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Potential Reversibility of the Antifertility Effects of Papaya (*Carica papaya*) Seed Extract on Sperm Quality and Testicular Histopathology in Male Mice (*Mus musculus*)

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ABSTRACT

Indonesia is experiencing population growth, with contraceptive use predominantly undertaken by women due to the limited male options. Studies on papaya (*Carica papaya*) seeds have shown antifertility potential in animal models, but data regarding the systemic toxicity and reversibility remain limited. Therefore, this post-test-only experimental study aims to evaluate the potential reversibility of antifertility effects on sperm quality and testicular histopathology. The sample comprised 25 male ICR (Institute of Cancer Research) mice (*Mus musculus*), aged 2–3 months and weighing 20–30 g, divided into five groups (n=5), namely control, treatment (10% and 20% papaya seed extract), and reversibility groups. The extract was prepared by maceration and administered orally for 36 consecutive days, followed by a 36-day withdrawal period for the reversibility groups. Parameters assessed were sperm quality, testicular morphometry and histopathology, and liver function (AST, ALT). Testicular morphometry and liver enzyme levels showed no significant changes ($p > 0.05$). The extract significantly reduced sperm concentration in a dose-dependent manner ($p < 0.05$). Furthermore, histopathological evaluation showed widened interstitial spaces, dilatation of the seminiferous tubule lumen, and disorganization of germ cells in treated groups. Potential reversibility of sperm quality and histopathological evaluation was observed following extract withdrawal, particularly at a 20% concentration. These results suggested that papaya seed extract induced antifertility effects by disrupting spermatogenesis and testicular histology, with reversible outcomes and no significant hepatic toxicity. In conclusion, papaya seed extract showed safe, dose-dependent, and reversible antifertility effects in male ICR mice, supporting its potential as a male contraceptive.

Keywords: *Carica Papaya*, Antifertility, Testicular Histopathology, Sperm Quality, Reversibility

Introduction

The population of Indonesia is experiencing annual growth, with over 278 million people in 2023. Family Planning (FP) programs have effectively controlled birth rates, but participation is predominantly female, with injectable hormonal contraceptives being the most commonly used contraceptive (62.42%).[1] However, long-term use of hormonal contraceptives is associated with health risks, such as cervical cancer,[2] hypertension,[3] and vaginal epithelium Langerhans cells reduction.[4] Male contraceptive options remain limited to condoms and vasectomy. Previous studies have explored alternative male contraceptives, including natural substances, such as papaya (*Carica papaya*) seeds. Papaya seed extract has been shown to reduce sperm quality and alter testicular histology in animal models. Its secondary metabolites, including alkaloids, steroids, and triterpenoids, may disrupt hypothalamic-pituitary feedback, affecting FSH, LH, spermatogenesis, Leydig cell function, and testosterone synthesis.[5–8]

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Papain enzyme may also impair nutrient absorption necessary for sperm maturation. Disruption of androgen receptor signaling in Leydig cells further contributes to impaired spermatogenesis and histological changes in seminiferous tubules.[9,10] A previous study reported that papaya seed extract at certain doses did not induce hepatic toxicity.[11] However, data regarding the systemic toxicity and potential reversibility remain limited. Considering the need for safe and reversible male contraceptives, this study aims to evaluate the antifertility effects of papaya seed extract on sperm quality and testicular histopathology in male mice, as well as its toxicity and reversibility during extract administration and withdrawal.

Materials and Methods

Taxonomical Identification

Carica papaya L. cv Calina samples were collected from Gowa Regency, South Sulawesi, Indonesia (5°33′–5°34′ S, 120°33′–120°38′ E) in December 2024. The taxonomical identification of both the plant and animal models was carried out by Devi Armita, M.Sc., from the Biology Laboratory at the Faculty of Science and Technology, State Islamic University Alauddin, Indonesia (identification numbers 124/FST-BIO-LAB/PP.00.9/12/2024 and 125/FST-BIO-LAB/PP.00.9/12/2024). No voucher specimens were prepared or deposited because all the seeds were immediately processed for extraction after collection.

Extraction Preparation

A total of 6 kg of dried papaya seed powder was macerated in 96% ethanol for 24 hours at a ratio of 1:4 (w/v). The mixture was stirred

occasionally for 24 hours and filtered. The filtrate was concentrated using a rotary evaporator at 50°C to obtain a crude extract, which was then diluted using distilled water to achieve 10% and 20% concentrations.

