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LEYDIG CELL COUNT IS INCREASES IN OLD WISTAR RATS (RATTUS NORVEGICUS) BY ANTIOXIDANT ETHANOL EXTRACT OF MORINGA OLEIFERA (EEMO)

Jumlah Sel Leydig Meningkat pada Tikus Wistar Tua (Rattus Norvegicus) dengan Antioksidan Ekstrak Etanol Moringa Oleifera (EEMO)

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ABSTRACT
Oxidative stress is the result of an imbalance between antioxidant production and the production of reactive oxygen species (ROS). Leydig cells have a high content of PUFAs in their cell membrane, making it easy to bind ROS. Most natural antioxidants are found in plants, including Moringa. In this work, aged Wistar rats' Leydig cells will be used to test the antioxidant activity of an ethanol extract from the leaves of Moringa oleivera (Rattus norvegicus). The study involved the division of 36 elderly rats, who were between the ages of 18 and 19 months, into two groups. For thirty days, the control group received the same quantity of 0.5% CMC every day, whereas the treatment group received 50 mg/kgBW/0.5 mL of Moringa leaf ethanol extract daily. The rats were healthy and free of physical impairments. The Independent Samples T-Test is used to assess the data in order to find any variations between the treatment and control groups. The significant difference ($p < 0.00$) in Leydig cell counts between the extract-treated group and the control group suggests that the ethanol extract of Moringa leaves can have a major effect on the number of Leydig cells in Old Wistar rats (Rattus Norvegicus).

***Keywords**: Moringa Leaf Ethanol Extract, Leydig cells, White Rat*

ABSTRAK
Ketidakseimbangan antara pembentukan spesies oksigen reaktif (ROS) dan antioksidan disebut sebagai stres oksidatif. Sel Leydig memiliki kandungan PUFA yang tinggi di membran selnya, sehingga mudah mengikat ROS. Sebagian besar antioksidan alami ditemukan pada tanaman, termasuk Kelor. Dalam penelitian ini, sel Leydig tikus Wistar yang sudah tua akan digunakan untuk menguji aktivitas antioksidan ekstrak etanol dari daun Kelor (Rattus norvegicus). Penelitian ini melibatkan pembagian 36 tikus tua, yang berusia antara 18 dan 19 bulan, menjadi dua kelompok. Kelompok perlakuan diberi 50 mg/kgBB/0,5 mL CMC 0,5% per hari sebagai ekstrak etanol daun Kelor, sedangkan kelompok kontrol diberi jumlah yang sama yaitu CMC 0,5% per hari selama 30 hari. Tikus-tikus tersebut tidak mengalami gangguan fisik dan dalam kondisi sehat. Data dievaluasi menggunakan Uji-T Sampel Independen untuk mengidentifikasi perbedaan antara kelompok perlakuan dan kelompok kontrol. Hasil penelitian menunjukkan bahwa ekstrak etanol daun kelor dapat memberikan pengaruh yang signifikan terhadap jumlah sel Leydig pada tikus Wistar Tua (Rattus Norvegicus), terbukti dari adanya perbedaan yang signifikan ($p < 0,00$) jumlah sel Leydig antara kelompok yang diberi ekstrak dan kelompok kontrol.

Kata kunci: Ekstrak Etanol Daun Kelor, Sel Leydig, Tikus Putih

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INTRODUCTION

Married couples experiencing infertility can be divided into three categories idiopathic factors (15%), female factors (45%), and male factors (45%) (Lestari & Sari, 2015). According to (Kumar & Singh, 2018), Male factor is the cause of 40–50% of occurrences of infertility in couples (Aziz & Agarwal, 2017). According to (Leslie et al., 2023), There are many different reasons why men become infertile, but they can all be broadly categorised by a common underlying aetiology. Endocrine diseases (typically resulting from hypogonadism) account for 2% to 5% of these, sperm transport disorders (such as vasectomy) account for 5%, and idiopathic (i.e., infertile men with normal sperm and semen characteristics) account for 10% to 20%. Between 65 and 80 percent of these cases are caused by primary testicular abnormalities, or aberrant sperm parameters without a known cause. Men become infertile for around half of the reasons that stem from poor sperm production, such as irregular sperm motility, low sperm concentration, spermatogenesis-related problems, and low sperm concentration (Widiastini, Karuniadi, & Tangkas, 2022a). Some of the risk factors for male infertility are obesity, smoking, alcohol, stress, and age (aging) (Okonofua et al., 2022).

