

Ethanol Extract of Indian jujube (*Ziziphus mauritiana*) Leaves Decrease Motility without Affecting Sperm Morphology in Mice (*Mus musculus* L.)

(EKSTRAK ETANOL DAUN BIDARA (*ZIZIPHUS*
MAURITIANA) DAPAT MENEKAN MOTILITAS TANPA
MENGUBAH MORFOLOGI SPERMA MENCIT (*MUS MUSCULUS* L.))

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ABSTRACT

Male infertility is one of the major causes of infertility in couples and is closely associated with a decline in spermatozoa quality due to oxidative stress. Indian jujube (*Ziziphus mauritiana*) leaves are known to possess antioxidant potential due to the presence of bioactive compounds; however, their effects on the male reproductive system have not yet been comprehensively understood. This study was aimed to evaluate the effect of ethanol extract of Indian jujube leaves on sperm quality parameters in healthy adult mice (*Mus musculus* L.). The study employed a completely randomized design consisting of three treatment groups with three mice in each group (n = 3): a control group, and treatment groups receiving Indian jujube leaf ethanol extract at doses of 200 mg/kg body weight and 400 mg/kg body weight for 14 days. The observed parameters included body weight, sperm morphology and sperm motility. The obtain data were analyzed using a one-way Analysis of variance at a significance level of 5%. The results showed that administration of Indian jujube leaves ethanol extract had no significant effect on body weight and sperm morphology. However, the extract significantly decreased normal sperm motility and increased abnormal sperm motility compared with the control group. These findings indicate that ethanol extract of Indian jujuba leaves reduces sperm motility in mice in a dose-dependent manner, without significantly affecting body weight and sperm morphology.

Keywords: male infertility; Indian jujube (*Ziziphus mauritiana*); sperm quality; antioxidant

ABSTRAK

Infertilitas pria merupakan salah satu penyebab utama kemandulan pada pasangan, yang berkaitan erat dengan penurunan kualitas spermatozoa akibat stres oksidatif. Daun bidara (*Ziziphus mauritiana*) diketahui memiliki potensi antioksidan karena kandungan senyawa bioaktif di dalamnya. Namun, pengaruhnya terhadap sistem reproduksi jantan belum sepenuhnya dipahami secara komprehensif. Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian ekstrak etanol daun bidara terhadap parameter kualitas spermatozoa pada mencit (*Mus musculus* L.) dewasa yang sehat. Penelitian ini menggunakan rancangan acak lengkap yang terdiri atas tiga kelompok perlakuan dengan tiga ekor mencit pada setiap kelompok ($n = 3$), yaitu kontrol, perlakuan ekstrak etanol daun bidara dosis 200 mg/kg berat badan, dan 400 mg/kg berat badan selama 14 hari. Parameter yang diamati meliputi bobot badan, morfologi spermatozoa dan motilitas spermatozoa. Data dianalisis menggunakan uji sidik ragam satu arah pada tingkat signifikansi 5%. Hasil penelitian menunjukkan bahwa pemberian ekstrak etanol daun bidara tidak memberikan pengaruh yang nyata terhadap bobot badan dan morfologi spermatozoa. Namun, ekstrak tersebut secara nyata menurunkan motilitas normal spermatozoa dan meningkatkan motilitas abnormal dibandingkan dengan kelompok kontrol. Temuan ini menunjukkan bahwa ekstrak etanol daun bidara menurunkan motilitas sperma pada mencit dan bergantung pada dosis (*dose-dependent*) yang diberikan, tanpa memengaruhi bobot badan dan morfologi spermatozoa.

Kata-kata kunci: infertilitas pria; daun bidara (*Ziziphus mauritiana*); kualitas sperma; antioksidan

INTRODUCTION

Infertility in human is a global health problem, with nearly 50% of cases of infertility being caused by male factors (Talin *et al.*, 2024; Simanjuntak, 2020). One of the causes of decreased sperm quality and quantity is oxidative stress, which is the production of reactive oxygen species (ROS) and an imbalance in the body's antioxidant system (Wang *et al.*, 2025) which damages the cell membrane, DNA and mitochondria of sperm, resulting in decreased motility and fertilization ability (Indriyani *et al.*, 2021; Barbagallo *et al.*, 2020).

