Moringa Leaf Extract Improves Spermatozoa Motility and Viability Through an Antioxidant Mechanism in Older Wistar Rats

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ABSTRAK

Widiastini LP, Karuniadi IGAM. 2025. Ekstrak daun kelor meningkatkan motilitas dan viabilitas spermatozoa melalui mekanisme antioksidan pada tikus Wistar tua. JITV 30(4):212-218. DOI: http/dx.doi.org/10.1443/jitv.v30i3.3405.

Proses penuaan reproduksi ditandai dengan penurunan secara progresif integritas fisiologis yang dapat memicu kerusakan testis, epididimis maupun organ reproduksi lainnya. Stress oksidatif berkaitan erat dengan penuaan serta infertilitas pada pria yang menyebabkan penurunan volume semen, abnormalitas morfologi, penurunan viabilitas serta motilitas sperma. Penanganan terhadap stress oksidatif salah satunya dilakukan dengan menggunakan senyawa antioksidan seperti tanaman kelor. Tujuan penelitian ini, adalah untuk mengetahui mekanisme antioksidan ekstrak etanol daun kelor terhadap motilitas dan viabilitas spermatozoa pada tikus Wistar tua. Jenis penelitian ini adalah randomized post-test only control group design. Jumlah sample sebansyak 36 tikus Wistar tua (umur 18-19 bulan), yang dirandomisasi menjadi 2 kelompok, kelompok perlakuan (18 tikus) diberikan ekstrak etanol daun kelor (EEMO) 50 mg/kgBB + CMC 0.5%, pada dosis 0,5 ml/hari. Kelompok kontrol (18 tikus) diberikan CMC 0,5% sebanyak 0,5 ml/hari, selama 30 hari. Terminasi tikus menggunakan metode cervical dislocation. Selanjutnya dilakukan pemeriksaan motilitas dan viabilitas spermatozoa. Analisa data menggunakan independent sample t-test dan Mann Whitney dengan nilai signifikansi P= 0,05. Hasil penelitian menunjukkan bahwa kandungan antioksidan EEMO 50 mg/kg BB selama 30 hari pada tikus Wistar tua meningkatkan progressive motility, menurunkan non progressive motility dan immotility, serta meningkatkan viability spermatozoa, secara bermakna pada kelompok perlakuan dibandingkan kelompok kontrol dengan nilai P<0,05. Kesimpulan pada penelitian ini adalah, ekstrak daun kelor dapatmeningkatkan motilitas dan viabilitas spermatozoa melalui mekanisme antioksidan pada tikus Wistar tua.

Kata Kunci: Antioksidan, Daun Kelor, Motilitas, Tikus Tua, Viabilitas

ABSTRACT

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The reproductive aging is characterized by a progressive decline in physiological integrity that triggers in damaging to the testicle, epididymis and other reproductive organs. An oxidative stress is associated with aging and infertility in men which causes a decrease in semen volume, abnormal morphology, decreased sperm viability and motility. One of the treatments for oxidative stress is the use of antioxidant compounds such as Moringa plants. The purpose of this study was to determine the antioxidant mechanism of moringa leaf ethanol extract in sperm motility and viability in older Wistar rats. This study is a randomized post-test only control group design. The total sample was 36 older Wistar rats (age 18–19 months), which were divided randomly into 2 groups, including the treatment group (18 rats) was given ethanol extract of Moringa oleifera (EEMO) 50 mg/kg BW+CMC 0.5%, 0.5 ml /day, and the control group (18 rats) was given CMC 0.5%, 0.5 ml/day, for 30 days. Rats were terminated by cervical dislocation method. Motility and viability of spermatozoa were examined. Data analysis using an independent sample t-test and Mann Whitney with a significance value of P= 0.5. The results showed that the antioxidant content of EEMO 50 mg/kg BW+CMC 0.5%, for 30 days in old Wistar rats increased progressive motility, decreased non-progressive motility and immotility, and increased spermatozoa viability, significantly in the treatment group compared to the control group with a P<0.05. In conclusion, Moringa leaf extract could improves spermatozoa motility and viability through an antioxidant mechanism in old wistar rats.