Animal Model
The animals used were male ICR (Institute of Cancer Research) mice (*Mus musculus*) aged 2-3 months, weighing 20-30 g. Each mouse was under controlled laboratory conditions at a temperature of 22 ± 2°C with a 12-hour light/dark cycle, and given free access to food and water (*ad libitum*). Sawdust bedding was provided in each cage and replaced every 2 days, then mice were acclimatized for 7 days. Male and female mice were paired in the estrus phase at a 1:1 ratio and cohabited overnight to assess male fertility. The presence of a vaginal plug in female was recorded as evidence of successful copulation, and male was then separated. Pregnancy in female was monitored for 18–21 days, as shown by abdominal distension, weight gain, and behavioral changes, which confirmed the fertility of male animal models. All procedures followed the ethical standards approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University (No. 58/UN4.6.4.5.31/PP36/2025).

Experimental Design
Mice were randomly divided into five groups (n=5), namely control/C (no administration), two treatment groups, P1 & P2 (10% and 20% concentrations), and two reversible groups (R1 & R2) corresponding to each treatment. The treatment and reversible groups were treated orally at 10% and 20% concentrations (0.5 mL per animal/day) for 36 consecutive days. On day 37, treatment groups were euthanized while reversible groups started day 1 of the withdrawal period without extract administration for 36 days. The reversible groups were euthanized on day 37 of the withdrawal period. Euthanasia was carried out by injecting a mixture of ketamine (50 mg/kgBW) and xylazine (10 mg/kgBW) intramuscularly, followed by cervical dislocation. Blood, bilateral testes, and epididymis were collected for liver function assessment, histological examination, and sperm analysis, respectively.

Morphometry Evaluation
Morphometric evaluation of testes included weight, diameter, and volume. After removal of blood and connective tissue, each testes was weighed using a calibrated scale, and the diameter was determined with a digital caliper at the equatorial circumference. Each testis was immersed in a tube containing 0.9% NaCl, and the difference between the final and initial fluid volumes was calculated. Volume was measured by fluid displacement, and data for the right and left testes were recorded separately.

Sperm Analysis
Epididymal sperm were collected by mincing the cauda epididymis in 0.9% NaCl, followed by incubation at 37°C for 10–15 minutes to allow spermatozoa to diffuse. Samples were evaluated immediately under a light microscope. Normal reference values were motility >40–50%, abnormal morphology <20%, and concentration ≥8.11 ± 2.7 × 10^6/mL.[12] To assess sperm motility, a 10 µL drop of sperm suspension was placed on a slide, covered with a cover glass, and observed at 200× magnification. The proportion of motile spermatozoa was recorded as a percentage. Furthermore, a 20 µL drop of sperm was smeared, fixed in methanol, and stained with eosin–nigrosine to assess sperm morphology, and abnormal forms were assessed under 100× magnification. Sperm concentration was counted by diluting sample 1:10 with 0.9% NaCl, loading onto a hemocytometer, and counting spermatozoa in five large squares at 400× magnification.

Liver Function Assessment
Liver function was assessed by evaluating liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Blood samples were collected through the periorbital vein and cardiac puncture immediately following euthanasia, then centrifuged to obtain serum. AST and ALT serum levels were measured using an automated biochemical analyzer. The normal values used in this study were AST : > 70.40±27.63 U/L, ALT : > 30.30±20.10 U/L.[13]

Histopathological Evaluation
Testes were fixed in 10% neutral buffered formalin, followed by dehydration in graded alcohols, clearing in toluene, and paraffin embedding. Transverse sections of 3–4 µm thickness were cut using a microtome, mounted on slides, deparaffinized, and rehydrated. The sections were stained with hematoxylin and eosin (H&E), dehydrated, and mounted. Histological evaluation was carried out under a light microscope connected to a computer monitor.

Statistical Analysis
Statistical analysis was carried out using SPSS software version 27 for Windows. All data were tested for normality using the Shapiro–Wilk test because the sample size was less than 50. After the normality test, hypothesis testing was conducted using a One-Way Analysis of Variance (ANOVA) for normally distributed data, or the Kruskal–Wallis test when the data were not normally distributed. A post hoc test was applied to determine the differences between groups, and statistical significance was determined at $\alpha = 0.05$.