Natural antioxidants are widely utilized and developed as an approach to suppress the impact of oxidative stress on the male reproductive system (Marcucci *et al.*, 2023). Indian jujube (*Ziziphus mauritiana*) leaves are known to have antioxidant activity by containing flavonoids, saponins, alkaloids, and phenolic compounds (Lestari *et al.*, 2022; Haeria *et al.*, 2016) and are known to capture free radicals and neutralize ROS

(Dhuha *et al.*, 2019). However, several compounds such as saponins and alkaloids, have also been reported to potentially affect reproductive functions depending on the dosage and duration of exposure. (Shai *et al.*, 2022; Nurlily *et al.*, 2022; Raad *et al.*, 2024).

Despite the known antioxidant properties of Indian jujube, limited studies have investigated its direct effects on sperm functional parameters *in vivo*, particularly sperm motility and morphology (Rudini *et al.*, 2021). Sperm motility is a crucial parameter in male fertility because it determines the ability of spermatozoa to reach and fertilize the ovum. Therefore, this study was aimed to evaluate the effect of ethanol extract of Indian jujube leaves on sperm motility and morphology in mice (*M. musculus* L.). It was hypothesized that administration of Indian jujube ethanol extract may influence sperm motility and morphology in mice. This study provides new information regarding the *in vivo* effects of Indian jujube ethanol extract on sperm functional para-

meters, particularly motility, which has not been widely reported in previous studies.

RESEARCH METHODS

The study was conducted experimentally in a laboratory using male mice of BALB/c strain (*M. musculus* L.) aged 2–3 months with a body weight of 25–35 g, obtained from UD Wistar Experimental Animal Breeding Laboratory, Yogyakarta. The mice were housed in cages containing two mice per cage, separated by individual partitions, under controlled environmental conditions at a temperature of 22–25°C with a 12 h light and 12 h dark photoperiod, and were provided pellet feed and drinking water *ad libitum*. The study used a quantitative approach to evaluate the effect of administering ethanol extract of Indian jujube (*Ziziphus mauritiana*) leaves on spermatozoa quality, including sperm motility and morphology. A Completely Randomized Design (CRD) was used as the research design, with all experimental units receiving treatment and the research environmental conditions being relatively homogeneous with equal opportunities (Nurlely *et al.*, 2022).

This study used three treatment groups with three mice per group ($n = 3$), thus nine mice are required. Each mouse was considered one experimental unit. The sample size was determined based on the minimum requirement for experimental animal studies while considering ethical principles for the use of laboratory animals. The first group, a control group, was administered distilled water, the second group, a treatment group, was administered ethanol extract of Indian jujube leaves prepared using a 70% ethanol extraction method at a dose of 200 mg/kg body weight, while the third group was administered the extract at a dose of 400 mg/kg body weight. The treatments were administered orally via a gastric tube once daily at 07:30 a.m. for 14 days. The treatment dosage was determined by considering the safe dose and its biological relevance to the reproductive system of mice

(Indriyani *et al.*, 2021). All experimental procedures have been analyzed and previously approved by the Health Ethics Commission of Dr. Moewardi Hospital, Surakarta, Indonesia with approval number No. 1.320 / VI / HREC / 2025.

Research Instruments

This study measured mouse body weight, spermatozoa morphology, and spermatozoa motility. Sperm motility was observed using a microscope equipped with an Optilab digital camera system at 40× magnification. The Optilab device was connected to a computer to capture and display microscopic images, allowing clearer observation and documentation of sperm movement. The motility of spermatozoa was then classified into normal (motile) and non-motile spermatozoa (Chakraborty and Saha, 2022). The morphology of spermatozoa was analyzed through smear preparations stained with Giemsa solution, by calculating the percentage of normal and abnormal spermatozoa from 100 observed cells (Shufyani and Dominica, 2022).

Extract Preparation.

A total of 500 g of dried Indian jujube leaves were macerated using 70% ethanol for 2 x 24 hours. The maceration results were filtered to obtain a filtrate, then using a rotary evaporator, the filtrate was evaporated to obtain a thick extract. The extract was stored in a closed container until used in the treatment (Nurlely *et al.*, 2022).

