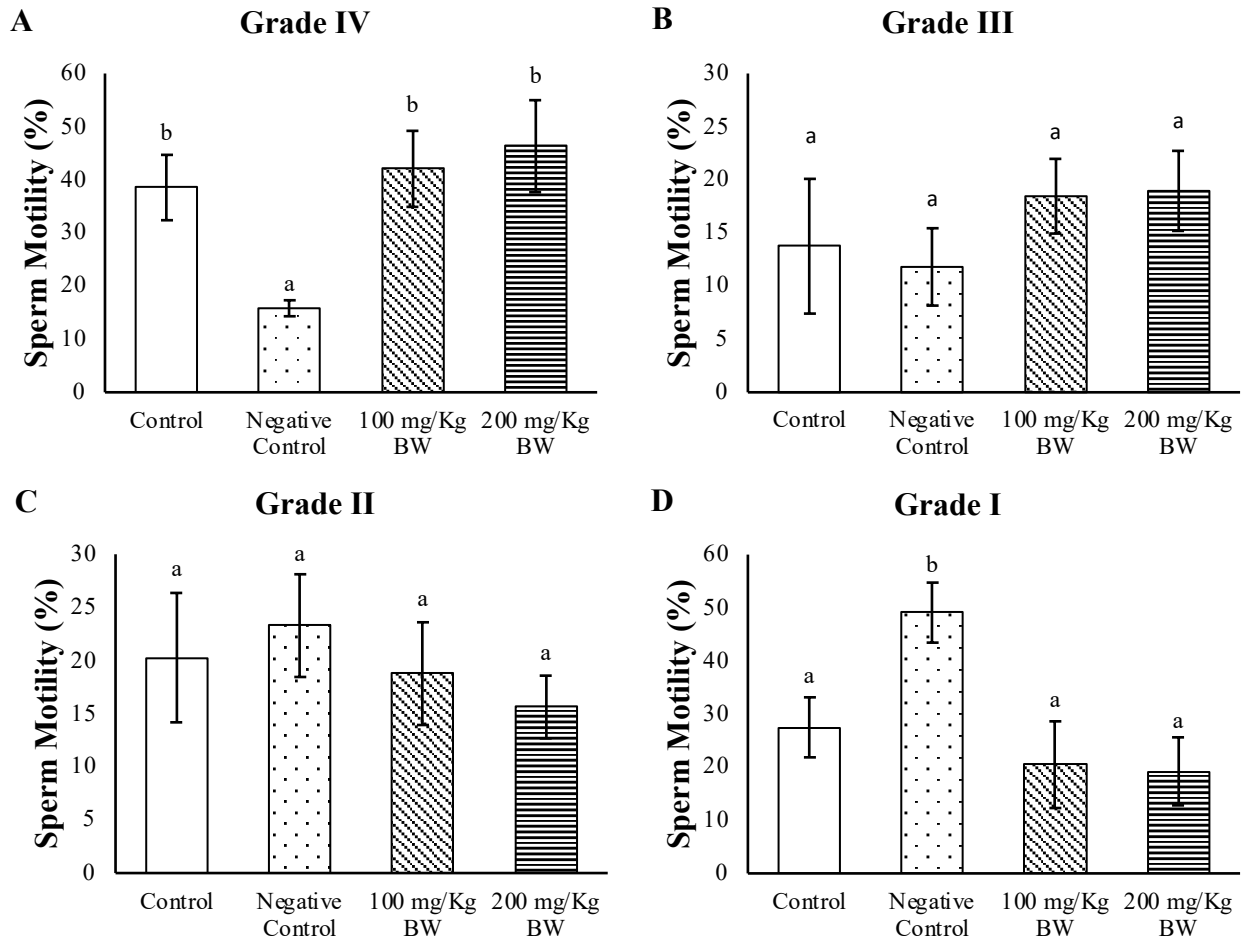

Figure 2. Histogram of sperm motility Grade IV (A); Grade III (B); Grade II (C); Grade I (D). C (0 mg/Kg BW extract and no HFF), NC (HFF), T1 (HFF and 100 mg/Kg BW extract), T2 (HFF and 200 mg/Kg BW extract). Mean ± SD, superscript a-b indicates significant differences between treatments ($p \leq 0,05$).