Aging is a physiological process in the body that occurs due to increasing age (Han et al., 2017; Widiastini, Karuniadi, & Tangkas, 2022b). Due to the process of reproductive ageing, the testes, epididymis, and other reproductive organs gradually lose all of their physiological capabilities (Widiastini et al., 2023). The aging process is an accumulation of cellular damage that can be caused by stress, environmental influences, and poor nutrition. Free radicals in the body are directly linked to ageing and can lead to oxidative stress (Balin & Allen, 2018; Liguori et al., 2018).

According to (Henkel et al., 2018), Numerous diseases, including ageing and male infertility, are directly associated with oxidative stress. An imbalance between the generation of reactive oxygen species (ROS) and antioxidants leads to oxidative stress (Luceri et al., 2018). Sperm capacitation, acrosome response, hyperactivation, and sperm-oocyte fusion are all regulated by physiological levels of ROS (Fatima, 2018; Lee et al., 2017). ROS levels exceeding physiological limits can affect spermatogenesis, reduce spermatozoa motility, and damage mitochondria and DNA integrity. Increased ROS refers to the main process of cell aging, and one of the etiological factors of male infertility. Men who are older may experience aberrant morphology, decreased sperm motility, and decreased semen volume (Fatima, 2018; Lucio et al., 2013; Morielli & O'Flaherty, 2015).

Leydig cells work in conjunction with the pituitary gonadotropins to create testosterone through the release of luteinizing hormone (LH), which is involved in the process of spermatogenesis and influences the characteristics of male secondary sex (Neves et al., 2017). Decreased testosterone production is an effect of ROS on the endocrine system. The decrease in testosterone disrupts spermatogenesis resulting in rudimentary spermatozoa. In addition, there is also a failure to maintain normal growth of auxiliary reproductive organs (Sertoli cells and Leydig cells) that play an important role in sperm maturation (Darbandi et al., 2018).

Leydig cells can easily bind to ROS because most of their cell membranes contain PUFAs. Oxidative damage that occurs in the testes and sperm also depends on oxidative defenses in the testes. The activity of drug-metabolizing enzymes is found in the Testes, but the mechanism of scavenging or destroying free radicals either enzymatically or by direct chemical reactions of oxidative metabolites in the Testes is low. In Leydig cells, antioxidant enzymes are found in the cytoplasm, but the amount is only small to be able to neutralize ROS (Prayoga, 2015).

Antioxidants are necessary for the body to fight off free radicals, shield cells from damage caused by free radical attacks, stop chain reactions from happening so that more damage is not done, and repair tissues and cells that have been harmed by free radical attacks (Parwata, 2015). By giving hydrogen or electrons to molecules that have undergone oxidation, reductants and antioxidants work to stop oxidation or neutralize it (Henkel et al., 2018).

Most natural sources of antioxidants come from plants, one of which is the

Moringa plant (Razis et al., 2014). An analysis of the phenolics, flavonoids, tannins, ascorbic acid, alkaloids, and saponins found in Moringa leaves in Bali's South Denpasar area has demonstrated the leaves' antioxidant potential (Widiastini et al., 2021). Based on the background information provided, scientists are curious to know how the administration of Moringa leaf ethanol extract (Moringa oleifera) affects the number of Leydig cells in senior Wistar rows of white rats (Rattus Norvegicus).

MATERIALS AND METHODS

A randomised post-test-only control group design was used in this study as an experimental research design. Adult male Wistar rats (Rattus Norvegicus) were used as the study's sample; the age and body weight requirements were 18–19 months and 200–250 grammes, respectively. Rats that displayed symptoms of disease and remained still were eliminated. Rats that died during the trial and lost more than 10% of their body weight following the laboratory acclimatization phase were considered exclusion samples. The treatment group (18 rats) and the control group (18 rats) were the two groups of the 36 rats used in this investigation. The sample was chosen by the researchers using random sampling techniques.