Preparation of Extract Suspension Solution.

The extract was weighed according to the specified dose and dissolved in a 1% Sodium Carboxymethyl Cellulose (Na CMC) suspension until homogeneous. This solution was aimed to ensure uniform dosing for each test animal (Nurlely *et al.*, 2022).

Experimental Treatment Design.

The mice were then randomly divided into three treatment groups: a control group given distilled water, a treatment of

ethanol extract of Indian jujube leaves group given a dose of 200 mg/kg body weight, and a treatment group given a dose of 400 mg/kg body weight. The treatment was administered orally using a gastric tube once daily at 07:30 a.m. for 14 days. The mice were then weighed daily to monitor their physiological condition during treatment (Indriyani *et al.*, 2021).

Sampling.

At the end of the treatment period, the mice were euthanized by cervical dislocation following ethical guidelines, a method that induces rapid death and minimizes pain and distress, after which the reproductive organs were removed for analysis. The epididymis was removed and soaked in 0.9% physiological NaCl solution to obtain a spermatozoa suspension. This suspension was observed under a microscope equipped with an Optilab digital camera at 40× magnification. Sperm motility and morphology were evaluated by counting spermatozoa in five different microscopic fields, with a total of at least 100 spermatozoa observed for each sample. Sperm motility was classified into motile and non-motile spermatozoa, while sperm morphology was categorized as normal or abnormal according to morphological characteristics (Indriyani *et al.*, 2021).

Data Analysis.

All data obtained were systematically analyzed statistically using the Analysis of Variance test at a 5% significance level. If the analysis results showed significant differences, a post-hoc Least Significant Difference (LSD) test was conducted to determine significant differences between treatment groups (Indriyani *et al.*, 2021).

RESULTS AND DISCUSSION

Mice body weight.

The average body weight of mice between the control group (K) and treatment

groups (P1 and P2) is presented in Table 1. Based on Table 1, there was an increase in the average body weight of mice during the 14 days of observation in the control group (K) and treatment 1 (P1), while in treatment 2 (P2) it was relatively stable. Overall, the average body weight of mice during the study was 32.77 ± 1.47 g (K); 33.55 ± 1.28 g (P1) and 33.88 ± 1.25 g (P2). Repeated one-way analysis of variance showed that the body weight of mice between treatments was not significantly different ($F = 2.899$; $p = 0.121$), as presented in Table 2.

Administration of Indian jujube leaf ethanol extract at doses of 200 mg/kg body weight and 400 mg/kg body weight did not show a significant effect on the body weight of mice ($p > 0.05$), which indicates the absence of systemic toxicity effects. This result is in line with the findings of Mostafa *et al.* (2025) who reported that moderate intake of polyphenol-rich plant extracts did not significantly alter body weight. Indian jujube leaves are known to contain several bioactive compounds, including polyphenols, flavonoids, and saponins, which may influence metabolic processes depending on the administered dose and duration of exposure. In the present study, administration of ethanol extract of Indian jujube leaves at doses of 200 and 400 mg/kg body weight for 14 days did not result in significant changes in body weight. This finding may indicate that the administered doses were still within a physiological tolerance range and were not sufficient to induce metabolic alterations (Krisna *et al.*, 2022). Deng *et al.* (2023) reported that the metabolic effects of saponins tend to appear only after prolonged administration, approximately 30 days, suggesting that the relatively short treatment duration in this study may explain the absence of significant body weight changes.

Morphology of Mice Spermatozoa

The following are the results of a study on the effects of administering ethanol extract of Indian jujube leaves on the morphology of mouse spermatozoa.