Number of Rat Sperm

The results demonstrated a significant difference in sperm count between NC and T1 and T2 ($p \leq 0,05$), wherein the count is higher in the treatment groups (Figure 3). The decrease in the number of sperm cells occurs due to incomplete sperm differentiation leading to increased dead sperm and apoptosis. The hormone FSH is unable to work together with testosterone to stimulate sertoli cells to form Androgen Binding Protein (ABP) effective for transporting and concentrating testosterone for the spermatogenesis (Arief, 2011). Low FSH and LH hormones cause disturbances in sertoli cells and Leydig cells producing dysfunctional sperm and contributing to reduced sperm counts (Iryani et al., 2019). Disrupted Sertoli cells, supporting cells that provide nutrients for spermatogenic cells, impede the development of spermatogenic cells (Nita et al., 2019). According to Rompis et al. (2018), decreased sperm counts in obesity cases may also result from elevated gonadal heat due to increased scrotal adiposity. Wedelia leaf ethanol extract's flavonoids and tannins support spermatogenesis by neutralizing obesity-related free radicals, enhancing sperm production (Sitohang et al., 2015). Additionally, flavonoids protect sperm

lipids from oxidation reactions (ROS), thus preserving sperm quality (Pakaya, 2014).

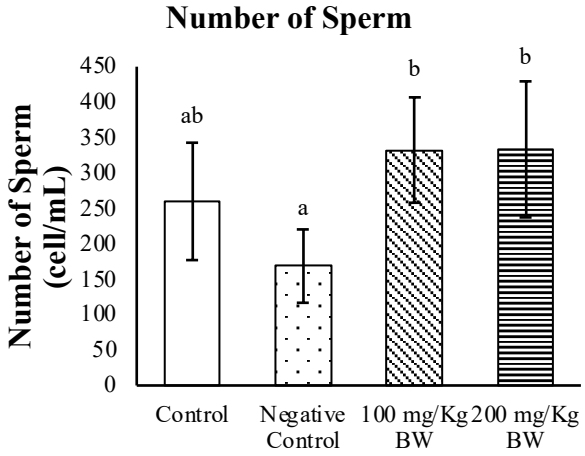

Figure 3. Histogram number of sperm. C (0 mg/Kg BW extract and no HFF), NC (HFF), T1 (HFF and 100 mg/Kg BW extract), T2 (HFF and 200 mg/Kg BW extract). Mean ± SD, superscript a-b indicates significant differences between treatments ($p \leq 0,05$).

Morphology and Viability of Rat Sperm

The results of the morphology analysis distinguished normal and abnormal forms, showing significant differences between NC and T1 and T2 ($p \leq 0,05$). Normal sperm showed an increase in T1 and T2 compared to NC (Figure 5A). The treatment groups showed a low value of abnormal sperm (Figure 5B). Morphologically, normal sperm of Wistar rats have a hook-like head structure, tail, and straight neck (Figure 4A).