Ethical Approval

This study was carried out at Udayana University's Faculty of Medicine's Integrated Biomedical Laboratory from March to May of 2022, with ethical test from KEPK STIKES Bina Usada Bali, No: 048/EA/KEPK-BUB-2022.

Preparation of Moringa Leaf Ethanol Extract

To make moringa leaf extract, up to 50 grams of dried moringa leaves were macerated. The leaves were then pulverized in a blender, mixed with 96% ethanol solvent, placed in a container, sealed, and kept out of direct sunlight for two days. The macerate was obtained by filtering this combination. The same process was used to macerate the leftovers with 96% ethanol. The process of maceration was continued until a clear macerate was achieved. A vacuum rotary evaporator operating at 40 0C was used to evaporate the macerate (Cahyani & Sukadana, 2017; Putra et al., 2017; Wasonowati et al., 2019; Widiastini et al., 2021).

Research procedure

The investigation's initial stage is to weigh every experimental animal. The treatment group received a dose of up to 50 mg/kgBW of moringa leaf ethanol extract, diluted with 0.5 mL of 0.5% CMC every day. A daily dose of 0.5 mL of 0.5% CMC was administered to the control group. The medication was administered for 30 days in a round, from 08.00-09.00 Wita.

Male Wistar rats (R. norvegicus) were put to death on the thirty-first day of the treatment period. The rats were put to sleep with ketamine and xylazine intramuscular (IM) doses of 100 mg/kg and 10 mg/kg (10:1), respectively, before being put to death by cervical dislocation. After being cut free from the cauda epididymis, the testes were placed in a petri plate with five millilitres of 0.9% NaCl. The bodies of rats were buried.

Leydig cell examination

The testis organ was first fixed for 24 hours in a 10% formalin buffer solution, and then for three hours in Bouin's solution to prepare the histological preparations. In addition, the testis was submerged in a toluene solution for 24 hours to clean the preparation after being repeatedly rinsed with a 70% alcohol solution and dehydrated using alcohol solutions of varying strengths. The testis was immersed in a solution containing toluene and paraffin for thirty minutes in order to perform paraffin infiltration into the tissue. The testis was then embedded in solid paraffin during the embedding step. The testis-containing paraffin block was cut into 3–5 µm-thick slices using a microtome. To ensure it was sufficiently strong, the slices were affixed to a glass object that had been covered in Mayer albumin and left for a full day. Hematoxylin-Eosin stain was applied to the histology preparations, which were then coated and adhered to with per mount. Quantitative information from both sample groups expressed as the quantity of Leydig

cells. A 40x10 magnification Olympus light microscope and an OptiLab camera were used for the observations. To obtain the five best fields of view on the right and left testes, the observation technique was applied by sweeping the histological preparation in a spiral motion from the top-left corner to the bottom-right.

Data Analysis

A descriptive analysis of the data was performed, and the frequency distribution and mean of Leydig cells were displayed. The Shapiro-Wilk test was used to determine whether the data were regularly distributed; a p-value of less than 0.05 indicated as much. In a comparative study, an Independent sample t-test was used to look for any differences between the treatment and control groups at a significance threshold of $\alpha = 0.05$. A 95% confidence level or $p < 0.05$ is used for data evaluation.

RESULTS AND DISCUSSIONS

Table 1. Body weight characteristics of male Wistar rats (Rattus Norvegicus) aged 18-19 months

Grup	Mean	Std	Min	Max	Homogeneity Test	*T-Test*
Treatment	237.33	7.452	224	250	0.495	0.54
Control	238.78	6.522	224	248		

