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Activity of Zuriat Fruit Extract (*Hyphaene thebaica*) on The DNA Fragmentation and Spermatozoa Quality of Male Mice (*Mus musculus*) Given Balinese Arak

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Abstract: Alcohol in the body is metabolized into acetaldehyde with the assistance of the enzyme alcohol dehydrogenase (ADH). Acetaldehyde, the metabolic byproduct of alcohol, plays a role in the formation of Reactive Oxygen Species (ROS). The effects of alcohol can lead to damage and death of spermatogenic cells caused by acetaldehyde, which is produced through the breakdown of alcohol by liver cells. Increased ROS levels generated by free radicals in sperm producing tissues may cause damage to the sperm membrane, thereby disrupting its stability and function. High concentrations of ROS may also exert toxic effects on sperm quality and function. This study aimed to examine the activity of Zuriat fruit (*Hyphaene thebaica*) extract on sperm quality and DNA fragmentation in male mice (*Mus musculus*) administered Balinese Arak. This research employed an experimental study design using a post-test only control group design. The results show that Zuriat fruit (*Hyphaene thebaica*) extract at doses of 1 g/kg body weight and 2 g/kg body weight significantly influenced sperm count, motility, viability, morphology, and DNA fragmentation (p < 0,05).

Keywords: Alcohol; Male mice (*Mus musculus L.*); Sperm quality; Zuriat fruit extract (*Hyphaene thebaica*)

Introduction

Indonesia, located within the tropical belt, is renowned for its rich biodiversity and abundant natural resources, which play a vital role in sustaining various forms of life. Indonesia possesses a rich diversity of traditional foods and beverages, with each region maintaining distinct culinary practices that have been preserved as part of the nation's cultural heritage. Traditional Balinese beverages that have been preserved to the present day include Balinese Arak, tuak, and brem. Balinese Arak is produced using conventional methods involving fermentation followed by distillation to obtain ethanol. The primary raw materials utilized in the production process include lontar (*Borassus flabellifer*) fruit, aren (*Arenga pinnata*) fruit, coconut water, white rice (*Oryza sativa*), and red rice that have different amounts of alcohol percentage (Pranatayana & Pratiwi Arcana, 2021)

Balinese Arak is a traditional alcoholic beverage that holds cultural significance and is predominantly used in various traditional rituals and religious ceremonies (Zayani et al., 2023) Balinese Arak is classified as a category C alcoholic beverage due to its alcohol concentration greater than 25% (Dewi Ratih & Prabawati, 2022). The effects of alcohol consumption may result in the degeneration and apoptosis of spermatogenic cells, caused by the presence of acetaldehyde, a toxic byproduct generated through hepatic metabolism of ethanol. An increase in reactive species (ROS) induced by free radicals in tissues responsible for spermatozoa production can lead to damage of sperm membrane, altering its stability and function. Excessive levels of ROS may also exert toxic effects on the equality and functionality of spermatozoa(J. M. Wang et al., 2022). Alcohol consumption can interfere with testosterone production and the process of sperm maturation. In chronic alcohol

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users, there is a significant decline in sperm count, motility, and morphology (Wael et al., 2023).

A study conducted by Antari et al. (2017), entitled *Provision of Balinese Arak Reduces Spermatozoa Quality of White Rats (Rattus norvegicus),* found that administration of Balinese Arak containing 40% alcohol significantly reduces the quality of spermatozoa in white rats (*Rattus norvegicus*), as evidenced by decreased motility, morphology, viability, membrane integrity, and overall spermatozoa function.

Indonesia possesses a wide variety of plant species that can be utilized as herbal medicine. As a tropical country, Indonesia holds significant potential for the cultivation of plant species originating from other Asian regions, including the Zuriat fruit (*Hyphaene thebaica*). Zuriat fruit originates from Africa, particularly Egypt. This fruit contains a diverse range of bioactive compounds, including flavonoids and polyphenols, as well as macronutrients such as proteins, carbohydrates, and fats. Additionally, it is rich in amino acids and essential minerals, including magnesium (Mg), iron (Fe), sodium (Na), potassium (K), sulfur (S), and copper (Cu)(Saber et al., 2023)

Zuriat fruit is considered as a beneficial herbal remedy that may support fertility programs in women. Several studies investigating the effects of Doum fruit on reproductive health have demonstrated positive outcomes in males, particularly in increasing spermatozoa count (Maroto et al., 2025). In Indonesia, Zuriat fruit (*Hyphaene thebaica*) has begun to be cultivated, and its extract is increasingly being commercialized for use in herbal medicine. One such product is Zuriat fruit powder, which is in antioxidants and is utilized in herbal treatments related to antifertility in women .