Key Words: Antioxidant, Moringa Leaf, Motility, Old Rat, Viability

INTRODUCTION

Aging is a physiological process in the body that occurs due to increasing age (Han et al. 2017). The aging process is an accumulation of cellular damage that can be caused by stress, environmental influences, and poor nutrition. Aging is closely related to free radicals in the body, which can induce oxidative stress (Balin & Allen 2018; Liguori et al. 2018)

Some studies state that aging in male rats may induce a decrease in testicular size, the number of germ cells, Sertoli cells, and Leydig cells, as well as peritubular cells. Based on the ultrastructural analysis, sperm maturation in spermatogenesis stops at the

spermatogonium and spermatocyte stages in the testes and there is a decrease in the percentage of motility and viability of spermatozoa in the cauda epididymis (Lucio et al. 2013). Increased reactive oxygen species (ROS) can produce DNA damage and lead to decrease motility, viability and plasma membrane integration of spermatozoa (Lucio et al. 2013; Morielli & O'Flaherty 2015; Fatima 2018; Luceri et al. 2018; Alahmar 2019; Kurkowska et al. 2020)

Reactive oxygen species (ROS), which are free radicals produced from oxygen, cause increased cellular damage in a condition known as oxidative stress (OS). Reactive oxygen species (ROS) generation and the body's antioxidant capacity are out of balance under oxidative stress (Agarwal et al. 2014; Luceri et al. 2018). Oxidative stress is closely related to aging and male infertility, causing decreased semen volume, abnormal morphology, decreased sperm viability and motility (Lucio et al. 2013; Morielli & O'Flaherty 2015; Fatima 2018; Alahmar 2019; Kurkowska et al. 2020).

In the study carriedout by (Mehrotra et al. 2013), showing that an increased of ROS production was correlated to the decreased spermatozoa motility, due to lipid peroxidation of the spermatozoa membrane. It may then affect the fluidity and mobility of the spermatozoa axoneme, inactivation or reduction of enzymes on the membrane, fragmentation of structured DNA and cell death (Dias et al. 2020). The amount of sperm DNA fragmentation and sperm vitality are correlated each other. High sperm DNA fragmentation is linked to the decreased of sperm viability, whereas low sperm DNA fragmentation is linked to the increased of sperm vitality. Male fertility was decreased by high DNA fragmentation levels and low viability; DNA fragmentation levels >30% were an indicative of subfertility (Samplaski et al. 2015).

Sperm motility is a reflection of the normal development and maturity of spermatozoa in the epididymis, while viability is the vitality of spermatozoa (Carson & Kallen 2021). Sperm viability examination can be used as an indicator of the integrity of the spermatozoa membrane structure (Solihati et al. 2016), while motility is used as an indicator of the strength of the spermatozoa plasma membrane (Zhou et al. 2015).

One of the treatments for oxidative stress is the use of antioxidant compounds (Sumit et al. 2013; Avula et al. 2014; Webb et al. 2019), antioxidants that come from outside the body or from food and drink are called exogenous antioxidants (Parwata 2015). One of them is the Moringa plant (Razis et al. 2014). The results of the analysis of moringa leaf content have antioxidant capacity, including phenolics, flavonoids, tannins, ascorbic acid, alkaloids and saponins (Widiastini et al. 2021). The use of moringa leaf extract can prevent free radical damage to sperm and preserve the spermatozoa membrane, thereby increasing the viability and motility of spermatozoa (Wahjuningsih et al. 2019; Carrera-Chávez et al. 2020).

The purpose of this study was to determine the antioxidant mechanism of moringa leaf ethanol extract (EEMO) on sperm motility and viability in mature Wistar rats.

MATERIALS AND METHODS

Ethical clearance

This study was carriedout under an animal ethic clearance No 094/EA/KEPK-BUB-2023 approved by KEPK STIKES Bina Usada Bali.

Research design

This study used an experimental design, namely a randomized post-test only control group design (Pocock 2013). This study used 36 heads of older Wistar strain male rats (*Rattus norvegicus*), aged 18-19 months old of 200-250 grams body weight. The animals were then divided into two groups, including the control group given 0.5 ml CMC (Carboxy Methyl Cellulose) 0.5%, and the treatment group was given 0.5 mL of moringa leaf ethanol extract 50 mg/kg BW + CMC 0.5%, The administration of both control and treatment solutions was performed via oral gavage (sonde) once daily for 30 consecutive days

Preparation of Ethanol Extract Moringa Oleifera (EEMO)

Preparation of moringa leaf extract was followed a method described by (Cahyani and Sukadana 2017; Putra et al. 2017; Wasonowati et al. 2019). Approximately 50 grams of dried Moringa leaves were used to produce EEMO. The leaves were crushed in a blender, mixed with 96% ethanol solvent, placed in a jar, sealed, and dried for two days under of the sunlight. The macerate was obtained by filtering this combination. The same process was used to macerate the filtrates in 96% ethanol until a clear solution was obtained. The maceration process was performed three times. The macerate was evaporated by using a vacuum rotating evaporator at 40°C. Moringa leaf extract was then subjected to phytochemical screening to detect plant compounds based on their classification.

