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Spermatogenic effect of myricetin on ethyl-alcohol induced sperm toxicity in pre-pubertal male Wistar rats

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ABSTRACT

Consistent ethanol beverage exposure adversely affects male reproductive function by oxidative stress-related mechanisms. This study was designed to investigate the spermatogenic and spermiographic effects of myricetin on ethyl-alcohol-induced sperm toxicity in pre-pubertal male Wistar rats. Thirty-five (35) pre-pubertal male Wistar rats were divided into seven (7) groups of five (5) rats each. Group 1 was given distilled water only, group 2 was given 40 % ethanol only, groups 3, 4, and 5 were given 40 % ethanol + 75 mg, 150 mg, and 300 mg myricetin respectively, while Groups 6 and 7 were given 75 mg and 300 mg myricetin respectively. On completion of treatment, animals were ketamine sedated and blood was collected through intra-cardiac puncture. Epidydimal tissues were eviscerated for spermiography and semen analysis. Descriptive statistics was used to determine the mean and standard error of the mean, and One-way analysis of variance was used to determine whether there were statistically significant differences between the means of independent groups. Tukey's post hoc test was used to compare differences among the groups. Tests were considered statistically significant at $p<0.05$. Results showed that Sperm motility, sperm concentration, and the number of normal sperm cells increased in myricetin-treated groups compared to the control and ethanol-induced groups. In conclusion, these finding suggests that myricetin protected the spermatic cells from oxidative stress and ameliorated ethanol-based toxicities to the epidydimal sperm cells, hence promoting its fertility function.

Keywords: Myricetin, Ethyl-alcohol, Semen analysis, Spermiography

1. INTRODUCTION

Infertility involves couples not having the capacity to bear a child within one year or more of unprotected sexual intercourse, a situation affecting about 15 % of couples. Infertility linked to the male gender is documented to be in about half of all reported cases. Male-based infertility is regarded as when a sexually active man cannot impregnate a sexually active and fertile woman. Considering infertility cases in humans, it is recorded for about 40 to 50 percent (Pandruvada et al., 2021). It is judged to affect almost 7 percent of all males. The primordial reason for male infertility cases involves inadequate seminal production, and this seminal quality is used as a basis of surrogate indicator for male fertility. In recent times, technically advanced seminal assays to examine intracellular sperm compositions and activities have been established to ease early detection and fertility monitoring (Turner et al., 2020).

Sperm motility level is seen to increase from pubertal age to the mid-thirties of such individuals. It is evaluated that spermatid levels at ages 20 and 30 are 90% within seminiferous tubules, whereas, 50% and 10% in the ages 40 and 50, and age 80 and above respectively (Isaiah et al., 2011). A random international paternity sample considering 11,548 biological fathers indicated the oldest confirmed father to be about 66 years old, information drawn after DNA paternity testing (DNA-rejection to DNA-confirmation paternity ratio) was consistent with a generalized view of 65-66 years of male infertility period (Forster et al., 2015). In about 10-30% of couples with infertility cases, their anti-sperm antibodies (ASA) are implicated to cause infertility. ASA production can stop the fertilization process by direct contact with the surface antigen of sperm cells, disrupting sperm motility, acrosome reaction, passage through the female reproductive tract, and embryonic development and growth.

ASA formation in men can be influenced by the following factors including; interference in the blood-testis barrier, infection effects, surgery, prostatitis, orchitis, varicocele, immunosuppression failure, and unprotected oral or anal sex with men (Restrepo and Maya, 2013). The conditions with inadequate support of testes to its hormonal activity and poor general health are referred to as pre-testicular factors. These conditions include: In varicocele, the veins in the testicles enlarge. It affects roughly 40% of men with infertility and 15% of normal men. It occurs in about 35% of primary infertility cases and 69% - 81% of later infertility cases. Obesity increases the incidence of hypogonadotropic hypogonadism. Obesity, as demonstrated by animal models, causes leptin insensitivity in the hypothalamus, lowering Kiss1 expression and altering gonadotropin-releasing hormone (GnRH) secretion (Kupis et al., 2015).