A review of the existing literature indicates that no prior studies have investigated the effects of Zuriat fruit (*Hyphaene thebaica*) extract on the spermatozoa quality of male mice (*Mus musculus*) exposed to Balinese Arak. Therefore, further research is warranted to evaluate the potential impact of *Hyphaene thebaica* on spermatozoa quality in male mice subjected to Balinese Arak administration.(Sukma Antari et al., 2017)

Method

Materials and Equipment

The present study utilized the following equipment, plastic cages (rat cages) with wire mesh lids, specialized mouse water bottles, oral gavage needles, 1 cc syringes, styrofoam supports, droppers, petri dishes, microscope slides, coverslips, analytical balance, drying oven, measuring cup, erlenmeyer flasks, rotary evaporator, filter paper, a blender, a microscope, sterile scissors, gloves and stopwatch. The main ingredients used were Balinese arak with 40% alcohol content and Zuriat fruit (Hyphaene thebaica) (Y. Wang et al., 2025). Chemical reagents included chloroform, aquadest (distilled water), 0,9% NaCL solution, 1% Eosin stain, 96% Ethanol, standard feed, 2 N HCL, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Mg powder, concentrate HCL, FeCL3, concentrated H2SO4, and glacial CH3COOH (acetic acid).

Research Procedures

1. *Sample Collection and Preparation*

Zuriat fruit or Gingerbread tree (*Hyphaene thebaica*) was selected based on its intact, elongated shape and dark brownish-black color. The fruits were thoroughly washed under running water and then grated. The grated material was subsequently dried in an oven at 50°C for approximately 7 hours. After drying, the sample was finely ground using a blender. The dried and ground sample was then subjected to maceration using 96% ethanol as the solvent until the solvent became clear. The resulting extract was filtered using filter paper and then evaporated using a rotary evaporator at 78°C until the extract reached a slightly viscous consistency. Finally, the concentrated extract was placed in an oven at 40°C for further processing.

2. *Phytochemical* Screening

Phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids. The sample used for this screening was the extract of Zuriat fruit (*Hyphaene thebaica*).

3. *Preparation* of Balinese Arak

Balinese Arak used in this study was sourced from Tri Eka Buana Village, Karangasem Regency, Bali. Specifically, this study used Barong brand arak, a commercially available product that has received BPOM (Indonesian Food and Drug Authority) approval and possesses an alcohol content of 40%.

4. *Animal Preparation*

In this study, thirty male mice (Mus musculus), aged three months and weighing between 20 and 30 grams, were utilized as experimental subjects. The mice were subsequently divided into five groups : the normal control group (K1), the negative control group (K2), and three treatment groups (K3, K4 and K5). the experimental animals underwent a seven-day acclimatization period prior to the administration of treatments. Throughout the study duration, the male mice were provided with standard concentrate feed and water *ad libitum.*

5. *Administration of Treatment*
1) Normal control group (K+) : received standard feed + drinking water daily.
2) Negative control group (K-) : received standard feed + drinking water + 0.1 mL of *Balinese Arak* for 35 days.
3) Treatment groups 1 (K1) : received standard feed + drinking water + 0.1 mL of *Balinese Arak* + 0.5 g/kg body weight of Zuriat fruit (*Hyphaene thebaica*) extract for 35 days.
4) Treatment group 2 (K2) : received standard feed + drinking water + 0.1 mL of *Balinese Arak* + 1 g/kg body weight of Zuriat fruit (*Hyphaene thebaica*) extract for 35 days.
5) Treatment group 3 (K3) : received standard feed + drinking water + 0.1 mL of *Balinese Arak* + 2 g/kg body weight of Zuriat fruit (*Hyphaene thebaica*) extract for 35 days.

6. *Surgery of Experimental Animals*

On day 36, the mice were sacrificed and subjected to surgery. Dislocation was performed using chloroform, followed by an abdominal incision. The testes were then cleaned of surrounding tissues, and the cauda epididymis was collected. The cauda epididymis was minced and homogenized in 1 cc of 0,9% NaCL solution with pH of 7,2-7,4 using scissors until a spermatozoa suspension was formed. 5 minutes after mincing, sperm quality analysis was conducted.