Results and Discussion

Effect of Carica papaya seed extract on testicular morphometry
Testicular morphometry data (Table 1) showed reductions in weight, diameter, and volume in P groups, particularly in the P2 group. Significant reduction was observed in left testicular weight, particularly between P2 and C ($p = 0.022$). The R groups showed weights close to the control values, suggesting potential reversibility of the effect. Testicular diameter suggested no significant differences for either left or right testes. The right testicular volume showed statistically significant differences ($p=0.022$), but the post hoc test was significant between P2:R1, which was excluded due to different extract concentrations. Left testicular volume also showed significant differences ($p = 0.033$). However, the post hoc Bonferroni correction showed no significant differences among groups. These results suggested that papaya seed extract induced testicular morphometry changes in a dose-dependent manner, which were reversible upon withdrawal of treatment. Administration of papaya seed extract led to a reduction in both the weight and diameter of testes. Nita et al. (2019) similarly observed a decrease in sperm concentration and seminiferous tubule diameter in rats receiving papaya seed extract, supporting the observed reduction in testicular weight and diameter in group P.[5] There was seminiferous tubule atrophy due to decreased spermatogenic activity, as evidenced by a reduction in germ cell populations (Table 4b). The results were consistent with the report of a previous study by Wiryawan et al. (2016), which reported significant reductions in sperm motility and concentration in male rats treated with papaya seed extract.[14]
Increased testicular weight was supported by improved sperm quality (Table 3). In the R1 group, the weight and diameter of testes continued to decline, while R2 showed improvement. This result could be explained by the concept of hormesis (stress-response hormesis), where exposure to higher doses leads to stronger recovery responses following withdrawal. High-dose exposure induces oxidative stress, thereby triggering stronger antioxidant defense and tissue repair mechanisms post-exposure.[15]
Testicular diameter measurements showed a similar dose-dependent trend. The smallest diameters were observed in the P2 group. Reduced diameter suggested seminiferous tubule atrophy and germinal epithelial degeneration.[16] However, statistical analysis showed no significant differences among the groups. Direct measurement of testicular diameter was limited in previous studies of papaya seed extract, making this data a valuable addition to testicular morphometry assessment.

Table 1: Result of testicular morphometry assessment

GROUPS	RTW (mg)	LTW (mg)	RTD (mm)	LTD (mm)	RTV (mL)	LTV (mL)
C	79.80±13.03	85.00±14.46	4.65±0.30	4.67±0.24	0.09±0.01	0.09±0.03
P1	74.00±9.49	70.20±10.66	3.80±0.88	3.66±0.67	0.11±0.04	0.10±0.07
P2	56.80±15.82	54.50±15.16[a]	3.50±1.39	3.26±1.29	0.08±0.05	0.08±0.05
R1	67.20±12.01	64.40±10.64	3.83±0.40	4.01±0.44	0.16±0.04	0.18±0.03
R2	77.00±14.92	71.40±19.00	3.49±1.12	3.56±1.08	0.12±0.03	0.12±0.02
p-value	0.083	0.042	0.290	0.124	0.022	0.033

Values are presented in Mean ± SD, significance level p<0.05. a : Tukey's post hoc test, significant difference relative to control, C: control, P1: 10% extract treatment group, P2: 20% extract treatment group, R1: reversible group of 10% extract treatment, R2: reversible group of 20% extract treatment. RTW : right testicular weight, LTW : left testicular weight, RTD : right testicular diameter, LTD : left testicular diameter, RTV : right testicular volume, LTV : left testicular volume

Testicular volume, which reflects a three-dimensional organ size provided a more comprehensive assessment than weight or diameter alone. P groups did not differ significantly from C, while R showed an increase in volume compared to C and P ($p < 0.05$). However, testicular volume showed a non-significant increasing trend after Bonferroni correction. This result was consistent with the study conducted by Ortego-Pacheco et al. (2010), who reported no significant decrease in testicular volume in dogs treated with 50 mg/kg/day papaya seed extract for 120 days, despite microscopic changes.[17] The increased testicular volume observed in R groups reflected tissue recovery, following withdrawal of treatment, supported by data of sperm quality (Table 3) and histopathological evaluation (Tables 4a & 4b).