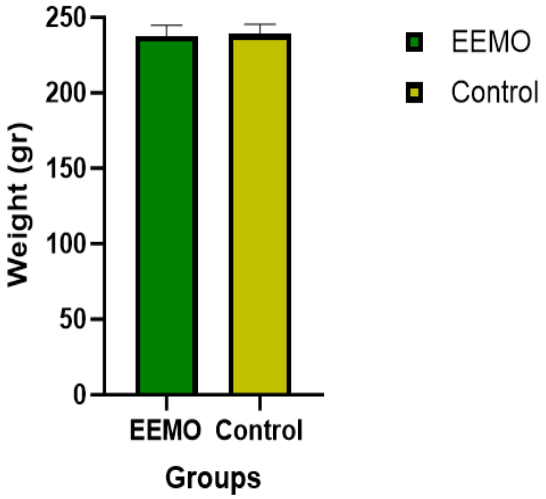

Figure 1. Body Weight in Male Wistar Rats (Rattus Norvegicus) (n=18) (mean)

Based on the table and figure above, it can be seen that there is no significant difference between the variance of rat body weight data in the treatment and control groups with a $p>0.05$ value.

Table 2. Number of Leydig Cells in Male Wistar Rats (Rattus Norvegicus) After Given Moringa Leaf Ethanol Extract

Grup	Mean ± SD	95% CI	Min	Max	Normality Test	T-Test
Treatment	18.61 ± 3.25	16.99-20.03	13	24	0,301	0,000
Control	11.22 ± 1.66	10.39-12.05	8	14	0,503	

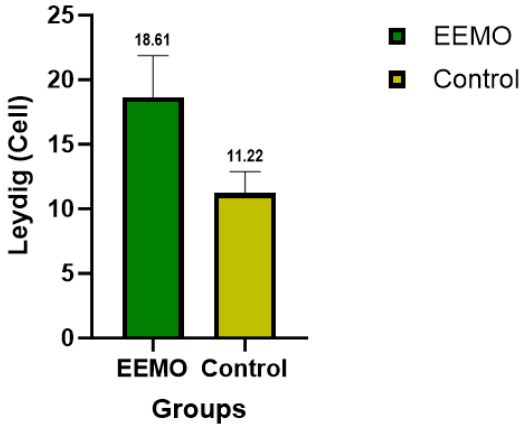

Figure 2. Differences in Leydig cells in male Wistar rats (Rattus Norvegicus) (n=18) (mean)

From the table above, it can be seen that the treatment group has a good Leydig Cell Count with the largest mean of 18.61, 95% CI 16.99-20.03, the largest minimum number is 13, the highest maximum number is 24. Data on the number of Leydig cells in both the treatment and control groups were normally distributed with a p value of 0.301 for the treatment group and 0.503 for the control group, so an Independent sample T Test analysis was carried out and the results obtained a p value of 0.000 (p value <0.05), which means there is a significant difference in the number of Leydig cells between the group given moringa leaf ethanol extract and the control group that was not given, so it can be said that moringa leaf extract can have a significant effect on the number of Leydig cells.

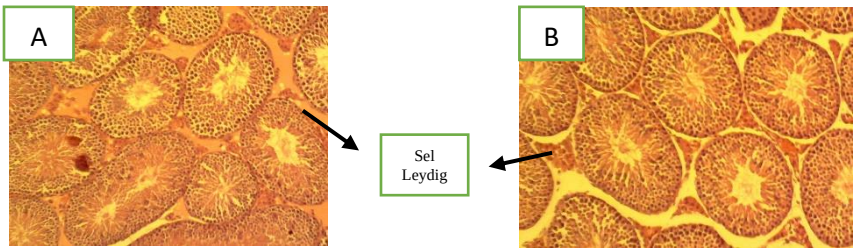

Figure 3. Leydig cells, with 400 times magnification
A: Control Group, B: Control Group

Male germ cells, Sertoli cells, and peritubular myoid cells are the three main cell types that comprise the seminiferous tubules. The connective tissue situated between the Leydig cells and other seminiferous tubules is referred to as the "testicular stroma" (Zhou et al., 2019). Free radicals produced by oxidative stress, genetic changes, and environmental factors combine to create a progressive loss in physiological integrity that leads to reduced function and ageing. The rate of aging-induced alterations will be slowed down by interventions that restrict or block free radical reactions; this should slow the rate of ageing and the pathogenesis of disease (Zalukhu et al., 2016). One of the impacts of ageing on the male reproductive system is changes in testicular morphology. One testicular aging-related alteration is a reduction in the quantity of Leydig cells (Gunes et al., 2016).