Undetected and unmanaged celiac disease. Celiac disease-related infertility in men may be reversed. Even when there are no gastrointestinal symptoms, the CD can cause a wide range of non-gastrointestinal symptoms that can impact practically any organ system. Consequently, there is a potential that the diagnosis will be ignored, resulting in long-term complications (Kupis et al., 2015). According to Teerds et al., (2011), CD in males can impair the quality of their sperm and cause immature secondary sex characteristics such as hypogonadism and hyperprolactinemia, leading to impotence and diminished libido. Restoring fertility may arise from having a gluten-free diet and making required dietary changes. Examination for undiagnosed celiac disease in both men and women is likely to be the most useful component of a thorough infertility evaluation.

Medications that impact spermatogenesis, including chemotherapy, anabolic steroids, fluoxetine, spironolactone, and cimetidine; those that lower FSH levels, including phenytoin; and those that reduce sperm motility, including nitrofurantoin and sulfasalazine (Freeman, 2010). There is growing evidence that the toxic byproducts of tobacco use might hurt testicles and destroy sperm, but it is unclear how this will affect male fertility. Manufacturers are required by some governments to include warnings on packs. Due to the tobacco plant's absorption of the metal, smoking tobacco increases cadmium intake. Because cadmium and zinc share similar chemical properties, cadmium may take the place of zinc in DNA polymerase, an enzyme essential to the development of sperm. Testes may be especially harmed by DNA polymerase that substitutes cadmium for zinc (Harlev et al., 2015).

Plant families, including the Myricaceae, Primulaceae, Polygonaceae, Anacardiaceae, and Pinaceae, contain myricetin (Borck et al., 2018). This flavonoid is more frequently found in fruits, vegetables, wine, and teas. Myricetin, identified as a free molecule, is glycosidically bounded in the following forms: myricetin-3-O-β-d-galactopyranoside, myricetin-3-O-(4″-acetyl)-α-l-arabinopyranoside, myricetin-3-O-(3″-acetyl)-α-l-arabinopyranoside, myricetin-3-O-β-d xylopyranoside, myricetin-3-O-(6″-galloyl)-β-d-galactopyranoside, myricetin 3-O-α-l-arabinofuranoside, myricetin-3-O-(2″-O-galloyl)-α-l-rhamnoside, and myricetin-3-O-α-l-rhamnoside (Cao et al., 2018). Despite myricetin having strong solubility in various chemical media like tetrahydrofuran, dimethylacetamide, acetone, and acetone dimethylformamide, it is observed to be partially hydrophilic (16.6 μg/ml) (Chang et al., 2012).

Moreover, a study on the degradation of this compound showed that it is quite stable even at pH 2, but it can differ with temperature. In the late eighteenth century, this compound was isolated from the bark of the *Myrica nagi* Thunb. Numerous pharmacological properties were also demonstrated, including hepato-protective, antitumor, anti-inflammatory, analgesic, and antidiabetic effects. Compared to other flavonoids, myricetin has a higher level of antioxidant activity because its ring B has three hydroxyl groups. According to in vitro research, the double-bonded oxygen group and the two hydroxyl groups are responsible for the mineral chelation. Myricetin's pro-oxidative impact is caused by the catechol groups in its structure, which result in the formation of semi-quinone radicals.

When the 4-hydroxyl group on the C ring and the 4-hydroxyl group on the B ring combine to produce a quinine, this radical gets oxidized. Myricetin has been linked to numerous health advantages, including lowered hepatic triglyceride levels, prevented liver damage, lowered oxidative stress, and lower cholesterol levels (Guo et al., 2015; Semwal et al., 2016). Antioxidants are molecules present in fruits and vegetables that have been demonstrated to protect against some forms of cancer and cardiovascular disease. Reactive oxygen species (ROS) can cause oxidative stress to biomolecules and cell structures when they are present and active. Aerobic respiration, a process involved in cellular metabolism, produces ROS such as •OH, •O2−, and H2O2. Proteins, DNA, and lipids can all be harmed by ROS (Barzegar, 2016).

Numerous illnesses and ailments, such as atherosclerosis, diabetes, cancer, persistent inflammation, and thrombosis, might arise as a result of the slow but continuous accumulation of such damage. Myricetin is one of the flavonoids that can scavenge reactive oxygen species (ROS) and chelate intracellular transition metal ions, which in turn create ROS. Moreover, myricetin amplifies the benefits of other antioxidants. Glutathione S-transferase (GST) is an enzyme that myricetin can activate. GST has been suggested to have cell-protective activity against oxidative stress by protecting the cells against free radicals. In vitro studies have shown that myricetin significantly increased GST activity (Barzegar, 2016). Myricetin has been shown in numerous studies to possess the ability to function as a pro-oxidant because of its propensity to experience autoxidation in response to environmental factors.