Effects of Carica papaya seed extract on liver function

The data in Table 2 showed no significant differences in AST and ALT levels between P and R groups compared to C ($p>0.05$). These results were consistent with previous reports showing the absence of hepatotoxic effects and histopathological examinations of liver, kidney, and cardiac tissue in Wistar rats treated with papaya seed extract.[11,18] Another study reported that papaya seed extract improved liver injury, suggesting a potential hepatoprotective effect. This was attributed to the antioxidant activity of bioactive compounds in papaya seeds.[19]

Table 2: Result of liver function assessment

GROUPS	AST (U/L)	ALT (U/L)
C	160.00±42.66	84.40±20.68
P1	197.20±62.97	64.20±33.37
P2	198.20±56.86	68.80±38.22
R1	196.40±52.43	73.40±19.07
R2	143.40±56.82	62.20±18.02
p-value	0.385	0.715

Values are presented in Mean ± SD, significance level p<0.05

In this study, serum AST and ALT levels in all groups, including controls, were approximately two-fold higher than the normal values. The elevation showed the presence of non-treatment-related factors, such as animal handling stress, the use of ketamine–xylazine anesthesia, or injection-associated muscle injury. Handling procedures of mice could induce acute stress, increasing cortisol and corticosterone, thereby altering hepatocyte membrane permeability and slightly increasing AST and ALT levels.[20] Hernández-Godínez et al. (2019) showed that intramuscular administration of ketamine (4 mg/kg) combined with xylazine (0.5-1 mg/kg) could elevate AST and ALT levels within the first hour,[21] while Chen et al. (2020) showed transient hepatic metabolic disturbance and sinusoidal obstruction after the peritoneal injection of a mixture a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg). Ketamine-xylazine anesthesia, metabolized mainly in the liver, adds an acute hepatocyte load and can transiently increase ALT.[22] Xylazine's cardiovascular depressant effects can reduce hepatic perfusion, further contributing to cellular stress. Furthermore, AST elevation can partly originate from local muscle trauma at the intramuscular injection site, as AST is abundant in skeletal muscle. [21]

Effects of Carica papaya seed extract on sperm quality

Sperm quality parameters measured in this study included motility, abnormalities, and concentration. The results in Table 3 showed a decrease in sperm motility and concentration, accompanied by increased abnormalities in P1 and P2 groups in a dose-dependent manner, compared with the control. A statistically significant reduction ($p<0.05$) was observed in sperm motility in the R1 group ($p=0.006$) and concentration in P and R compared with the C group ($p=0.000$). These results were corroborated by histological evaluation showing reduced germ cell populations, characterized by thinning of the epithelium and widening of the seminiferous tubule lumen. Based on the results, antifertility effects of papaya seed extract were observed at concentrations of 10% and 20%. This result was consistent with previous studies, which concluded the antifertility effect of papaya seed extract.[5,14,23] Papaya seed extract impaired male reproductive function through both hormonal and oxidative stress mechanisms. This extract was also shown to inhibit steroidogenic enzymes essential for testosterone synthesis, such as aromatase and 17β-hydroxysteroid dehydrogenase.[24,25] Alkaloids and triterpenoids in the extract are known to disrupt hypothalamic-pituitary feedback mechanisms, thereby affecting LH and FSH production.[5,23] The major compound (benzyl isothiocyanate, BITC) promotes reactive oxygen species (ROS) formation, leading to mitochondrial dysfunction and oxidative stress.[26,27] Excessive ROS disrupts spermatogenesis, reduces sperm motility through lipid peroxidation and mitochondrial abnormalities, and increases morphological defects due to protein damage and DNA fragmentation, leading to reduced fertilization capability.[28,29] In addition, 1,2,3,4-tetrahydropyridin-3-yl-octanoate (B5C1) further reduced sperm motility and viability while increasing abnormal morphology.[30] In the R groups, R1 exhibited a further decline in sperm motility and concentration compared with P1, while R2 showed a potential reversibility relative to P2. This pattern suggested that low-dose exposure caused persistent germ cell damage without effectively stimulating compensatory or repair mechanisms, thereby delaying reversibility after treatment withdrawal. In this context, a higher dose induces stronger adaptive responses after the stressor removal. This paradoxical improvement could be explained by the concept of stress-response hormesis. The acute oxidative injury at higher doses activates robust antioxidant defenses and repairs pathways in the testes, facilitating spermatogenic recovery post-treatment.[15]

Effects of Carica papaya seed extract on testicular histology

The data in Table 4a showed a trend of reduced seminiferous tubule numbers in group P and an increase in group R compared to controls. The decrease observed in P groups could be attributed to the effects of benzyl isothiocyanate (BITC), which induced ROS, impaired spermatogenesis, and reduced testosterone synthesis.[26] Meanwhile, the increase in R groups suggested the activation of regenerative processes, consistent with the improvements of germ cell populations (Table 4b) and sperm quality (Table 3) observed in this study.