Table 1. Average body weight of mice during 14 days of treatment of ethanol extract of Indian jujube leaves

Treatment Group	Average Mouse Weight (g) by Time (Weeks)			Mean $\pm$ SD
	0	1	2	
K (no treatment)	33.33	35.33	35.66	32.77 $\pm$ 1.47
P1 (200 mg/kg)	34.33	34.66	35.33	33.55 $\pm$ 1.28
P2 (400 mg/kg)	30.66	30.66	30.66	33.88 $\pm$ 1.25

Description: K= given 0 mg/kg bw of ethanol extract of Indian jujube leaves; P1= given 200 mg/kg bw; P2= given 400 mg/kg bw

Table 2 One-way repeated measures of Analysis of variance test results

Sousces		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	5.852	2	2.926	2.899	.084
	Greenhouse-Geisser	5.852	1.115	5.247	2.899	.121
	Huynh-Feldt	5.852	1.168	5.012	2.899	.119
	Lower-bound	5.852	1.000	5.852	2.899	.127
Error (W)	Sphericity Assumed	16.148	16	1.009		
	Greenhouse-Geisser	16.148	8.923	1.810		
	Huynh-Feldt	16.148	9.340	1.729		
	Lower-bound	16.148	8.000	2.019		

Description: W (Label for within-subject factor, e.g. "Time"; F (Ratio of between group variance to within-group variance); df (degrees of freedom); sig (significance).

Table 3. The average normal and abnormal morphology of mouse spermatozoa in the control group and treatment group along with the results of statistical analysis

Treatment	Normal Morphology of Mouse Sperm (%)	Abnormal Morphology of Mouse Sperm (%)
K (No Treatment)	85.66 $\pm$ 1.76	14.33 $\pm$ 1.76
P1 (200 mg/kg body weight)	76.66 $\pm$ 5.23	23.33 $\pm$ 5.23
P2 (400 mg/kg body weight)	79.00 $\pm$ 6.55	21.00 $\pm$ 6.55

Description: K= no treatment; P1= treatment one; P2=treatment two.

Table 4. Average normal and abnormal motility of mouse spermatozoa in the control group and treatment group along with the results of statistical analysis

Treatment	Normal Motility of Mouse Sperm (%)	Abnormal Motility of Mouse Sperm (%)
K (No Treatment)	69.33 $\pm$ 5.36 ^a	30.66 $\pm$ 5.36 ^a
P1 (200 mg/kg body weight)	29.00 $\pm$ 5.13 ^b	71.00 $\pm$ 5.13 ^b
P2 (400 mg/kg body weight)	14.00 $\pm$ 1.73 ^b	86.00 $\pm$ 1.73 ^b

Description: Numbers followed by different letters indicate a significant difference based on the LSD Post-Hoc Test at the 5% level ($P \leq 0.05$).

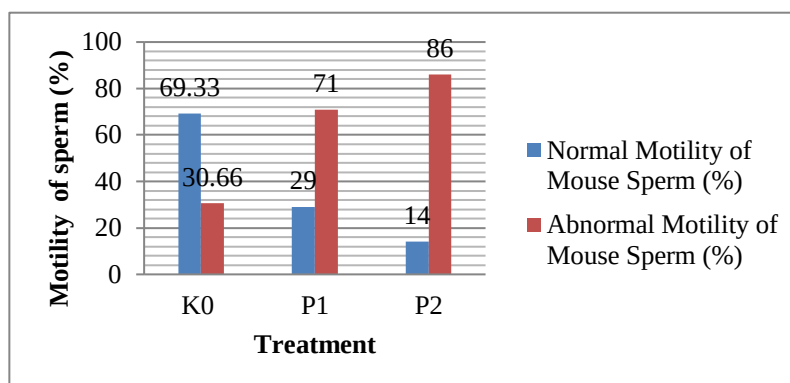


Figure 1. Bar chart of normal and abnormal morphology of mouse spermatozoa in the control group and treatment group (K = No Treatment; P1 = Treatment 1; P2 = Treatment 2)