Research procedure

The animals were acclimatized for 7 days with 12 hours of light and 12 hours of darkness, at a temperature of 25±0.5°C and a humidity of 50–60%. The treatment

group was orally administered EEMO at a dose of 50 mg/kg BW combined with 0.5% CMC, in a total volume of 0.5 mL per day. The control group was administered 0.5% CMC (0.5 mL/day) orally. The administration was conducted between 08.00–09.00 WITA (Eastern Indonesian Time) for 30 days. At day-30 of the termination period, the animals were sacrificed by disclocation method of the cervical region of rats. It was then followed by an open orchidectomy. The testes were separated from the cauda epididymis and collected in a media container.

Spermatozoa motility examination

The examination is carried out immediately when the spermatozoa are taken from the caudal epididymis, by dripping a drop of spermatozoa on the glass object. Each droplets were approximately to be the same size for each examination. Observations were made under a microscope with a magnification of 400 times. The analysis was defined as (1) Progressive motility (PR): Spermatozoa move freely, either straight or in large circles, at any speed. (2) Non-progressive motility (NP): all types of spermatozoa that do not have progressive criteria, such as swimming in small circles, tail / flagellum that is difficult to move the head or only the tail moves. And (3) Immotility (IM) : does not move at all (World Health Organization (WHO) 2021)

Spermatozoa viability examination

The examination was carried out by making thin section preparations using eosin-negrosin staining which was then observed with an Olympus microscope at 1000X magnification. Live spermatozoa will not be stained by the dye, while dead spermatozoa will be stained.

Data analysis

The difference between the treatment and control groups was determined using the Statistical Program for Social Science (SPSS) 23 (IBM Corporation, Armonk, NY, USA) Independent Sample T Test and Mann Whitney test at the level of significance P= 0.05.

RESULTS AND DISCUSSION

Table 1 shows result of phytochemical screening of EEMO of moringa leaf ethanol extract in the South Denpasar area of Bali contains Antioxidant Capacity, including Phenol, Flavonoids, Tannin, Vitamin C, Saponins, and Alkaloids (Widiastini et al. 2021). Table 2 and Figure 1a show the treatment group that showed an average progressive motility of spermatozoa of 66.16±3.653% (mean±SD), while the control group showed an average of 45.55±6.073%. According to the Mann–Whitney test analysis, the results obtained a p-value of 0.000 ($P<0.05$), indicating a significant difference in the percentage of progressive motility between the group given Moringa leaf ethanol extract and the control group without Moringa extract. Therefore, it can be concluded that the ethanol extract of Moringa leaves has a significant effect on increasing the percentage of spermatozoa with progressive motility.

Table 2 and Figure 1b show that the treatment group has lower number of Non-Progressive Motility with an average of 21.54±3.393%. The independent sample T Test analysis was carried out and found that a P value was 0.000 ($P<0.05$), and shows that there is a significant difference in number of Non-Progressive Motility between the group given moringa leaf ethanol extract and the control group without moringa extract. This was then lead to a significant effects of moringa extract in the number of Non-Progressive Motility.

Table 1. Phytochemical screening of Ethanol Extract Moringa Oleifera (EEMO**)**

No	Phytochemical Test	Unit	Result
1	Antioxidant Capacity	mg/L GAEAC	1812.95
2	IC_{50}	Ppm	353.09
3	*Fenol*	mg/100 g	1556.52
4	*Flavonoid*	mg/100 g	23546.12
5	*Tannin*	mg/100 g	1300.34
6	Vit C	mg/100ml	23798.077
7	*Saponin*	-	Positif (+)
8	*Alkaloid*	-	Positif (+)

Table 2. Motility and viability of spermatozoa in older male Wistar rats (*Rattus norvegicus*) after Ethanol Extract Moringa Oleifera (EEMO) administration

Parameters	Treatment	Control	P-value
Progressive Motility (%)	66.16±3.653	45.55±6.073	0.000
Non-Progressive Motility(%)	21.54±3.393	29.02±3.380	0.000
Immotility (%)	12.30±1.184	25.44±5.829	0.000
Viability Spematozoa (%)	88.27±1.019	73.41±5.842	0.000