Table 3: Result of sperm analysis

GROUPS	Motility (%)	Abnormality (%)	Consentration (x10^6/mL)
C	51.68±7.23	14.00±5.29	12.71±2.59
P1	35.74±7.97	24.40±7.80	6.31±1.88[a]
P2	39.65±11.50	20.40±6.23	5.76±1.41[a]
R1	33.38±4.15[a]	14.80±2.28	4.71±0.95[a]
R2	40.85±2.79	20.80±6.87	7.01±2.22[a]
p-value	0.006	0.062	0.000

Values are presented in Mean ± SD, significance level p<0.05, a : Tukey's post hoc test, significant difference relative to control

Blood vessel count (Table 4a) showed no statistically significant differences among groups (p>0.05), but P and R exhibited an upward trend compared to C. Although papaya seed extract contains pro-oxidant compounds (such as BITC) that can induce oxidative stress, testes possess adaptive defense mechanisms to maintain oxygen and nutrient supply to germ cells. The data showed a compensatory angiogenesis process as part of tissue regeneration. Oxidative stress affects VEGF (Vascular Endothelial Growth Factor) signaling pathway through both VEGF-dependent and VEGF-independent mechanisms. Treatment withdrawal showed a revascularization process as part of the tissue recovery mechanism, and oxidative stress decreased, allowing VEGF angiogenic pathway to promote endothelial regeneration and new blood vessel formation.[31]

Interstitial space appeared normal in the control group, consistent with healthy testes in which seminiferous tubules were tightly packed and interstitial tissue was narrow and intact (Figure 1). The increased interstitial space in the P group, specifically P2 (p<0.05), showed intertubular damage, possibly due to interstitial edema from increased vascular permeability and local inflammation induced by oxidative stress.[32,33] Simultaneously, degeneration and desquamation of the germinal epithelium led to a reduction in the seminiferous tubule diameter, resulting in a relative widening of the surrounding interstitial compartment.[18] In R2, the interstitial space was reduced relative to the P2 group, while an increase was observed in R1 of the left testis. This reduction was attributed to the resolution of edema during recovery, alongside the regeneration of the germinal epithelium, which restored the seminiferous tubule diameter and consequently narrowed the intertubular distance, further supported by improvements in sperm quality (Table 3) and the increased germ cell population (Table 4b).

The results in Table 4b showed that papaya seed extract (P1 and P2 groups) reduced the number of spermatogenic cells at different developmental phases, particularly spermatocytes and spermatids, compared with the C group. The reduction was statistically significant for right and left spermatocytes (p=0.010 and p=0.048) and spermatids (p=0.017 and p=0.001), while spermatogonia showed no significant difference (p>0.05). This result showed that the extract predominantly disrupted the middle and late phases of spermatogenesis. Previous studies reported similar outcomes, showing that papaya seed extract decreased spermatocyte and spermatid populations without markedly affecting spermatogonia or Sertoli and Leydig cells.[34,35] This reduction was attributed to oxidative stress, a process that was implicated in the alterations of sperm quality described earlier. Excessive ROS can damage DNA, proteins, and cellular organelles in germ cells, thereby impairing spermatogenesis in parallel with the decline in sperm quality.[5,23-29]

Table 4a: Result of testicular histopathological observation

GROUPS	Seminiferous Tubules		Blood Vessel		Interstitial Space (%)	
	Right	Left	Right	Left	Right	Left
C	209.20 ± 40.15	219.40 ± 52.46	105.00 ± 37.67	103.40 ± 35.80	3.00 ± 4.47	1.20 ± 1.64
P1	206.60 ± 21.55	212.20 ± 15.45	117.60 ± 15.18	119.80 ± 26.75	6.20 ± 3.56	5.20 ± 2.86
P2	202.80 ± 32.85	169.80 ± 22.15	113.00 ± 58.63	100.40 ± 34.36	18.00 ± 13.04[a]	21.00 ± 17.46[a]
R1	234.80 ± 40.38	211.40 ± 31.43	148.60 ± 20.44	126.40 ± 37.78	6.60 ± 3.21	6.60 ± 3.21
R2	252.20 ± 51.00	201.40 ± 31.16	141.60 ± 54.01	124.00 ± 47.43	6.00 ± 2.24	12.00 ± 10.37[a]
p-value	0.224	0.100	0.117	0.507	0.330	0.040