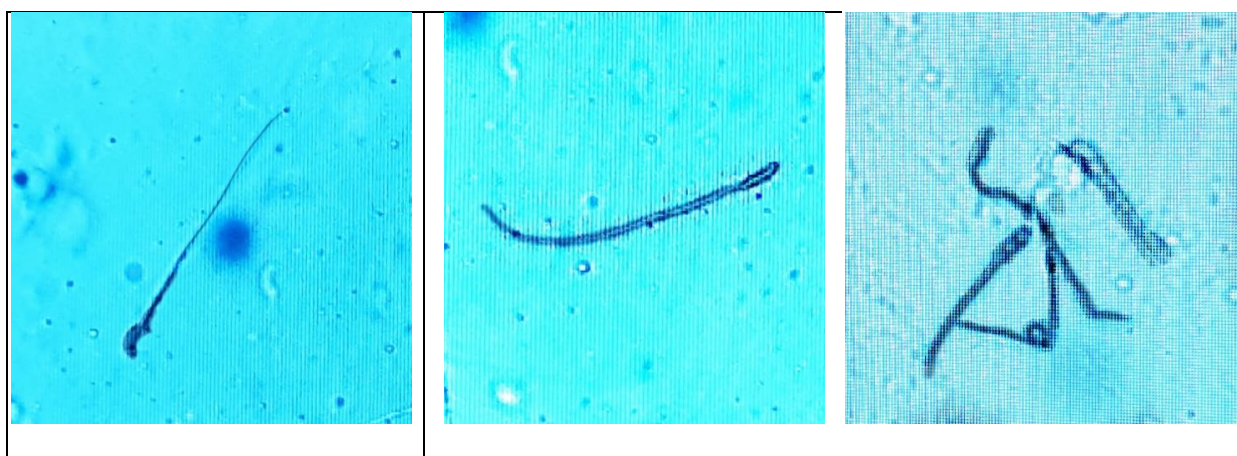


Figure 2. Normal sperm (left), abnormal sperm (shrinking head (centre & right), agglutinated sperm (bottom left), magnification 40x.

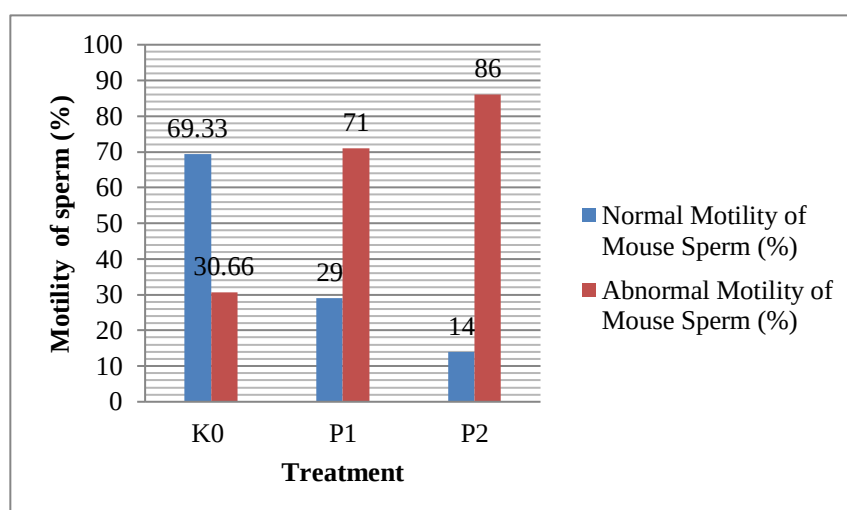


Figure 3. Bar chart of normal and abnormal motility of mouse spermatozoa in the control group and treatment group (K = No Treatment; P1 = Treatment 1; P2 = Treatment 2)

The average morphology of normal and abnormal mouse spermatozoa between the control group (K) and the treatment groups (P1 and P2) tested using ANOVA is presented in Table 3.

Based on Table 3, the control group had the highest percentage of normal spermatozoa morphology ($85.66 \pm 1.76\%$), while in treatment groups P1 and P2 it was lower, $76.66 \pm 5.23\%$ and $79.00 \pm 6.55\%$, respectively. In contrast, groups P1 ($23.33 \pm 5.23\%$) and P2 ($21.00 \pm 6.55\%$) had a higher percentage of abnormal spermatozoa than the control ($14.33 \pm 1.76\%$).

The assumption of normality of the percentage data of spermatozoa morphology was met and homogeneity ($P > 0.05$), so the analysis was continued with one-way ANOVA. The ANOVA results showed that there was no significant effect of administering ethanol extract of Indian jujube leaves at doses of 200 and 400 mg/kg body weight on the morphology of mouse spermatozoa ($P > 0.05$), so further testing was not carried out; the normal and abnormal morphological images are shown in Figure 2.