The group that received 50 mg/kg BW of moringa leaf ethanol extract had a higher number of Leydig cells than the control group (p-value <0.05). This implies that the ethanol extract from moringa leaves can increase the quantity of Leydig cells. It is estimated that moringa contains approximately ninety different kinds of nutrients, including important minerals, vitamins, and amino acids as well as substances with anti-inflammatory and anti-aging properties. Moringa

has been utilised in traditional medicine to prevent over 300 ailments since it has 539 components that are recognised in African and Indian traditional medicine (Toripah et al., 2014).

Ascorbic acid, tannins, alkaloids, saponins, phenolics, and flavonoids are among the antioxidants found in the content analysis of moringa leaves found in Bali's South Denpasar area (Widiastini et al., 2021). The antioxidants in moringa leaves neutralise free radicals, preventing oxidative damage to the majority of biomolecules and producing a considerable protective effect against oxidative damage (Vergara-jimenez et al., 2017).

Variations in the thickness of tubule epithelial cells and the diameter of seminiferous tubules are indicators that the flavonoid content of Moringa leaves can affect spermatogenesis. Flavonoids are powerful antioxidants that can prevent oxidative stress (OS), combat free radical damage, and enhance spermatogenesis. Flavonoids can aid in the promotion of cell regeneration by removing free radicals, providing unsaturated lipids in membranes with a competitive substrate, and/or accelerating the process of repairing damaged cell membranes (Laoung-on et al., 2021)

The water-soluble vitamin C, also known as ascorbic acid, is present in moringa leaves and is essential for the hydroxylation and amidase reactions that they catalyse (Ahmadi et al., 2016). As an electron transporter, vitamin C is involved in several bodily chemical processes. By contributing an electron to make ascorbyl radical, also known as semidehydro ascorbic acid, vitamin C works against free radicals. These more stable radicals have the ability to interact with other free radicals to scavenge or squench them, turning them into less reactive radicals (Padayatty & Levine, 2016). Based on the findings of a review of the literature carried out by (Widiastini, Karuniadi, Karmaya, et al., 2022), It was discovered that moringa, particularly the leaves, have a favourable impact on sexual behaviour, particularly libido. Moreover, it improves spermatogenesis and sperm quality, particularly by boosting sperm motility, count/volume, and germ cell count. It also lowers ROS levels, renews endogenous antioxidant enzyme activity, and protects against injury to testicles and Leydig cells.

CONCLUSION

The results of this study show that, Compared to the control group, which received CMC 0.5%, 0.5 mL, the treatment group in White Rats (Rattus Norvegicus) Old Age Wistar Rats that received up to 50 mg / kg BW of Moringa leaf ethanol extract (Moringa Oleifera) for 30 days generated more Leydig cells. The difference in the number of Leydig cells between the group receiving the extract and the unextracted control group was statistically significant, with a p value of 0.000 (p value <0.05), indicating that Moringa extract can have a significant impact on the quantity of Leydig cells.

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REFERENCES

Ahmadi, S., Bashiri, R., Ghadiri-Anari, A., & Nadjarzadeh, A. (2016). Antioxidant supplements and semen parameters: An evidence based review. *Int J Reprod BioMed*, *14*(12), 729–736.

Aziz, N., & Agarwal, A. (2017). The Diagnosis and Treatment of Male Infertility. In N. Aziz & A. Agarwal (Eds.), *Springer* (Vol. 38, Issue 6). Springer International Publishing AG 2017. https://doi.org/10.1007/978-3-319-56547-7

Balin, A. K., & Allen, R. G. (2018). Oxidative stress and aging. *Encyclopedia of Endocrine Diseases*, *2014*, 659–663. https://doi.org/10.1016/B978-0-12-801238-3.03872-1

Cahyani, S. L., & Sukadana, I. M. (2017). Skrining Fitokimia dan Aktivitas Penangkapan Radikal Bebas DPPH Ekstrak Etanol Daun Kelor (Moringa Oleifera) di Ende. *Jurnal Poltekkes Kupang*, 410–416.

Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., Durairajanayagam, D., Henkel, R., & Sadeghi, M. R. (2018). Reactive oxygen species and male reproductive hormones. *Reproductive Biology and Endocrinology*, *16*(1), 1–14. https://doi.org/10.1186/s12958-018-0406-2

Fatima, S. (2018). Role of Reactive Oxygen Species in Male Reproduction. *Novel Prospects in Oxidative and Nitrosative Stress*. https://doi.org/10.5772/intechopen.74763

Gunes, S., Hekim, G. N. T., Arslan, M. A., & Asci, R. (2016). Effects of aging on the male reproductive system. *Journal of Assisted Reproduction and Genetics*, *33*(4), 441–454. https://doi.org/10.1007/s10815-016-0663-y

Han, J. D. J., Xia, X., Chen, W., & McDermott, J. (2017). Molecular and phenotypic biomarkers of aging. *F1000Research*, *6*(0). https://doi.org/10.12688/f1000research.10692.1

Henkel, R., Sandhu, I. S., & Agarwal, A. (2018). The excessive use of antioxidant therapy: A possible cause of male infertility? *Andrologia*, *51*(1), 1–8. https://doi.org/10.1111/and.13162

Kumar, N., & Singh, A. K. (2018). Reactive oxygen species in seminal plasma as a cause of male infertility. *Journal of Gynecology Obstetrics and Human Reproduction*, *47*(10), 565–572. https://doi.org/10.1016/j.jogoh.2018.06.008

Laoung-on, J., Saenphet, K., & Jaikang, C. (2021). Effect of Moringa oleifera Lam . Leaf Tea on Sexual Behavior and Reproductive Function in Male Rats. *National Library of Medicine*, 1–17. https://doi.org/10.3390/plants10102019

Lee, D., Moawad, A. R., Morielli, T., Fernandez, M. C., & O'Flaherty, C. (2017). Peroxiredoxins prevent oxidative stress during human sperm capacitation. *Molecular Human Reproduction*, *23*(2), 106–115. https://doi.org/10.1093/molehr/gaw081

Leslie, S. W., Soon-Sutton, T. L., & Khan, M. A. (2023). *Male Infertility*. StatPearls Publishing LLC.

Lestari, S. W., & Sari, T. (2015). Fragmentasi DNA Spermatozoa: Penyebab, Deteksi, dan Implikasinya pada Infertilitas Laki-Laki. *EJournal Kedokteran Indonesia*, *3*(2). https://doi.org/10.23886/ejki.3.5050.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *13*, 757–772. https://doi.org/10.2147/CIA.S158513

Luceri, C., Bigagli, E., Femia, A. Pietro, Caderni, G., Giovannelli, L., & Lodovici, M. (2018). Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative injury. *Toxicology Reports*, *5*(November 2017), 141–145. https://doi.org/10.1016/j.toxrep.2017.12.017

Lucio, R. A., Tlachi-López, J. L., Eguibar, J. R., & Ågmo, A. (2013). Sperm count and sperm motility decrease in old rats. *Physiology and Behavior*, *110–111*, 73–79. https://doi.org/10.1016/j.physbeh.2012.12.015

Morielli, T., & O'Flaherty, C. (2015). Oxidative stress impairs function and increases redox protein modifications in human spermatozoa. *Reproduction*, *149*(1), 113–123. https://doi.org/10.1530/REP-14-0240

Neves, B. V. D., Lorenzini, F., Veronez, D., de Miranda, E. P., Neves, G. D., & De Fraga, R. (2017). Numeric and volumetric changes in leydig cells during aging of rats. *Acta Cirurgica Brasileira*, *32*(10), 807–815. https://doi.org/10.1590/s0102-865020170100000002

Okonofua, F. E., Favour, L., Ntoimo, C., Omonkhua, A., Ayodeji, O., Olafusi, C., Unuabonah, E., & Ohenhen, V. (2022). Causes and Risk Factors for Male Infertility : A Scoping Review of Published Studies. *International Journal of General Medicine*, *15*,

5985–5997. https://doi.org/10.2147/IJGM.S363959

Padayatty, S. J., & Levine, M. (2016). Vitamin C: the known and the unknown and Goldilocks. *Oral Diseases*, *22*(6), 463–493. https://doi.org/10.1111/odi.12446

Parwata, O. A. (2015). *Uji Bioaktivitas : Antioksidan*. Universitas Udayana.