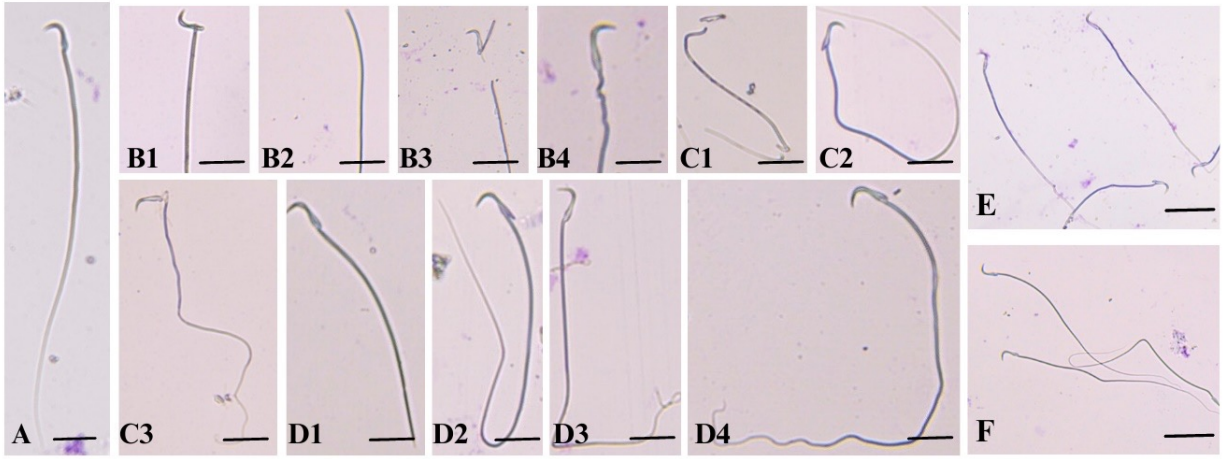

Figure 4. Morphological classification of rat epididymal sperm. Normal sperm (A); sperm with different patterns of abnormal heads (B1-4); sperm with both abnormal head and tail (C1-3); sperm with different patterns of abnormal tails (D1-4); viable sperm (E); non-viable sperm (F). The scale bar shown in the figure represents 10 µm.

Abnormal sperm morphology is characterized by broken heads, broken tails, bent heads, and twisted tails. Sperm morphology abnormalities can affect the spermatogenesis, causing infertility. According to research by Annisaroh & Wibowo (2019), the morphological defect of spermatozoa is characterized by a lump on the neck of the spermatozoa and the attachment of the tail to the head of the spermatozoa. HFF consumption increases Reactive Oxidative Stress (ROS) and lipid peroxidation, which can harm the spermatogonial cell membrane, Sertoli, and Leydig cells in testes, further disrupting sperm morphology (Rahayu et al., 2019). This can directly interfere with the function of the epididymis as a place for sperm maturation (Greenspan & Hall, 2014). The release of Follicle Stimulate Hormone (FSH) by the anterior pituitary which runs normally can determine normal sperm morphology (Setiawan et al., 2020). The presence of natural antioxidants from Wedelia Leaf extract, such as flavonoids, demonstrates its effectiveness in repairing damaged sperm morphology against obesity-induced free radicals. Flavonoid compounds play a role in neutralizing ROS in sperm and can protect sperm membranes from lipid peroxidation damage, resulting in normal sperm morphology (Adamkovicova et al., 2016).