7. *Spermatozoa Quality Assessment*
1) *Spermatozoa Count*

Spermatozoa were counted by first drawing sperm into an erythrocyte pipette to the 0,5 mark. This sample was diluted with a specific solution up to the 101 mark and agitated for 15-20 minutes to ensure thorough mixing. A drop of this diluted mixture was then applied to an improved neubauer hemocytometer. The spermatozoa were visualized and enumerated under a microscope using a 10x objective lens, with a stopwatch and counter used to aid in the process (Wuwungan et al., 2017)

2) *Spermatozoa motility*

A drop of spermatozoa suspension was drawn using a dropper pipette and placed onto the chamber of the improved Neubauer counting grid. Spermatozoa motility speed was measured under a microscope at 10x magnification. The distance moved by each spermatozoon was recorded at 10-second intervals using a stopwatch. The motility speed was then calculated by dividing the distance traveled by the corresponding time interval. Observations were repeated three times, and a total of 100 spermatozoa were assessed (Mamajang et al., 2023). The observable criteria included immotile sperm, slowly motile sperm, sperm exhibiting circular motion, and sperm exhibiting linear progressive motion (Wattimena et al., 2023).

3) *Spermatozoa Viability*

Spermatozoa viability was assessed by mixing 1 drop of spermatozoa suspension with 1 drop of 1% Eosin stain. The evaluation was based on the absorption of the stain, non-viable spermatozoa absorbed the dye and appeared red, whereas viable spermatozoa did not absorb the stain and remained clear. Observations were conducted using a light microscope at 40x magnification.(Antari et al., 2021)

4) *Spermatozoa Morphology*

Spermatozoa morphology was assessed by placing 1 drop of spermatozoa suspension onto a microscope slide and spreading it evenly. The resulting smear was fixed with 96% ethanol for 5 minutes, followed by the addition of 1 drop of 1% Eosin stain. Observations were then conducted with a light microscope at 40x magnification. Spermatozoa were categorized as morphologically normal if they exhibited an intact head and neck, and a straight tail. Abnormal morphology was characterized by features such as a small head, absence of a head, enlarged head, double heads, coiled tail, broken tail and double tails.

Result and Discussion

Phytochemical Screening Test

Phytochemical screening of *Hyphaene thebaica* fruit extract revealed the presence of alkaloids and flavonoids. These results are summarized in Table 1.

Table 1. Phytochemical Analysis Result of Zuriat fruit (*Hyphaene thebaica*)

Type of test	Reagent	Result	Information
Alkaloid	Mayer	+	Formation of a yellowish white precipitate
	Dragendrof	+	Formation of a orange precipitate
	Wagner	+	Formation of a brown precipitate
Flavonoid	Concentrated HCL	+	Formation of pink ring
Tanin	10% Chloride solution	-	No color change to dark green
Steroid	H_2SO_4	-	No color change to violet, blue or green
Terpenoid	H_2SO_4	-	No color change
Saponin	HCL 2N	-	No form formation

Descriptions: (+) indicates the presence of the corresponding compound, (-) indicates its absence.

DNA Fragmentation

Based on the results of the analysis using the Anova test regarding the average difference in the value of spermatozoa DNA Fragmentation, the control group, P1 P2 and P3 showed that the administration of zuriat fruit extract can have a good effect on spermatozoa DNA Fragmentation, this is shown by the results of a significant analysis between the Control group (K-) and the treatment group (P) (P>0.05).

Table 2. Mean Values of DNA Fragmentation Spermatozoa

Variable	Treatment Group	Mean ± SD	P-value	Description
Normal DNA	K+	68.73 ± 2.227	< 0.001	Significant
	K-	41.00 ± 1.873		
	P1	64.00 ± 2.910		
	P2	75.83 ± 1.414		
	P3	83.17 ± 0.334		
Abnormal DNA	K+	33.83 ± 2.227	< 0.001	Significant
	K-	65.50 ± 2.306		
	P1	34.00 ± 2.910		
	P2	27.83 ± 1.376		
	P3	10.17 ± 0.601		

The calculation of spermatozoa DNA fragmentation was carried out using a fluorescence microscope with a magnification of 400x on 100 cells. Observations on spermatozoa DNA are divided into 2 categories, namely A) orange which indicates spermatozoa with fragmented DNA, B) green which indicates intact DNA or no fragmentation.