Prayoga, P. R. (2015). The Effect of Tomato (Lycopersicum Esculentum Mill) to Amount, Motility, and Morphology of Spermatozoa In Cigarettes-Induced Infertilty Patients. *J Majority*, *4*(5), 60–66.

Putra, D. P., Dharmayudha, O., & Sudimartini. (2017). Identifikasi Senyawa Kimia Ekstrak Etanol Daun Kelor (Moringa oleifera L) di Bali. *Indonesia Medicus Veterinus*, *5*(5), 464–473.

Razis, A. F. A., Ibrahim, M. D., & Kntayya, S. B. (2014). Health Benefits of Moringa oleifera. *Asian Pacific Journal of Cancer Prevention (APJCP)*, *15*(20), 8571–8576.

Toripah, S. S., Abidjulu, J., & Frenly Wehantouw. (2014). *Aktivitas Antioksidan dan Kandungan Total Fenolik Ekstrak Daun Kelor (Moringa Oleifera Lam)*. *3*(4), 37–43.

Vergara-jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. *National Library of Medicine*, 1–13. https://doi.org/10.3390/antiox6040091

Wasonowati, C., Sulistyaningsih, E., Indradewa, D., & Kurniasih, B. (2019). Analisis Fitokimia Ekstrak Daun Kelor (Moringa oleiferaLamk) di Madura. *Prosiding SEMNASDAL (Seminar Nasional Sumber Daya Lokal)*, *2*(9), 421–427.

Widiastini, L. P., Karuniadi, I. G. A. M., Karmaya, I. N. M., & Widhiantara, I. G. (2022). The Potential of A Balinese Traditional Medicine Kelor Leaves (Moringa oleifera) For Male Infertility Treatment : A Mini Review. *Indian Journal of Forensic Medicine & Toxicology*, *16*(1), 734–742. https://doi.org/10.37506/ijfmt.v16i1.17579

Widiastini, L. P., Karuniadi, I. G. A. M., & Tangkas, M. (2021). Senyawa Antioksidan Ekstrak Etanol Daun Kelor (Moringa Oleifera) di Denpasar Selatan Bali. *Media Kesehatan Politeknik Kesehatan Makassar*, *XVI*(1), 135–139. https://doi.org/https://doi.org/10.32382/medkes.v16i1.2038

Widiastini, L. P., Karuniadi, I. G. A. M., & Tangkas, M. (2022a). Antioxidant Effect Of Moringa (Moringa Oleifera) Leaf Ethanol Extract On Spermatogenesis In Old Wistar Rats (Rattus Norvegicus). *Jurnal Bioteknologi Dan Biosains Indonesia*, *9*(October).

Widiastini, L. P., Karuniadi, I. G. A. M., & Tangkas, M. (2022b). Ethanol Extract Of Moringa Oleifera Increased The Number Of Spermatozoa And Improved Sperm Morphology Of Old Rattus Norvegicus. *Bioteknologi & Biosains Indonesia*, *9*(October 2021), 11–19.

Widiastini, L. P., Karuniadi, I. G. A. M., & Tangkas, M. (2023). The Impact of Aging on the Quality of Sperm in White Rats (Rattus Norvegicus) Wistar Strain. *JurnalInfo Kesehatan*, *21*(2), 248–256. https://doi.org/10.31965/infokes.Vol21Iss2.876

Zalukhu, M. L., Phyma, A. R., & Pinzon, R. T. (2016). Proses Menua, Stres Oksidatif dan Peran Antioksidan. *CDK Journal*, *43*(10), 733–736.

Zhou, R., Wu, J., Liu, B., Jiang, Y., Chen, W., Li, J., He, Q., & He, Z. (2019). The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. *Cellular and Molecular Life Sciences*, *76*(14), 2681–2695. https://doi.org/10.1007/s00018-019-03101-9