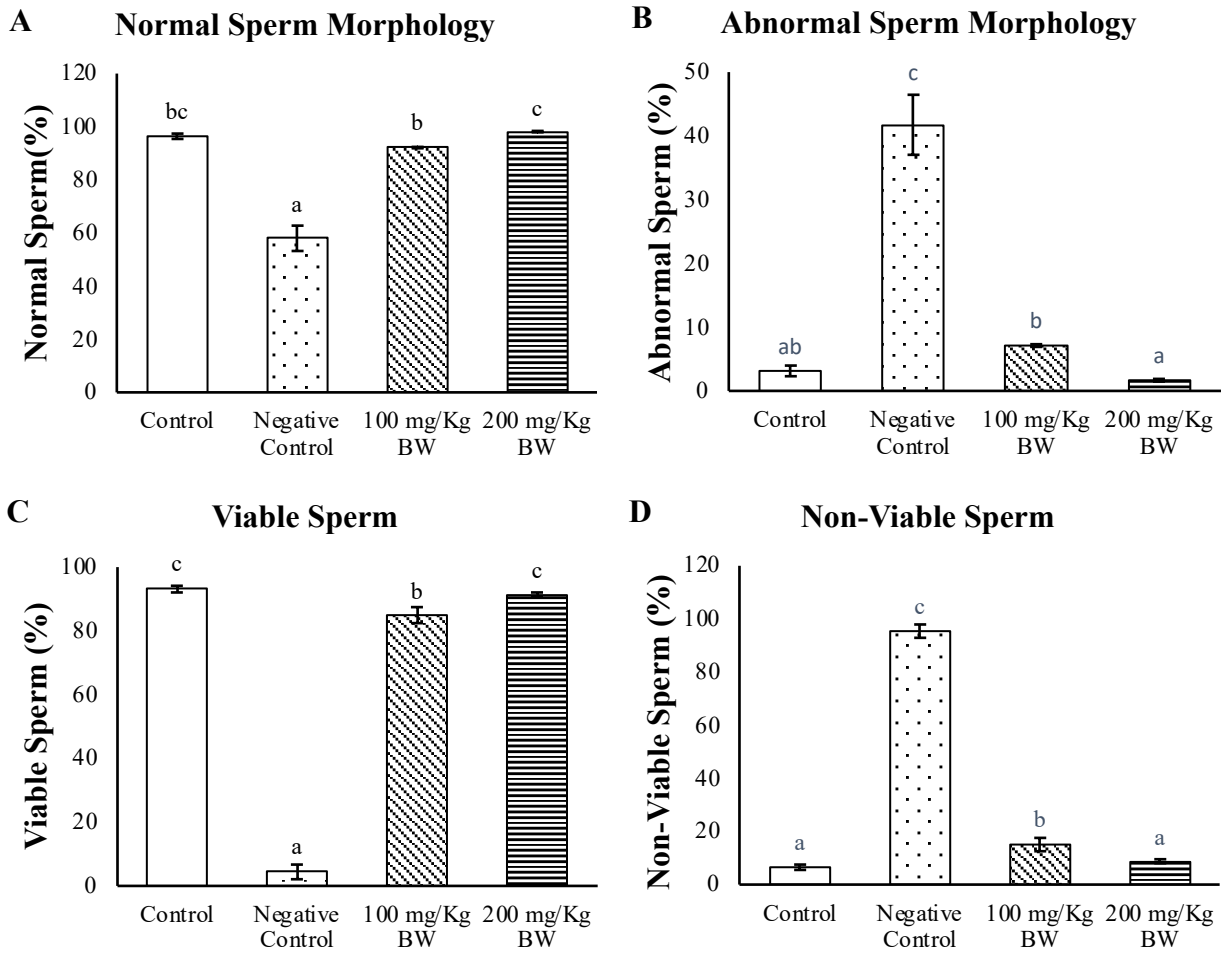

Figure 5. Histogram of normal sperm morphology (A); abnormal sperm (B); viable/live sperm (C); non-viable / dead sperm (D). C (0 mg/Kg BW extract and no HFF), NC (HFF), T1 (HFF and 100 mg/Kg BW extract), T2 (HFF and 200 mg/Kg BW extract). Mean ± SD, superscript a-c indicates significant differences between treatments ($p \leq 0,05$).

Sperm viability was divided into 2 categories, viable and non-viable sperm, showing significant differences between NC and T1 and T2 ($p \leq 0,05$). Viable sperm showed an increase in the T1 and T2 compared to NC (Figure 5C). The treatment showed a low value of non-viable sperm (Figure 5D). The disrupted spermatogenesis leads to abnormal sperm, including dead sperm (non-viable). Free radical formation is a primary cause of cellular and tissue damage; an increase in free radicals leads to the breakdown of unsaturated fatty acids into unstable lipid peroxides, disrupting metabolic function and damaging sperm structure. According to Susilo et al. (2018), flavonoid compounds can protect sperm cell membranes from oxidative stress caused by free radicals and prevent molecules affecting membrane integrity from entering.

CONCLUSION

Based on the results of the study, it can be concluded that Wedelia leaf plant extract can reduce obesity index, total cholesterol levels, and LDL levels, while improving the quality of sperm motility, count, morphology, and viability.

AUTHOR CONTRIBUTION

Y.N.I: collecting research data on number of sperm parameters, drafting the article; **D.K.W**: collecting research data on sperm morphology parameters; **A.G.H**: collecting research data on sperm viability parameters; **U.F.M**: collecting research data on sperm motility parameters; **HS**: drafting the research, drafting the article, revising the final manuscript.

ACKNOWLEDGMENTS

The research team would like to thank the Directorate General of Higher Education Research Technology through the Directorate of Learning and Student Affairs and Universitas Ahmad Dahlan for funding this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest with any parties involved in this research, which was funded by the Ministry of Education, Culture, Research, and Technology through the 2024 Student Creativity Program.