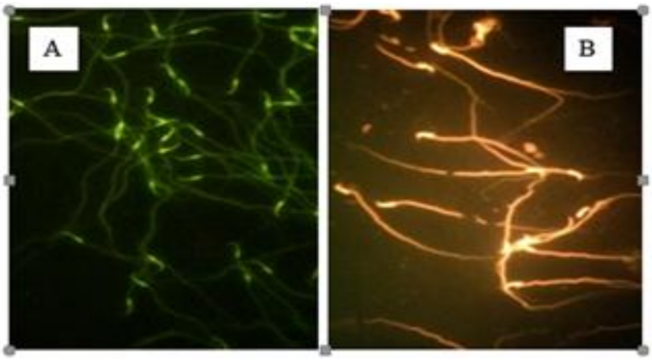

Figure 1. DNA Fragmentation Sperm with intact DNA are marked in green (A), fragmented DNA is marked in orange (B).

Spermatozoa viability loss is primarily attributed to DNA damage mediated by reactive oxygen species (ROS). Oxidative stress exacerbates ROS generation, resulting in extensive DNA fragmentation that triggers apoptotic pathways (Musfiroh & Gustari, 2015).This molecular damage underlies the elevated incidence of spermatozoa cell death observed. Furthermore, ROS-induced oxidative lesions contribute to genomic instability through DNA mutations and induce cytotoxic effects that compromise cellular integrity(Pinto et al., 2023). Cytotoxicity contributes to cellular apoptosis and a consequent decline in cell population within tissues. A key mechanism driving cytotoxicity is oxidative stress, which results from the overproduction of reactive oxygen species (ROS) coupled with the dysfunction of endogenous antioxidant defense systems. This redox imbalance precipitates extensive spermatozoa cell death through oxidative damage to cellular macromolecules and disruption of vital physiological processes which results from the overproduction of reactive oxygen species (ROS) coupled with the dysfunction of endogenous antioxidant defense systems. This redox imbalance precipitates extensive spermatozoa cell death through oxidative damage to cellular macromolecules and disruption of vital physiological processes (Antari, et al., 2017).

Spermatozoa Quality

1. *Spermatozoa count*

The analysis of the average spermatozoa count showed values of 74.00 in the normal control group (K1), 37.00 in the negative control group (K2), 74.00 in treatment group 1 (K3), 79.50 in treatment group 2 (K4), and 93.00 in treatment group 3 (K5) with a p value of < 0.001 (Table 3).

Table 3. Mean spermatozoa count

Variable	Treatment Group	Mean ± SD	P-Value	Description
Count of spermatozoa	K+	74.00 ± 1.342	< 0.001	Significant
	K-	37.00 ± 0.730		
	P1	74.00 ± 1.528		
	P2	79.50 ± 0.764		
	P3	93.00 ± 1.807		

The results of the study on the administration of Zuriat fruit (*Hyphaene thebaica*) in male mice (*Mus musculus*) exposed to Balinese traditional alcohol (Balinese Arak) demonstrated an effect on sperm quality, specifically an increase in spermatozoa count. The findings revealed a significant difference in the mean spermatozoa count among the groups, with treatment group 3 (K5) exhibiting a higher average spermatozoa count compared to the other groups.

The mean value in the negative control group was lower, attributed to the administration of alcohol in arak at dose of 0,1 cc for 35 days. This resulted in a decrease in spermatozoa count in the mice, alcohol being lipid soluble can easily cross cell membranes through protein and lipid mediated mechanisms, leading to increased respiratory activity that contributes to elevated levels of reactive oxygen species (ROS), which can damage cellular structure and function (Antari, et al., 2017).

Alcohol metabolism in the body can disrupt the hormonal system through the hypothalamic – pituitary – testicular. This process begins with a decrease in the levels of LHRH in the hypothalamus and LH in the pituitary gland, leading to reduced activity of Leydig cells in producing testosterone (Ratih & Habibah, 2022). Consequently, testosterone levels in the testes decline. Both acute and chronic alcohol exposure are associated with lowered hypothalamic LHRH and pituitary LH levels. The damage to Leydig cells caused by alcohol is attributed to the generation of free radicals and resulting oxidative stress(Finelli et al., 2022). Testosterone plays a crucial role in the process of spermatogenesis. A decrease in serum testosterone levels leads to reduced proliferation of spermatogonia, disruption of spermiation, and accelerated apoptosis, resulting in a decline in spermatozoa count. Additionally, reduced testosterone impairs the adhesion of germ cells and inhibits the release of mature spermatozoa from the seminiferous tubules(Ayad et al., 2022). In the absence of testosterone stimulation, mature spermatozoa are phagocytosed by sertoli cells (Ramaswamy, 2015).