It has been observed that autoxidation is promoted in the presence of cyanide, leading to the production of superoxide, a byproduct with the ability to harm cells. Nevertheless, it has been observed that catalase, sodium azide, and superoxide dismutase prevent myricetin from autoxidizing (Mandić et al., 2021). By reacting with Fe2+ or Fe3+-EDTA and hydrogen peroxide, myricetin can also function as a pro-oxidant by increasing the generation of hydroxy radicals. The resultant hydroxy radicals are frequently associated with DNA degradation; however, there are uncertainties regarding the significance of this damage when assessed in vivo, as in vitro experiments involving both bovine and human serum albumin have shown considerable protection against it (Jomová et al., 2019).

2. METHODS

Myricetin and other chemical purchase

Myricetin 3-Rhamnoside was ordered and purchased from Source Natural, USA. Phosphate buffer saline (PBS) and Phosphate buffered formalin (PBF) were ordered and purchased from Thermo Fisher, Germany.

Animal Care and Use

Thirty-five (35) pre-pubertal male albino Wistar rats of five (5) weeks old were obtained from an animal farm, department of Veterinary Medicine, University of Nigeria, Nsukka weighing 55g to 120g and used for the study. The animals were allowed to acclimatize for one week and given humane care following the Principle of Laboratory Animal Care and Use (National Institute of Health, 2011). The animals were stratified and selected by weight into seven (7) groups of five (5) animals each, housed in well-ventilated aluminum cages, and kept under conditions of temperature 25 ± 5% and 12hours' light/dark cycle. The rats were given standard chow (Vital feeds) and water *ad libitum* throughout the study. Treatment design included; Group 1 received distilled water only, group 2 received 40 % ethanol only, groups 3, 4, and 5 received 40 % ethanol + 75 mg, 150 mg, and 300 mg myricetin respectively, while Groups 6 and 7 received 75 mg and 300 mg myricetin respectively.

Experimental Design

The thirty (35) experimental animals were weighed and then randomly divided into six (6) experimental groups as follows (Table 1);

Table 1 Experimental Design

S/N	Treatment Group	Dosage	Duration
1	Control	2 ml/kg distilled water	4 weeks
2	Ethanol alone	5 ml/kg 40 % Et	4 weeks
3	Ethanol + Myricetin low dose	5 ml 40 % Et + 75 mg MYR	4 weeks

4	Ethanol + Myricetin middle dose	5 ml 40 % Et + 150 mg MYR	4 weeks
5	Ethanol + Myricetin high dose	5 ml 40 % Et + 300 mg MYR	4 weeks
6	Myricetin (low dose)	75 mg MYR	4 weeks
7	Myricetin (High dose)	300 mg MYR	4 weeks

CC = Clomiphene Citrate, Et = Ethanol, CP = *Carica papaya*, MYR = Myricetin.

Dosage of Administration

Drug Administration

Drugs were calculated and administered thus;

1 caplet of myricetin = 100 mg

100mg caplet dissolved in 100 ml of distilled water.

Therefore, 1.0 mg/ml $\longrightarrow$ Stock concentration.

100 mg of caplet of myricetin $\longrightarrow$ 70 kg body weight

Therefore 1.428 mg/kg $\longrightarrow$ Dosage.

By calculation, dosages of 75 mg, 150 mg, and 300 mg of myricetin were derived to be

$$\text{Administered dosage} = \frac{\text{Weight (g)}}{1000} \times \frac{0.714\ \text{mg/kg}}{0.5\ \text{mg/ml}}$$

The value derived in mills.

Ethanol Dosage

40% Ethanol = 40 ml of Ethanol + 60 ml Distilled Water

$$\text{Administered dosage} = \frac{\text{Weight (g)}}{1000} \times \frac{5\ \text{ml/kg}}{1}$$

Testicular Morphometry

The testicular indices were carried out according to the method by.

Testicular Weight

Testis is extracted from the animal. Cleaned off connective tissue or blood. Testis is placed on the weighing balance with a setting to measure in grams. The weights of both testes are recorded, and the average weight is documented. Weights were recorded in grams (g).