REFERENCES

Adamkovicova, M., Toman, R., Martiniakova, M., Omelka, R., Babosova R., Krajcovicova., Grosskopf, B., dan Massanyi, P. (2016). Sperma Motility and Morphology Changes in Rats Exposed to Cadmium and Diazinon. *Repductive Biology and Endocrinology* 14(42) : 1-7. DOI: 10.1186/s12958-016-0177-6

Afzal, S., & Rajesh, B. R. (2021). Phytochemical Screening and Pharmacological Evaluation of Leaf Extract *Sphagneticola trilobata*. *World Journal of Pharmaceutical Research* 10(9) : 1143 1154. DOI: 10.20959/wjpr20219-21137

Al-Sultani, Y. K., S. R. Al-Katib., and S. Al-Zayadi. (2013). Effect of Vitamin C on In-Vitro Sperm Activation of Asthenozoospermic Infertile Patients. *American Journal of Research Communication* 1(10) : 40-48.

Annisaroh, L. S., & Wibowo, D. A. (2019). Pengaruh Pemberian Jus Alpukat Terhadap Morfologi Spermatozoa Tikus Wistar yang Dipapar Asap Rokok.

Jurnal Kedokteran Diponegoro 8(2) : 802–810. DOI: 10.14710/dmj.v8i2.23801

Arief, Y. S. (2011). Stres dapat Menggangu Proses Spermatogenesis pada Mencit. *Jurnal Ners* 6(2) : 169-174. DOI: 10.20473/jn.v6i2.3987

Azizah, R. N., Putra, B., and Tobis, R. (2018). Aktivitas Hipolipidemik Ekstrak Etanol Herba Krokot (*Portulaca oleracea* L.) pada Tikus Obesitas dengan Parameter Trigliserida. *As-Syifaa* 10(1) : 66-73. DOI: 10.33096/jifa.v10i1.332

Billah, M. M., Khatiwada, S., Lecomte, V., Morris, M. J., & Maloney, C. A. (2022). Ameliorating High Fat Diet-Induced Sperm and Testicular Oxidative Damage by Micronutrient-Based Antioxidant Intervention in Rats. *European Journal of Nutrition* 61(7) : 3741–3753. DOI: 10.1007/s00394-022-02917-9

Badan Pengawas Obat dan Makanan (BPOM). (2020). Uji Farmakodinamik Praklinik Obat Tradisional. URL https://jdih.pom.go.id/download/product/1240/-/2021. Diakses tanggal 11 September 2024.

Budiawan, A., Purwanto, A., & Puradewa, L. (2021). Aktivitas Penyembuhan Luka Ektstrak Herba Krokot (*Portulaca oleracea*) Wound Healing Activity of Purslane Herb Extract (*Portulaca oleracea*). *Jurnal Ilmiah Kefarmasian* 3(1) : 1–8. DOI: 10.36760/jp.v3i1.270

Campbell, N. A. & Reece, J. B. (2010). *BiologiJilid3Edisike8*.Jakarta:Erlangga.

Craig, J. R., Jenkins, T. G., Carrell, D. T., & Hotaling, J. M. (2017). Obesity, Male Infertility, and The Sperm Epigenome. *Fertility and Sterility* 107(4) : 848–859. DOI: 10.1016/j.fertnstert.2017.02.115

Datu, O. S., Lebang, J. S., & Rumondor, E. M. (2021). Pengaruh Pemberian Sari Buah Salak (*Salacca zalacca*) terhadap Profil Lipid dan Berat Badan Tikus Model Hiperlipidemia dan Obesitasda. *Jurnal MIPA* 11(1) : 12–16. DOI: 10.35799/jm.v11i1.36530

Dias, M. de M. e, Reis, S. A. dos, Conceição, L. L. da, Sediyama, C. M. N. de O., Pereira, S. S., Oliveira, L. L. de, Peluzio, M. do C. G., Martinez, J. A., & Milagro, F. I. (2021). Diet-Induced Obesity in Animal Models: Points to Consider and Influence on Metabolic Markers. *Diabetology and Metabolic Syndrome* 13(32) : 1–14. DOI: 10.1186/s13098-021-00647-2

Greenspan, A. C., dan Hall, J. E. (2014). *Text Book of Medical Physiology*. USA: Saunder Elsevier.

Iryani, T., Carolina, N. and Saftarina. (2019). L.) The effect of Oral Leunca fruti (*Solanum nigrum* L.) Ethanol Extract to Decrease Sperma Count of White Rats (*Rattus norvegicus*) Sprague Dawley Strain. *Medula* 9(1) : 99–101.