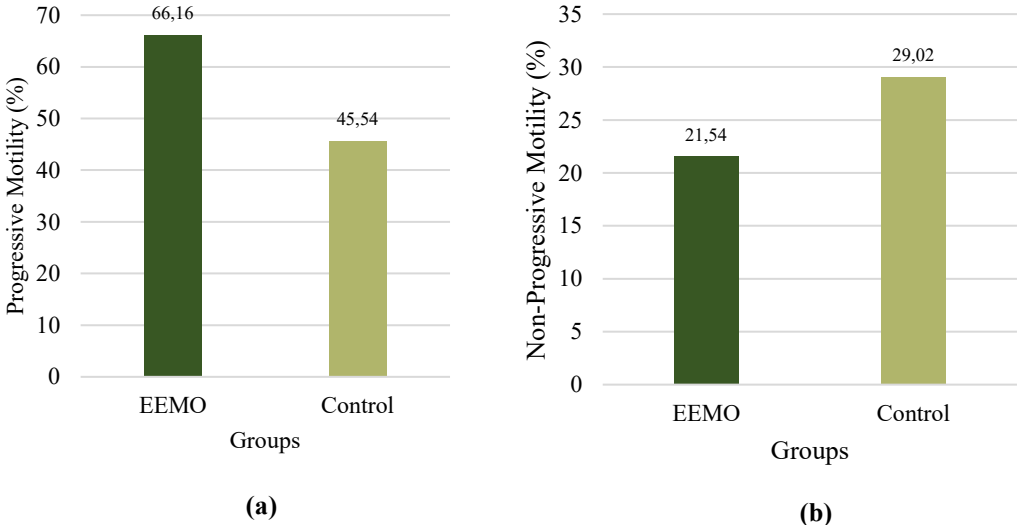

Figure 1. Progressive Motility (a) and non progressive motility (b) in older male Wistar rat (*Rattus norvegicus*) (n=18) (mean). EEMO= Ethanol Extract Moringa Oleifera

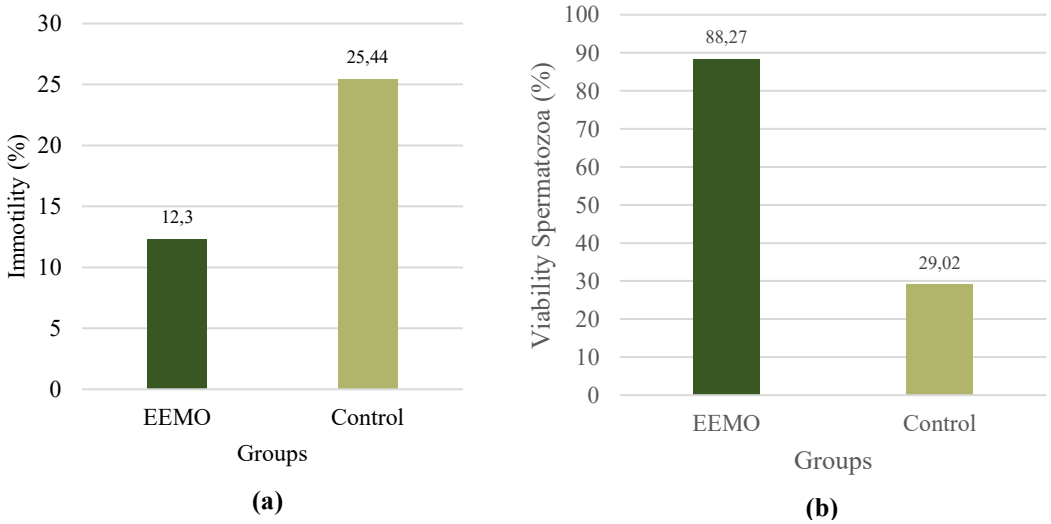

Figure 2. Immotility (a) and viability spermatozoa (b) in older male Wistar rat (*Rattus norvegicus*) (n=18) (mean). EEMO= Ethanol Extract Moringa Oleifera

Table 2 and Figure 2a shows that the treatment group has lower number of Immotility Spermatozoa with an average of 12.30±1.184% compares to control group. The independent sample T Test analysis shows that the P value was 0.000 (P<0.05), meaning that there is a significant difference in the number of Immotility

Spermatozoa between the group given moringa leaf ethanol extract and the control group without moringa extract. It appears that moringa leaf extract may have significant effect in the number of Immotility Spermatozoa.