Testicular length

Testis was extracted from the animal. Cleaned off connective tissue or blood. Cleaned testis is placed in a petri dish. The jaws of the digital vernier caliper were extended to touch both pools of the right and left testes. Values are observed and recorded. Lengths were recorded in millimeters (mm).

Testicular Diameter

Testis was extracted from the animal. Cleaned off connective tissue or blood. Cleaned testis is placed in a petri dish. Jaws of the digital vernier caliper were extended to touch the circumference of both the right and left testes at the equatorial pool. Values are observed and recorded. Diameters were recorded in millimeters (mm).

Sperm Motility

Caudal epididymis is incised to expose fluid. 5 µl of the epididymal fluid is collected with a micropipette**.** Fluid is delivered onto a glass slide and then covered with a 22 x 22 mm coverslip. Glass slide is taken to a light microscope with a magnification of X400. Motility estimation is carried out at room temperature between 24 – 28 ºC. Microscopic field is scanned systematically and each spermatozoon encountered is assessed. Motility is recorded in percentage and classified as Progressively motile, Non–progressively motile, and Non-motile. The procedure is repeated and the average is taken.

Sperm Morphology

Caudal epididymis is incised to expose fluid. 5 µl of the epididymal fluid is collected with a micropipette**.** Fluid is delivered onto a glass slide and then covered with a 22 x 22 mm coverslip. Glass slide is taken to a light microscope with a magnification of X100 and then X400. Microscopic field is scanned systematically and each spermatozoon encountered is assessed. Morphological changes of assessed sperm including; normal sperm, curved sperm, and spermatozoa with bent neck is recorded in percentage. The procedure is repeated and the average is taken.

Sperm Concentration

Harvested Epididymis is cut with anatomical scissors and minced in a petri dish. 50 µl of epididymal spermatozoa is diluted in 950 µl physiological saline. Well-mixed solution is pipette into both chambers of the hemocytometer. The hemocytometer is placed on the stage of the microscope. The objective of the microscope is adjusted to an X40 magnification. Hemocytometer is viewed and counting is done. Values of counts are recorded. Counting is repeated in each chamber and the average count is documented. The average number of cells, cell density, dilution factor, and cell concentration are now calculated by applying the formula;

$$\text{Average number of cells} = \frac{\text{sum of cells in each square}}{\text{Number of squares}}$$

Concentration (viable cells/ml) = Average number of cells / square X Dilution Factor X 104.

Spermiography

Caudal epididymis is incised to expose fluid. 5 µl of the epididymal fluid is collected with a micropipette**.** Fluid is delivered onto a glass slide and then covered with a 22 x 22 mm coverslip. Glass slide is taken to a light microscope with a magnification of X100 and then X400. Microscopic field is scanned systematically and morphological changes including; normal sperm, short tail, only head, head and neck, only tail, double head, and slender spermatozoa were snapped using a digital microscope camera.

Statistical Analysis

Data obtained from this study were analyzed using the Graph-pad 8.0 version II (San Diego, California) system package. The results of this study were expressed as mean ± standard error of the mean. One-way ANOVA, multiple comparisons, and a T-test were employed with a significance level of $p<0.05$.

3. RESULTS

Result on the effect of Myricetin on Testicular Morphometry

The result on the testicular length showed a non-significant decrease ($p>0.05$) in the control group when compared to all other treatment groups, and a non-significant increase ($p>0.05$) in the 40 % ethanol + 75 mg/kg myricetin treated groups when compared to the control and other treatment groups. Assessment of testicular diameter changes showed a significant increase ($p<0.05$) in the 40 % ethanol-treated group when compared to the control group, and a non-significantly increased ($p>0.05$) when compared to all other treated groups (Table 2).

Testicular weight result showed a significant increase ($p<0.05$) in the 40 % ethanol group and the 40 % ethanol + 75 mg/kg myricetin treated group when compared to the control, and a significant decrease ($p<0.05$) in the 75 mg/kg myricetin treated group when compared to the control group. A significant weight increase ($p<0.05$) was also observed in the 40 % ethanol group and the 40 % ethanol + 75 mg/kg myricetin-treated group when compared to the 75 mg/kg and 300 mg/kg myricetin-treated groups. However, a significant decrease ($p<0.05$) was observed in the 40 % ethanol + 300 mg/kg myricetin-treated group when compared to the 40 % ethanol + 75 mg/kg myricetin-treated group (Table 2).