Ismail, W. M. (2018). Histopatologi Epitel Tubulus Seminiferus Testis, Kualitas dan Kuantitas Sel Sperma Tikus Hiperkolesterolemia yang Diberi VitaminC.*JurnalBuletinFarmatera*3(3) : 181–192. DOI: 10.30596/bf.v3i3.1847

Kementerian Kesehatan Republik Indonesia (KEMENKES RI). (2023). Epidemi Obesitas. URL https://p2ptm.kemkes.go.id/uploads/N2VaaXIxZGZwWFpEL1V1RFdQQ3Z-RZz09/2018/02/FactSheet_Obesitas_Kit_Informasi_Obesitas.pdf. Accessed September 11th, 2024.

Kumar, N., & Singh, A. (2015). Trends of Male Factor Infertility, An Important Cause of Infertility. *Journal of Human Reproductive Sciences* 8(4) : 191–196. DOI: 10.4103/0974-1208.170370

Listiyana, A. D., Mardiana, M., & Prameswari, G. N. (2013). Obesitas Sentral dan Kadar Kolesterol darah Total. *Jurnal Kesehatan Masyarakat* 9(1) : 37–43. DOI: 10.15294/kemas.v9i1.2828

Lobstein, T., Jackson-Leach, R., Powis, J., Brinsden, H., Gray, M. (2023). World Obesity Federation. URL https://www.worldobesity.org/resources/resource-library/world-obesity-atlas-2023. Accessed September 11th, 2024.

Mardina, V., Ilyas, S., & Harmawan, T. (2020). Pengujian Aktivitas Anti Bakteri Daun *Sphagneticola trilobata* J.F (L.) Pruski terhadap *Salmonella typhi* dan *Escherichia coli*. *Prosiding Seminar Nasional Biotik* 1(1) : 364–369.

Martin, L. J., & Touaibia, M. (2020). Improvement of Testicular Steroidogenesis Using Flavonoids and Isoflavonoids for Prevention of Late-onset Male Hypogonadism. *Antioxidants* 9(3) : 1–17. DOI: doi.org/10.3390/antiox9030237

Mu, Y., Dai, H. G., Luo, L. B., and Yang, J. (2021). Irisin Alleviates Obesity-Related Spermatogenesis Dysfunction Via the Regulation of the AMPka Signaling Pathway. R*eprod Biol Endocrinal* 19(135) : 1-14. DOI: doi.org/10.1186/s12958-021-00821-1

Mutia, S., Fauziah, and Thomy, Z. (2018). Pengaruh Pemberian Ekstrak Etanol Daun Andong (*Cordyline fruticos*a (L.) A. Chev) Terhadap Kadar Kolesterol Total dan Trigliserida Darah Tikus Putih (*Rattus norvegicus*) Hiperkolesterolemia. *Jurnal Bioseluler* 2(2) : 29-35. DOI: 10.24815/bioleuser.v2i2.14883

Nashriana, N. J., Wirjatmadi, B., dan Adriani, M. (2015). Combined Food (Bekatul dan Lemak) Menurunkan Kadar Kolesterol Total, Trigliserida, dan LDL pada Tikus Galur Wistar. *Jurnal Kedokteran Brawijaya* 28(3) : 208-212. DOI: 10.21776/ub.jkb.2015.028.03.8

Nita, S., Setiawan, A., Inggarsih, R., Tantri, U., & Herdiana, M. (2019). Papaya (*Carica papaya* L.) Seed Extract as Male Contaception via Decreasing The Quality of Rat's (*Rattus norvegicus*) Sperm. *Bioscientia Medicina* 3(1) :14–26. DOI: 10.32539/bsm.v4i1.110

Rahayu, I., Usman, E., dan Reza, M. (2019). Effect of Vitamin C on Testosterone Level, Sperm Count and Sperm Mophology in Gentamicin-Induced Wistar Rats. *International Journal of Research in Medical Sciences* 7(2) : 451-456. DOI: 10.18203/2320-6012.ijrms20190352

Romadhoni, I., Racmawati, A., and Suyadi. (2014). Kualitas Semen Sapi Madura setelah Pengenceran dengan Tris Aminomethane Kuning Telur yang Disuplementasi α-tocopherol pada Penyimpanan Suhu Ruang. *Jurnal Ilmu-Ilmu Peternakan* 24(1) : 39-44.