Phytochemical screening of the Zuriat fruit (*Hyphaene thebaica*) tested positive for the presence of alkaloids and flavonoids. Flavonoids possess antioxidant properties that can inhibit oxidation reactions by effectively scavenging free radicals and other reactive molecules, thereby preventing the decline in spermatozoa count in male mice (*Mus musculus*) (Aninda, 2022). Antioxidants also function to neutralize or stabilize free radicals by donating electrons to compensate for the radicals electron deficiency, thus inhibiting the chain reactions involved in free radical formation that can cause cellular damage. (Ervi Husni, 2014) Consequently, damage to cell membranes caused by free radicals can be prevented, ensuring that the binding of hormones involved in spermatogenesis to their receptors remains unaffected, leading to a more optimal spermatogenesis process and an increase in spermatozoa count (Lefaan, 2014). The results in the normal control group did non different significantly from treatment group 1 (K3), which is attributed to the fact that the extract dose administered in K3 was the lowest 0,5 g/kg body weight compared to treatment group 2 (K4) at 1 g/kg body weight and treatment group 3 (K5) at 2 g/kh body weight.

2. *Spermatozoa Motility*

The results of the mean spermatozoa motility analysis are presented in Table 4.

Table 4. Mean Values of Spermatozoa Motility

Variable	Treatment Group	Mean ± SD	P-value	Description
Motility A	K+	30.67 ± 1.282		
(immotile sperm)	K-	20.17 ± 0.749		
	P1	28.33 ± 1.085	< 0.001	Significant
	P2	33.83 ± 0.601		
	P3	44.83 ± 0.792		
Motility B	K+	30.50 ± 2.377		
(slowly motile sperm)	K-	20.83 ± 0.872		
	P1	29.17 ± 2.810	< 0.005	Significant
	P2	31.50 ± 1.875		
	P3	27.50 ± 0.847		
Motility C	K+	25.17 ± 1.833		
(sperm exhibiting circular motion)	K-	28.83 ± 1.078		
	P1	26.83 ± 1.579	< 0.001	Significant
	P2	20.00 ± 1.342		
	P3	16.67 ± 0.715		
Motility D	K+	15.50 ± 0.957		
(sperm exhibiting)	K-	29.33 ± 1.542		
	P1	17.67 ± 1.978	< 0.001	Significant
	P2	14.67 ± 0.760		
	P3	11.00 ± 1.065		

The observation of spermatozoa motility in male mice (*Mus musculus L.*) demonstrated statistically significant differences across the experimental groups. The data indicated that the mean values for Motility A (progressive movement) and Motility B (non-progressive movement) were highest in Treatment Group 3 (K5), in contrast, the mean values of Motility B (non-progressive motility) and Motility C (circular motility) were higher in the negative control group compared to the other groups. This phenomenon is likely attributed to the administration of *Balinese Arak*, which contains ethanol that may disrupt the integrity of the thin cytoplasmic membrane in the sperm head, thereby impairing sperm motility and ultimately reducing the fertilization capability of the spermatozoa (Antari, et al., 2016).

Spermiogenesis is the process of formation and maturation of spermatozoa that occurs within the seminiferous tubules of the testes (F. Wang et al., 2021). Spermatozoa undergo differentiation as they exit the testes and enter the epididymis. During the process of spermiogenesis, the formation and development of the flagellum occurs, in which round spermatids begin to form the axoneme(Kaltsas, 2023). The axoneme is a microtubule-based structure that generates the force required for flagellar movement. During spermatogenesis, secondary structures such as the outer dense fibers (ODF), fibrous sheath, and mitochondrial sheath are also formed (Bahmanpour et al., 2022). The mitochondrial sheath is involved in the production of ATP, which provides the energy necessary for spermatozoa motility. The fibrous sheath contributes to the rigidity of the flagellum, which is essential for determining the waveform of flagellar beating. The ODF is rich in krestinin and facilitates sperm motility while providing structural stiffness to withstand shear forces during the passage through the female reproductive tract (Rosa et al., 2024).