Table 2 Testicular Morphometry

Testicular Morphometry			
Treatment groups	**Testicular Length (mm)**	**Testicular Diameter (mm)**	**Testicular Weight (g)**
Control	18.71 ± 0.6792	9.547 ± 0.8315	2.197 ± 0.1633
40% Ethanol	19.29 ± 0.6182	11.47 ± 0.1224*a	2.887 ± 0.1009*a
40% Eth. + 75 mg MYR	20.14 ± 0.3975	11.02 ± 0.2210	3.080 ± 0.04163**a
40% Eth. + 150 mg MYR	19.29 ± 0.1342	10.96 ± 0.3707	2.630 ± 0.1450
40% Eth. + 300 mg MYR	19.31 ± 0.1185	11.01 ± 0.3137	2.323 ± 0.05667*c
75 mg MYR	18.76 ± 0.4825	10.26 ± 0.1027	1.977 ± 0.2504**b***c*d
300 mg MYR	19.11 ± 0.3132	10.49 ± 0.1400	2.110 ± 0.04041*b**c
	P = 0.3867	P = 0.0545	P = 0.0003
	F = 1.146	F = 2.770	F = 9.538

Values are expressed in Mean ± SEM. *, **, ***, **** represents significant differences at p<0.05, p<0.01, p<0.001 and p<0.0001 respectively. a = indicating a significant difference to Control. b = indicating a significant difference to the 40% Ethanol group. c = indicating a significant difference to 40% Eth. + 75 mg MYR group. d = indicating a significant difference to 40% Eth. + 150 mg MYR group. e = indicating a significant difference to 40% Eth. + 300 mg MYR group.

Result on the effect of Myricetin on Sperm motility

Findings on the percentage of motile sperm showed a significant decrease (p<0.05) in the 40 % ethanol-treated group when compared to the 300 mg/kg myricetin and a non-significantly decrease (p>0.05) when compared to the control group and all other treatment groups. Assessment of the percentage of non-motile sperm showed a significant increase (p<0.05) in the 40 % ethanol-treated group when compared to the 300 mg/kg myricetin and a non-significantly increase (p>0.05) when compared to the control group and all other treatment groups (Table 3).

The result on the percentage of progressively motile sperm indicated that there was a non-significant decrease (p>0.05) in the 40 % ethanol-treated group when compared to the control group and all other treatment groups (Table 3). Also, the result on the percentage of non-progressively motile sperm indicated that there was a non-significant increase (p>0.05) in the 40 % ethanol-treated group when compared to the control group and all other treatment groups.

Table 3 Sperm motility

Sperm Motility				
Treatment groups	**% Motile**	**% None Motile**	**% Progressively motile**	**% None progressively motile**
Control	65.00 ± 5.401	35.00 ± 5.401	71.25 ± 5.154	28.75 ± 5.154
40% Ethanol	43.00 ± 11.79	57.00 ± 11.79	40.00 ± 15.08	60.00 ± 15.08
40% Eth. + 75 mg MYR	62.00 ± 8.155	38.00 ± 8.155	53.00 ± 8.456	47.00 ± 8.456
40% Eth. + 150 mg MYR	69.00 ± 5.099	31.00 ± 5.099	59.00 ± 4.301	41.00 ± 4.301
40% Eth. + 300 mg MYR	72.00 ± 4.637	28.00 ± 4.637	66.00 ± 6.782	34.00 ± 6.782
75 mg MYR	61.00 ± 4.301	39.00 ± 4.301	64.00 ± 5.788	36.00 ± 5.788
300 mg MYR	74.00 ± 5.099*b	26.00 ± 5.099*b	71.00 ± 3.317	29.00 ± 3.317
	P = 0.0640	P = 0.0640	P = 0.1138	P = 0.1138
	F = 2.297	F = 2.297	F = 1.920	F = 1.920

Values are expressed in Mean ± SEM. *, **, ***, **** represents significant differences at p<0.05, p<0.01, p<0.001 and p<0.0001 respectively. a = indicating a significant difference to Control. b = indicating a significant difference to the 40% Ethanol group. c =

indicating a significant difference to 40% Eth. + 75 mg MYR group. d = indicating a significant difference to 40% Eth. + 150 mg MYR group.