Rompis, S. A., Tendean, L. E. N., & Rumbajan, J. M. (2018). Pengaruh Kelebihan Berat Badan terhadap Kualitas Spermatozoa Tikus Wistar (*Rattus norvegicus*).*Jurnal E-Biomedik* 6(1):39–44. DOI: 10.35790/ebm.6.1.2018.18769

Rumianti, R. T. (2011). Efek Propolis Terhadap Kadar Kolesterol Total pada Tikus Model Tinggi Lemak. *JKM* 11(1) : 17-22.

Roy, P., Tomassoni, D., Traini, E., Martinelli, I., Micioni Di Bonaventura, M. V., Cifani, C., Amenta, F., & Tayebati, S. K. (2021). Natural Antioxidant Application On Fat Accumulation: Preclinical Evidence.*Antioxidants* 10(858) : 1–24. DOI: 10.3390/antiox10060858

Setiawan, H., Maliza, R., Maulana, S. A., & Hisbullah, M. I. (2020). The Effect of Coffee Fruit Skin Extract on Sperm Characteristics And Testicular of Mice With Ethanol-Induced. *Jurnal Biodjati* 5(2) : 259–270. DOI: 10.15575/biodjati.v5i2.9280

Setiawan, H., & Subagja, R. A. (2023). Pengaruh Pemberian Jus Pare (Momordica charantia L.) Terhadap Kualitas Sperma dan Histologi Testis Tikus Wistar. *Al-Kauniyah: Jurnal Biologi* 17(1) : 10–20. DOI:

10.15408/kauniyah.v16i2.1.23216

Sitohang, A. G., B. Wantouw., dan E. de Queljoe. (2015). Perbedaan antara Efek Pemberian Vitamin C dan Vitamin E terhadap Kualitas Spermatozoa Tikus Wistar (*Rattus norvegicus*) Jantan setelah diberi Paparan Asap Rokok. *Jurnal e-Biomedik* 3(1) : 65-71. DOI: 10.35790/ebm.v3i1.6608

Susilo, S., Akbar, B., & Pratinaningsih, I. (2018). Pengaruh Ekstrak Etanol Daun Sambiloto Terhadap Jumlah dan Motilitas Spermatozoa Mencit Jantan. *Jurnal Biodjati* 3(2) : 166–172. DOI: 10.15575/biodjati.v3i2.3505

Tamon, B. T., Tiho, M., & Kaligis, S. H. M. (2021). Efek Antioksidan pada Teh Hijau terhadap Kadar Kolesterol Darah. *Jurnal E-Biomedik* 9(2) : 151–159. DOI: 10.35790/ebm.v9i2.31869

UNICEF. (2019). Analisis Lanskap Kelebihan Berat Badan Dan Obesitas di Indonesia. In 01 Desember 2022. https://www.unicef.org/indonesia/id/laporan/analisis-lanskap-kelebihan-berat-badan-dan-obesitas-di-Indonesia

Unitly, A. J. A., Eddy, L., Nindatu, M., Jesmendy, R. (2022). Peningkatan Motilitas dan Viabilitas Spermatozoa Rattus norvegicus Terpapar Asap Rokok Pasca Diterapi Sirup Cengkeh. *Jurnal Biologi Edukasi* 14(1) : 14-20. DOI: 10.24815/jbe.v14i1.26345

Venigalla, G., Ila, V., Dornbush, J., Bernstein, A., Loloi, J., Pozzi, E., Miller, D., & Ramasamy, R. (2023). Male Obesity: Associated Effects on Fertility and the Outcomes of Offspring. *Andrology* 1(1) : 1–8. DOI: 10.1111/andr.13552

Wahyuni, T., & Diansabila, J. (2020). Persepsi Tubuh dan Bulimia Nervosa pada Remaja Putri. Muhammadiyah Journal of Nutrition and Food Science (MJNF) 1(2) : 48–53. DOI: 10.24853/mjnf.1.2.48-53

Yuliani, N. N., and Dienina, D. P. (2015). Uji Aktivitas Antioksidan Infusa Daun Kelor (*Moringa oleifera*) dengan Metode 1,1-diphenyl-2-picrylhydrazyl (DPPH). *Jurnal Info Kesehatan* 12(2): 1061-1082.