The results of statistical tests showed that the morphological parameters of spermatozoa among treatment groups were not significantly different ($p > 0.05$). However, a descriptive decrease in morphology from 85% to 76% was observed in the treatment group. Although this change was not statistically significant, the trend may indicate a biologically noticeable variation that could be influenced by limited statistical power. Previous studies have suggested that antioxidant compounds in Indian jujube leaves, particularly flavonoids, may contribute to maintaining sperm structural integrity (Tvrđá *et al.*, 2020). This finding is in line with Mishra *et al.* (2024); Mostafa *et al.* (2025), which indicated that flavonoids and polyphenols may help preserve the morphological integrity of spermatozoa. However, since oxidative stress markers were not measured in the present study, the protective mechanism cannot be confirmed. Therefore, further studies evaluating oxidative stress biomarkers are necessary to clarify the potential role

of antioxidant compounds in maintaining sperm morphology.

Motility of Mouse Spermatozoa.

The following are the results of a study on the effects of administering ethanol extract of Indian jujube leaves on the motility of mouse spermatozoa. Data from observations of mouse spermatozoa motility are presented in the Table 4. The average normal motility of mouse spermatozoa between the control group (K) and the treatment groups (P1 and P2) tested using ANOVA and Post-Hoc LSD tests are presented in Table 4.

Sperm motility with the administration of Indian jujube leaves ethanol extract experienced a significant decrease, with the highest normal motility in the control group ($69.33 \pm 5.36\%$) and decreased in P1 ($29.00 \pm 5.13\%$) and P2 ($14.00 \pm 1.73\%$). In contrast, abnormal motility increased from $30.66 \pm 5.36\%$ in the control group to $71.00 \pm 5.13\%$ in P1 and $86.00 \pm 1.73\%$ in P2 in a dose-dependent manner.

The assumptions of normality and homogeneity of spermatozoa motility data were met and one-way ANOVA analysis revealed that ethanol extract of Indian jujube leaves had a significant effect on normal and abnormal motility ($p < 0.05$). Post-hoc LSD showed that there was a significant difference between the control and treatment P1 and P2 groups, while P1 and P2 did not differ significantly ($p = 0.053$).

A significant decrease in sperm motility was observed in the treatment groups, declining from 69.33% in the control group to 29.00% in P1 and further to 14.00% in P2, indicating a pronounced dose-dependent effect. Such a substantial reduction suggests severe impairment of sperm motility, which is closely associated with mitochondrial function as the primary source of ATP required for flagellar movement. Previous studies have reported that mitochondrial dysfunction can reduce sperm motility without necessarily causing morphological alterations (Raad *et al.*, 2024; Barbagallo *et al.*, 2020). However, the magnitude of the decline observed in the present study may

also indicate potential antifertility effects or mitochondrial toxicity associated with the extract. Bioactive compounds present in Indian jujube leaves extract, particularly alkaloids and saponins, have been reported to interfere with cellular bioenergetic processes through interactions with ATPase activity and disruption of ion gradients, which may contribute to decreased sperm motility (Patalas-krawczyk *et al.*, 2021). Therefore, while the results highlight impaired sperm motility, they also raise the possibility of reproductive toxicity at the tested concentrations. Further studies evaluating mitochondrial activity, oxidative stress markers, and fertility outcomes are required to better clarify the underlying mechanisms and potential reproductive implications.

CONCLUSION

The administration of ethanol extract of the leaves of Indian jujube (*Z. mauritiana*) over a duration of 14 days did not impact on body mass and the morphology of spermatozoa; however, it was reduced motility in a dose-dependent manner. These observations imply a potential impairment of the functional activity of sperm rather than any structural modifications.

SUGGESTION

Further studies should evaluate sperm concentration, viability, and DNA integrity to provide a more comprehensive assessment of reproductive function. Assessment of mitochondrial activity, ATP production, and oxidative stress biomarkers is also necessary to confirm the proposed mechanism of motility impairment. In addition, longer treatment durations and histopathological analysis of testicular and epididymal tissues are recommended to determine potential reproductive toxicity or antifertility effects of the extract.

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