Result on the effect of Myricetin on Sperm Morphology and Sperm Concentration.

Assessment of the concentration of sperm showed a significant decrease ($p<0.05$) in the 40 % ethanol-treated group when compared to the other treatment groups and a non-significantly decrease ($p>0.05$) when compared to the control group and the 40 % ethanol + 75 mg/kg myricetin treated group (Table 4). The result on the percentage of normal sperm cells showed a significant decrease ($p<0.05$) in the 40 % ethanol-treated group when compared to the control group and other treatment groups.

Assessment of the percentage of bent neck sperm cells indicated that the 40 % ethanol-treated group had a non-significant increase ($p>0.05$) when compared to the control group and the other treated groups except for the group treated with 40 % ethanol + 75 mg/kg myricetin with no significant difference (Table 4). Further assessment of the percentage of curved sperm cells indicated a significant increase ($p<0.05$) in the 40 % ethanol-treated group when compared to the control group and other treatment groups.

Table 4 Sperm Morphology and Sperm Concentration

Sperm Concentration / Sperm Morphology				
Treatment groups	**Concentration (Cells x 106)**	**% Normal**	**% Bent Neck**	**% Curve**
Control	69.80 ± 6.537	72.50 ± 1.443	5.000 ± 0.000	22.50 ± 1.443
40% Ethanol	52.70 ± 6.723	62.00 ± 4.062*a	6.000 ± 1.000	32.00 ± 3.391*a
40% Eth. + 75 mg MYR	72.95 ± 3.213	72.00 ± 1.225*b	5.000 ± 0.000	23.00 ± 1.225*b
40% Eth. + 150 mg MYR	78.20 ± 2.757*b	72.00 ± 1.225*b	5.000 ± 0.000	23.00 ± 1.225*b
40% Eth. + 300 mg MYR	79.10 ± 4.881*b	72.00 ± 1.225*b	6.000 ± 1.000	22.00 ± 1.225*b
75 mg MYR	77.75 ± 2.524*b	75.00 ± 2.236**b	5.000 ± 0.000	20.00 ± 2.236**b
300 mg MYR	82.00 ± 5.851**b	76.00 ± 1.871**b	5.000 ± 0.000	19.00 ± 1.871**b
	P = 0.0049	P = 0.0026	P = 0.5827	P = 0.0022
	F = 4.041	F = 4.544	F = 0.7941	F = 4.681

Values are expressed in Mean ± SEM. *, **, ***, **** represents significant differences at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively. a = indicating a significant difference to Control. b = indicating a significant difference to the 40% Ethanol group. c = indicating a significant difference to 40% Eth. + 75 mg MYR group. d = indicating a significant difference to 40% Eth. + 150 mg MYR group.

Result of the effect of Myricetin on Semen Spermiography

Spermiograph of a control semen showing the absence of abnormal sperm cells (Figure A). Spermiograph of a 40 % ethanol-treated semen showing morphological abnormalities including Sperm head (red arrows), Short tail (black arrow), Sperm head and neck (yellow arrow) (Figure B). Spermiograph of a 40 % ethanol + 75 mg myricetin treated semen showing less morphological abnormalities, but having Sperm heads (red arrows) (Figure C). Spermiograph of a 40 % ethanol + 150 mg myricetin treated semen showing absence of abnormal sperm cells (Figure D). Spermiograph of a 40 % ethanol + 300 mg myricetin treated semen showing absence of abnormal sperm cells (Figure E). Spermiograph of a 75 mg myricetin-treated semen showing the absence of abnormal sperm cells (Figure F). Spermiograph of a 300 mg myricetin-treated semen showing the absence of abnormal sperm cells (Figure G).