Values are presented in Mean ± SD, significance level p<0.05, a: Tukey's post hoc test, significant difference relative to control

Table 4b: Result of testicular histopathological observation (continued)

GROUPS	SG		SC		ST		Lc	
	R	L	R	L	R	L	R	L
C	40.26± 3.67	40.90± 1.75	74.30± 8.21	79.20± 5.06	100.80± 18.13	111.80± 10.48	71.00± 17.96	71.40± 17.36
P1	32.70± 7.29	37.00± 14.83	52.00± 7.75	58.70± 22.35	72.80± 14.12	77.30± 27.90[a]	57.20± 21.83	45.40± 12.36
P2	32.50± 4.73	30.10± 10.13	49.10± 13.58[a]	46.20± 12.83[a]	66.90± 12.30	60.86± 9.97[a]	40.00± 12.14	39.40± 15.35[a]
R1	29.70± 5.67	29.60± 9.81	52.60± 11.70	57.10± 13.56	81.60± 13.35	77.50± 5.12[a]	64.40± 7.60	59.80± 13.61
R2	34.30± 11.48	40.30± 11.30	74.00± 17.24	67.10± 25.00	103.50± 17.05[b]	92.80± 15.88	69.80± 7.46	66.80± 13.59
p-value	0.233	0.192	0.010	0.048	0.017	0.001	0.064	0.010

Values were presented in Mean ± SD, significance level p<0.05, a: Tukey's post hoc test, significant difference relative to control, b: post hoc test, significant difference between P2:R2. SG:Spermatogonium, SC: Spermatocyte, ST: Spermatid, Lc : Leydig cell, R: Right, L: Left

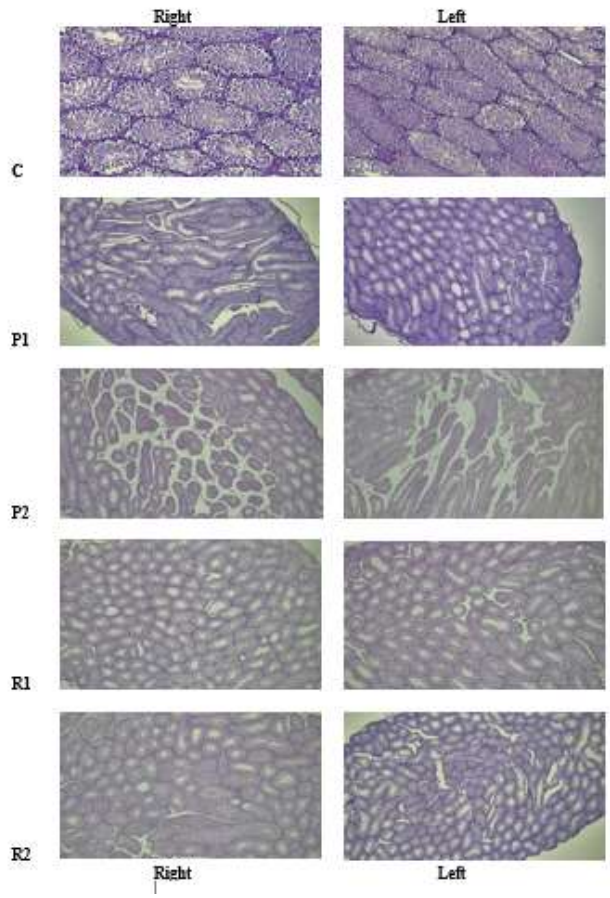

Figure 1: Representative histology of seminiferous tubules and interstitial spaces. C (magnification 100×) shows closely packed seminiferous tubules with germ cells arranged in concentric layers, and narrow lumens filled with spermatozoa. P1, P2, show widened interstitial spaces, whereas R1, R2 show reversibility, reflected by narrower interstitial spaces (magnification 40×)