Table 2 and Figure 2b show that the treatment group has better spermatozoa viability with an average of 88.27±1.019% compare to control group. Independent sample T Test analysis was carried out and the results obtained a p value of 0.000 (P<0.05), which means there is a significant difference in spermatozoa viability between the group given moringa leaf ethanol extract and the control group that is not given, so it can be said that moringa leaf extract can have a significant effect on spermatozoa viability.

Spermatozoa motility

The results showed that the administration of ethanol extract of moringa leaf (*Moringa oleifera*) at 50 mg/kg BW for 30 days to rats (*Ratus norvegicus*) of 18-19 months old increased significantly the progressive motility, decreased the non-progressive motility and the immotility in the treatment group compared to the control group with a value of P<0.05. The mean progressive motility in the treatment group was 66.16±3.653%, and the control group was 45.55±6.073%. The mean non progressive motility in the treatment group was 21.54±3.393% while the control group was 29.02±3.380%%, and the mean immotility of spermatozoa in the treatment group was 12.30±1.184% and the control group was 25.44±5.829%.

The antioxidants in moringa leaves (*Moringa oleifera*), including phenolic compounds, flavonoids, tannins, vitamin C, saponins, and alkaloids, neutralize free radicals, preventing oxidative damage to biomolecules and providing significant protection against oxidative damage. They have the ability to protect lipoproteins from peroxyl radicals, thereby increasing the progressive motility of spermatozoa. This is consistent with the findings of (Nayak et al. 2015), who showed that administering Moringa oleifera can increase sperm density and motility, reduce DNA damage, and significantly raise the levels of superoxide dismutase and catalase, along with a decrease in lipid peroxidation in testicular tissues. A study carried out by (Diao et al. 2019), showed that supplementation with Quercetin (Flavonoid) can protect Spermatozoa from sperm damage mediated by H_2O_2 (hydrogen peroxide) and increase spermatozoa motility. Vitamin C, as a key antioxidant, has been shown to play an important role in improving sperm quality. Bebas et al., (2015), demonstrated that vitamin C administration could maintain spermatozoa viability and motility, while the findings of Dimitriadis et al., (2023), further emphasized its role in enhancing sperm motility. Thus, vitamin C holds significant potential in improving male fertility parameters, particularly sperm motility. However, it should be noted that an excessive use of antioxidants may lead to pro-oxidants called antioxidant paradoxes, which can reduce endogenous oxidants that are important for the induction of physiological pathways, thereby inhibiting sperm capacitation, hyperactivation and acrosomal reactions of spermatozoa that can increase various pathological conditions such as aging and male infertility (Ali et al. 2020).

Spermatozoa viability

Viability of spermatozoa is the ability to live spermatozoa every time they are ejaculated. The normal percentage of spermatozoa viability is >58% regarded to be alive when spermatozoa do not absorb color, otherwise were died when spermatozoa absorb color (World Health Organization (WHO) 2021). Aging is also linked to decreased daily sperm production, total sperm count, and sperm viability as well as decreased semen volume brought on by diminished accessory gland activity (Gunes et al. 2016; Folorunsho et al. 2023).

The results showed that the administration of 50 mg/kg BW of moringa leaf ethanol extract (*Moringa oleifera*) for 30 days to older rats (*Ratus norvegicus*) of 18–19 months old may significantly increase spermatozoa viability when compared to the control group, with a value of P<0.05. The mean viability of spermatozoa in the treatment group was 87.70±1.183%, the control group was 74.57±5.828%, this is in line with the study of (El et al. 2022), that the administration of moringa extract, can improve sperm quality, and (Carrera-chávez et al. 2020), the supplementation of *M. oleifera* extract could increase antioxidant activity, sperm membrane integrity, viability, and progressive motility after thawing. Antioxidants contained in moringa leaves can react to and inactivate ROS, it is therefore the administration of moringa leaf ethanol extract may significantly increase the number and morphological quality of spermatozoa of older rats (Widiastini et al. 2022).

CONCLUSION

Ethanol extract *Moringa oleifera* (EEMO) has a significant effect as an antioxidant in improving male fertility. The results of phytochemical compounds from EEMO were effectively increasing the progressive motility of spermatozoa and spermatozoa viability, and reducing non-progressive motility and immotility of spermatozoa in older Wistar rats. To fully understand the impact of EEMO on the pathophysiology of male infertility brought on by aging, further study is required, particularly in regards to the role of EEMO in a number of molecular markers in testicular tissue.

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