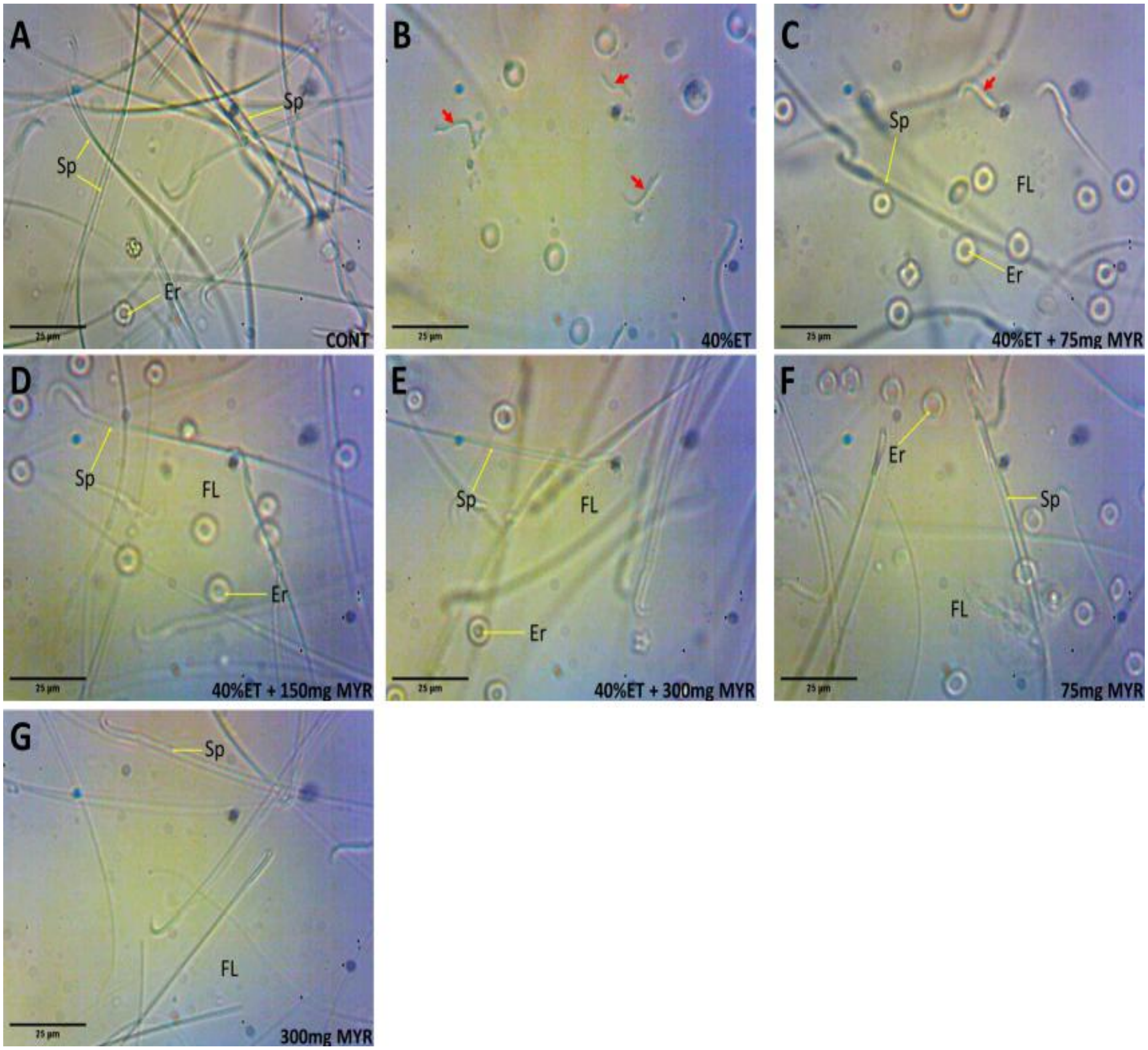

Figure A to G Spermiographs of seminal cells morphology in control and other treatment groups. Sp= Spermatozoa, FL = Semen fluid, C = Curved spermatozoa, Er = Erythrocytes, Sperm head (red arrows), Short tail (black arrow), Sperm head and neck (yellow arrow). Sp= Spermatozoa, Bn = Bent neck, Ct = Connective tissues, Ab = Air bubbles.

4. DISCUSSION

Results from this study show that the ethanol-administered group had an increase in testicular length, testicular diameter, and testicular weight, whereas, myricetin maintained the normal testicular morphology when compared to the control. These findings, however, contradict the position of Dosumu et al., (2014) who noted that alcohol intake has been documented to induce reproductive problems, including testicular atrophy and irregularities in the diameter of seminiferous tubules in men and experimental animals. Adaramoye et al., (2013) also observed degeneration and atrophy of seminiferous tubules leading to a reduction in cross-sectional areas of the testes in ethanol-treated rats. On the other hand, Han et al., (2024) did not observe any significant morphometric alteration in chronic ethanol-fed mice.