Testosterone plays a crucial role in the process of spermatogenesis; therefore, a decline in this hormone may disrupt the development and formation of the flagellum during spermatogenesis, potentially impairing spermatozoa motility. Abnormalities in sperm morphology may also lead to reduced motility, as sperm motility is directly correlated with the structural integrity of sperm morphology (Rosa et al., 2024).

The administration of *Balinese Arak* followed by Zuriat fruit (*Hyphaene thebaica*) extract in mice was found to enhance spermatozoa motility, with the highest increase observed in Treatment Group 3 (K5). This improvement is attributed to the presence of flavonoids in Zuriat fruit extract which act as potent antioxidants by protecting the cytoplasmic membrane in the sperm head, thereby preserving progressive forward movement of the spermatozoa (Antari, et al., 2016). Changes in spermatozoa motility represent the most prominent alterations during the maturation process in the epididymis. Spermatozoa released from the testes into the caput epididymis are immotile. The lumen of the epididymis provides an optimal environment for the activation of the spermatozoa motility, enabling spermatozoa located in the cauda epididymis to exhibit progressive motility. Testosterone influences protein synthesis, electrolyte transport, and gene expression in the epididymis to maintain appropriate luminal conditions, including pH and isotonicity.The findings of this study are consistent with those reported by Antari et al. (2016), which demonstrated that the administration of *Balinese Arak* reduced spermatozoa quality including motility, morphology, and viability in mice.(Amusan et al., 2024)

3. *Spermatozoa Viability*

The analysis results of spermatozoa viability are presented in Table 5.

Table 5. Mean spermatozoa viability

Variable	Treatment Group	Mean ± SD	P-value	Description
Normal Viability	K+	67.50 ± 1.857	< 0.001	Significant
	K-	33.83 ± 2.227		
	P1	34.33 ± 2.305		
	P2	64.67 ± 2.305		
	P3	89.00 ± 0.365		
Abnormal Viability	K+	32.50 ± 1.857	< 0.001	Significant
	K-	66.50 ± 1.473		
	P1	66.67 ± 2.305		
	P2	35.33 ± 2.305		
	P3	11.00 ± 0.365		

This study revealed a significant difference in the viability of live spermatozoa between the treatment groups and and the negative control groups. The data indicated that the mean viability of live spermatozoa in treatment group 3 (P3) was higher compared to the other groups. This increase is likely due to the administration of the highest extract dose, 2 grams/kg body weight, in group P3. According to the phytochemical screening results, the extract contains a high level of antioxidants, which play a role in protecting cell membranes, including the plasma membrane from damage caused by free radicals. Consequently, the extract helps maintain the integrity of the plasma membrane and prevents damage induced by oxidative stress (Sinaga, 2016).

The mean viability of live spermatozoa in the negative control group was lower, which may be attributed to the lack of protection of the spermatozoa heads by the plasma membrane that had been damaged by free radicals, likely as a result of the alcohol content in Balinese arak (Andini et al., 2022). When the membrane is compromised due to alcohol exposure, the heads of the spermatozoa become more susceptible to damage or structural alterations (Antari et al., 2021). The disruption of the plasma membrane in non-viable spermatozoa impairs the function of the sodium pump, which is responsible for regulating the circulation of substances. As a result, eosin dye is able to enter and remain within the cell, staining the spermatozoa (particularly the head) red. In contrast, viable spermatozoa possess an intact plasma membrane, allowing the sodium pump to function properly, therefore the spermatozoa head does not absorb the dye and remains unstained or appears white (Antari et al., 2021).

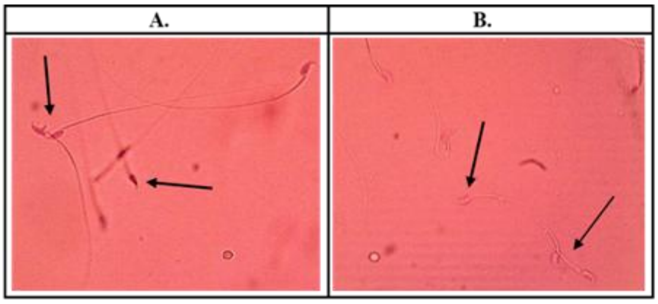

Figure 2. Spermatozoa Viability. Description: A: Dead Spermatozoa, characterized by red stained heads B: Live Spermatozoa, characterized by unstained (white) heads

The findings of the present study corroborate those reported by Ilyas, 2013, wherein male mice subjected to prolonged exposure and higher doses of Tuak exhibited a marked decline in spermatozoa quality parameters, including morphology and viability, accompanied by a significant reduction in progeny output. This suggests a dose and time dependent detrimental effect of Tuak on male reproductive function

4. Spermatozoa Morphology

The results of the mean spermatozoa analysis are presented in Table 6.