A significant reduction in Leydig cell numbers was observed in group P2 compared with C ($p=0.018$). This result could be explained by oxidative stress mechanisms as described by Tang et al. (2024) and Monageng et al. (2023). ROS-induced lipid peroxidation in plasma and organelle membranes impairs cholesterol transport essential for steroidenesis. Mitochondria, the primary site of steroid hormone synthesis, are particularly vulnerable. Oxidative injury disrupts cholesterol transfer for pregnenolone formation, decreases mitochondrial membrane potential, and promotes cytochrome c release, leading to apoptosis. ROS also damage nuclear and mitochondrial DNA, with limited mtDNA repair capacity leading to a reduced expression of steroidogenic acute regulatory (StAR) protein and impaired steroid biosynthesis. Furthermore, ROS activate inflammatory signaling pathways, such as NF-κB, exacerbating cellular injury.[36,37]
R groups of germ and Leydig cell numbers showed a reversibility trend, getting closer to normal values ($p>0.05$). This result showed the regenerative capacity of cells following the treatment withdrawal. Recovery was closely related to the reduction of oxidative stress. Cells activate endogenous defense systems to restore homeostasis when ROS levels return to physiological ranges. Intracellular antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), neutralize residual ROS and prevente further lipid peroxidation. Phospholipid repair enzymes contribute to the restoration of membrane integrity and facilitate cholesterol transport. Additionally, progenitor Leydig cells in the interstitial tissue can differentiate into mature Leydig cells, replacing those lost to oxidative injury. Restoration of steroidogenesis is further supported by renewed expression of StAR protein and other key enzymes in testosterone biosynthesis. [36]
The histopathological evaluation in Figure 2 showed several specific lesions observed in the P and R groups. Giant cells, which are multinucleated cells undergoing degeneration, are formed through cytoplasmic bridge rupture and fusion, processes triggered by oxidative stress and Sertoli cell dysfunction.[16,38] Residual bodies, basophilic spherical structures, are formed from spermatid cytoplasmic remnants, suggesting impaired cytoplasmic clearance, due to oxidative stress–induced disruption of Sertoli cell phagocytic function.[16,39] Tubular lumen dilation with minimal or absent spermatozoa in P and R groups showed germ cell depletion. Furthermore, intratubular vacuoles were found in cytoplasm of Sertoli cells. These structures show the cell membrane injury and organelles dysfunction, namely mitochondria and endoplasmic reticulum.[40] Cytoplasmic vacuolization in interstitial cells show steroidogenesis that is disrupted.[16] Desquamation is characterized by the premature detachment of germ cells into the seminiferous tubule lumen. Papaya seed compound, BITC, induces oxidative stress, promotes lipid peroxidation, and disrupts Sertoli cell cytoskeletal integrity, weakening junctional complexes.[16,38] This process contributes significantly to reduced sperm quality, as many germ cells are shed before completing maturation. According to Widyanjaya et al. (2020), there were abnormal histological features in mice treated with papaya seed extract (20–50 mg/mouse/day), such as reduced tubule diameter, widened interstitial space, sparse germ cell layers, and fewer sperm in the tubule lumen.[10]

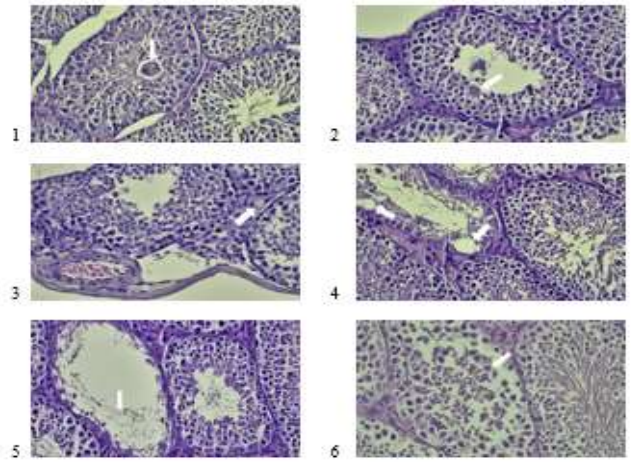

Figure 2: Representative histopathological lesions in testis observed in treated groups. 1. Giant cell (arrow), 2. Atypical residual body (arrow), 3. Intratubular vacuole (arrow), 4. Interstitial vacuole (arrow), 5. Dilatation of seminiferous tubule lumen, depletion of germ cells, absence of normal spermatozoa, only damaged spermatozoa (arrow), 6. Desquamation of germ cells (arrow). Magnification 400×

Conclusion

In conclusion, *carica papaya* seed extract induces safe, dose-dependent, and reversible antifertility effects in male ICR mice. This result shows the potential as a natural and temporary male contraceptive. Future studies focusing on oxidative stress, immunohistochemistry, and hormonal analyses are required for an advanced understanding of molecular mechanisms responsible for its antifertility effect and potential reversibility.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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