Asuquo et al., (2020) earlier noted that testis exposed to ethyl alcohol had no structural alteration, as effects were more evidenced in micro-structural changes. He further reasons that the ethanolic dosage may not have had a deleterious impact on its connective tissues, by exposing its tissues to inflammatory responses, including the tunica layer alteration. This suggests that ethanol's effects on the testis morphology may vary depending on factors linked to dosage of exposure, duration of exposure, and inter-species differences. This study, also, did not reveal any significant improvement in the testicular morphometric parameters in the ethanol + myricetin treated groups when compared to the ethanol-treated group. The morphology of spermatozoa, or the spermatic shape and structure, is a crucial parameter in semen analysis.

Abnormal sperm morphology can indicate underlying issues such as genetic defects, oxidative stress, or exposure to toxic substances (Moretti et al., 2022). Findings from this study show that ethanol consumption has detrimental effects on the spermatozoa. The spermiograph from this study reveals that the group administered with ethanol showed abnormal spermatozoa with short tails and malformed heads and necks. Previous researches indicate that alcohol consumption negatively impacts male reproductive function, particularly sperm parameters. Studies have shown that ethanol intake leads to decreased sperm count, motility, and normal morphology (Ricci et al., 2017). Pourentezari et al., (2016) also agreed that alcohol consumption in diabetic mice can intensify sperm chromatin/DNA damage, leading to abnormal sperm parameters and chromatin quality.

Ethanol significantly impacts sperm motility, vitality, and acrosome integrity in a dose-dependent manner (Bisconti et al., 2022). Sub-chronic ethanol exposure during stress can lead to reduced sperm viability, mortality, and increased apoptosis, potentially causing permanent male subfertility (Fozooni et al., 2021). In this study, myricetin reduced the population of abnormal spermatozoa in a dose-dependent manner in the groups treated with 40 % ethanol + myricetin. This finding shows that myricetin has a positive impact on sperm parameters. Previous studies have provided biochemical and histological evidence supporting myricetin's protective role on the spermatozoa. For example, myricetin treatment improved sperm count and motility and decreased sperm defects in ATZ-treated rats, indicating a restoration of normal reproductive function (Ikeji et al., 2023).

Lai et al., (2020) also reported that myricetin reduces the reproductive toxicity of cyclophosphamide in male mice, improving sperm density, movement, and vitality. Aquila et al., (2013) agreed that myricetin was found to potentiate sperm function, improving motility, viability, and other features even at low concentrations (up to 100 nM). The authors further added that myricetin has been shown to induce capacitation-associated biochemical changes, such as cholesterol efflux and tyrosine phosphorylation. These changes are essential for sperm to acquire the ability to fertilize an egg. Additionally, myricetin increases acrosin activity, glucose utilization, and fatty acid oxidation, which are vital for sperm energy metabolism and motility.

5. CONCLUSION

Ethyl alcohol is spermato-toxic and can destroy stored spermatozoa within the epididymis. Seminiferous tubular degeneration and atrophy result in a reduction in cross-sectional areas of the testes in ethanol-treated rats. Abnormal sperm morphology can indicate underlying issues such as genetic defects, oxidative stress, or exposure to toxic substances. Myricetin treatment improved sperm count and motility and decreased sperm defects in ethyl alcohol-treated rats, indicating a restoration capacity of myricetin against substance-based toxicity to normal reproductive function.

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Authors Contributions

EGA: Project administration, supervision, review, methodology, and validation. IEA: Investigation, conceptualization, writing-original draft, writing-review and editing, funding acquisition, resources, data curation, data analysis, and visualization. DIA: Resources and writing review and editing.

Ethical approval & declaration

In this article, the animal regulations followed as per the ethical committee guidelines of College of Medicine, University of Nigeria, Nsukka, Nigeria & Principle of Laboratory Animal Care and Use; the authors observed the spermatogenic effect of myricetin on ethyl-alcohol induced sperm toxicity in pre-pubertal male Wistar rats. Myricetin 3-Rhamnoside was ordered and purchased from Source Natural, USA. The Animal ethical guidelines are followed in the study for observation, identification & experimentation.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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