Table 6. The mean value of spermatozoa morphology

Variable	Treatment Group	Mean ± SD	P-Value	Description
Normal Morphology	K+	66.17 ± 2.227	< 0.001	Significant
	K-	34.50 ± 2.306		
	P1	65.83 ± 2.833		
	P2	72.17 ± 1.376		
	P3	89.83 ± 0.601		
Abnormal Morphology	K+	33.83 ± 2.227	< 0.001	Significant
	K-	65.50 ± 2.306		
	P1	34.00 ± 2.910		
	P2	27.83 ± 1.376		
	P3	10.17 ± 0.601		

The morphological analysis of spermatozoa revealed a statistically significant difference between the mean morphology of the negative control group and that of the treatment group. The data indicates that the mean percentage of normal morphology in treatment group 3 (P3) was higher than that in the other groups, whereas the mean percentage of abnormal morphology in the negative control group was. The result of the spermatozoa morphology analysis demonstrated that higher doses of Zuriat fruit extract (*Hyphaene thebaica*), administered following exposure to *Balinese Arak*, led to a reduction in the number of spermatozoa abnormalities.

Factors contributing to the increase in abnormal spermatozoa morphology include disruptions in the spermatogenesis process, which may be caused by hormonal imbalances, free radicals, and chemical exposures (Hussain et al., 2023). Excessive alcohol consumption can impair epididymal function in the maturation of spermatozoa, leading to disruption in the division of spermatogonia cells prior to the formation of the head, neck, and tail. Within the epididymis, spermatozoa undergo a series of morphological and functional changes, including alterations in size and shape (Antari et al., 2021).

The increase in abnormal sperm morphology observed in the negative control group was attributed to damage occurring during spermatogenesis, which was induced by elevated levels of reactive stress can cause damage to the cell membranes of the seminiferous tubules, allowing toxic free radicals to penetrate into the seminiferous tubules.

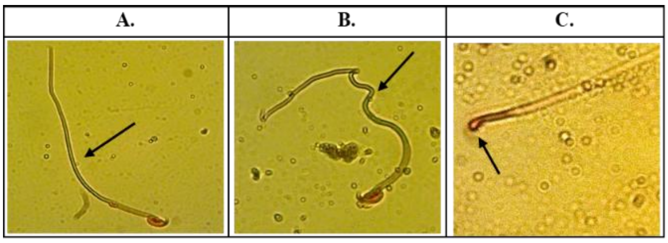

Figure 3. Spermatozoa morphology stained with Eosin. Description: A – Normal spermatozoon; B – Spermatozoon with coiled tail; and C – Spermatozoon with a small (shrunken) head.

The head and tail of the spermatozoon are the most critical components of sperm morphology. Sperm morphology is classified into two categories: primary and secondary abnormalities. Primary morphological abnormalities occur during the process of spermatogenesis and are characterized by features such as a head that is smaller or larger than normal, double head, flattened head, double tails, coiled tails, and detached tails (Antari et al., 2023). Secondary morphological abnormalities occur during the maturation of spermatozoa in the epididymis and can be identified by features of cytoplasmic droplets, and detached acrosomes (Rosa et al., 2024). In this study, primary abnormal sperm morphology observed included coiled tails, detached head, and bent necks. Secondary abnormalities were identified as broken tails

Conclusion

The administration of Zuriat fruit extract (*Hyphaene thebaica*) at dosages of 1 g/kg and 2 g/kg body weight enhanced spermatozoa quality (encompassing count, motility, viability, and morphology) in male mice (*Mus musculus*) exposed to Balinese traditional alcohol (Balinese Arak). An increase in ROS levels induces oxidative stress due to an imbalance between elevated ROS concentrations and insufficient antioxidant capacity, leading to cellular tissue, and organ damage.

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Author Contributions
Conceptualization, N.W.S.A. and I.A.M.D.; methodology, N.W.S.A.; software, N.W.S.A.; validation, N.W.S.A., I.A.M.D.; formal analysis, N.W.S.A.; investigation, N.W.S.A.; resources, N.W.S.A.; data curation, N.W.S.A.; writing original draft preparation, N.W.S.A.; writing review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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