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Reversible Impairment of Male Reproductive Function and Acrosomal Enzyme Activity Following Exposure to a Benzene-Eluted Fraction from the Chloroform Extract of *Carica papaya* Seeds

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ABSTRACT

This study investigated the reversible effects of a benzene-eluted fraction of *Carica papaya* seed chloroform extract on male reproductive function and acrosomal enzyme activity. Phytochemical screening identified alkaloids as the predominant constituents, alongside flavonoids, anthraquinones, tannins, cardiac glycosides, and saponins. Seventy-two male rats (190-210 g) were randomly assigned into three groups: control (vehicle), 100 mg/kg, and 200 mg/kg. Treatments were administered orally for 21 consecutive days, followed by a 21-day withdrawal period, with evaluations conducted at 7-day intervals up to Day 42. Exposure resulted in significant ($p < 0.05$), dose- and time-dependent reductions across all assessed parameters. At the highest dose by Day 21, sperm count, motility, viability, and normal morphology decreased by approximately 51.4%, 54.2%, 53.3%, and 41.1%, respectively. Acrosomal enzyme activities were similarly suppressed, with acrosin and hyaluronidase reduced by up to 40.3% and 39.8%, respectively. Serum testosterone, acid phosphatase, and seminal sialic acid levels also showed marked dose-dependent declines, with maximum reductions of approximately 57.9%, 35.4%, and 48.3%, respectively. During the withdrawal phase (Days 28–42), all parameters exhibited progressive recovery toward control values; however, the high-dose group remained significantly lower than control at Day 42. The Composite Fertility Index further confirmed a substantial decline in overall fertilizing capacity during exposure, with incomplete restoration following cessation. Across all endpoints, the 200 mg/kg group consistently showed greater impairment than the 100 mg/kg group, indicating a clear dose-response relationship. These findings demonstrate that the benzene-eluted fraction of *Carica papaya* seed chloroform extract induces reversible, dose-dependent impairment of male reproductive function, likely mediated through coordinated disruption of spermatogenesis, acrosomal enzyme activity, and hormonal–biochemical processes.

Keywords: *Carica papaya*; benzene-eluted fraction; phytochemicals; spermatogenesis; acrosomal enzymes; hormonal–biochemical regulation; reversible toxicity

INTRODUCTION

Infertility continues to represent a major global concern in reproductive health, with male related factors accounting for a substantial proportion of cases [1]. Impairment of male fertility may result from a wide range of conditions, including congenital defects, endocrine abnormalities, environmental exposures, lifestyle influences, and idiopathic origins [2]. These factors can disrupt spermatogenesis or compromise sperm performance, leading to reduced sperm concentration, impaired motility, abnormal morphology, and decreased fertilizing capacity. While semen analysis is routinely employed in fertility evaluation, there is increasing reliance on functional and biochemical assessments to better characterize sperm competence [3].

Interest in plant derived substances as potential male fertility regulators has grown considerably in recent years [4], [5], [6]. Bioactive compounds from medicinal plants have been reported to influence reproductive processes through mechanisms involving oxidative balance, hormonal regulation, and direct effects on germ cell development and sperm maturation [7], [8]. Seeds of *Carica papaya* have long been utilized in traditional medicine for fertility control, and experimental findings indicate that their extracts can adversely affect sperm parameters and

reproductive potential [9], [10], [11], [12], [13]. These actions are thought to occur largely at the level of sperm maturation and post testicular functional modification.

Despite these observations, many studies have concentrated mainly on conventional sperm indices, with comparatively little attention given to acrosomal function and enzyme mediated fertilization processes. The acrosome contains enzymes such as acrosin and hyaluronidase that are essential for oocyte penetration, and disruption of their activity can hinder fertilization even when standard sperm parameters appear adequate [14], [15], [8], [16], [17]. In addition, the impact of chemically fractionated components of *Carica papaya* seed extracts on reproductive biochemical markers, including testosterone, acid phosphatase, and seminal sialic acid, remains insufficiently defined. These indices reflect androgenic activity, accessory gland function, and seminal fluid composition, and together provide a more comprehensive view of male reproductive status.

Another limitation of earlier investigations is the reliance on crude extracts, which contain complex mixtures of phytochemicals and may obscure the contribution of specific active constituents [10]. Fractionation using organic solvents enhances the resolution of bioactive components and allows for more precise evaluation of their biological effects. The benzene eluted fraction obtained from the chloroform extract of *Carica papaya* seeds represents a more concentrated phytochemical preparation that may be responsible for the observed reproductive effects [12].

Therefore, this study examines the reversible influence of this fraction on male reproductive function, with particular focus on sperm quality, acrosomal enzyme activity, and selected biochemical indices in adult male Wistar rats.

MATERIALS AND METHODS

Chemicals

Chloroform, benzene, glacial acetic acid, sodium acetate, hydrochloric acid, N-α-benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA), Hepes (N-2 hydroxyethylpiperazine-N'-2-ethanesulfonic acid), Triton X-100, Ficoll, dimethylsulfoxide (DMSO), and hyaluronic acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Silica gel (60-120 mesh), tris salt, sodium citrate, citric acid, sodium chloride, and calcium chloride were procured from Loba Chemie Pvt. Ltd. (India). Potassium tetraborate, potassium carbonate, disodium hydrogen orthophosphate and sodium dihydrogen phosphate were purchased from BDH Chemicals Ltd. (UK). All other chemicals and reagents used were of analytical grade.

Plant material

Ripe mature fruits of *Carica papaya* were collected in January from Basawa village, Zaria (11°04′00″ N, 7°42′00″ E), Kaduna State, Nigeria. The plant was authenticated, and a voucher specimen (No. 755) was deposited at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Seeds were separated from pulp, washed, air-dried under shade for two weeks, and pulverized using an electric blender (Waring Commercial, USA). The powdered sample was stored in airtight containers until use.

Preparation of benzene-eluted fraction

The benzene-eluted fraction was prepared from chloroform extract of *C. papaya* seeds according to [12] with slight modification. Seed powder (70 g) was extracted with 400 mL chloroform using Soxhlet apparatus at 58°C. Extraction was performed continuously in 12 successive cycles of 3 h each. The extract was filtered and concentrated at 45°C using a water bath. The crude extract was subjected to column chromatography on deactivated silica gel (60-120 mesh) using a glass column (1.5 × 30 cm). Elution with benzene yielded the benzene-eluted fraction, which was concentrated, dried, and stored for analysis.

Phytochemical screening

The benzene-eluted fraction of *Carica papaya* seed chloroform extract was subjected to qualitative and quantitative phytochemical analyses using standard procedures as described by [18].

Experimental animals

Sexually mature male albino Wistar rats (190 ± 20 g) were obtained from the National Veterinary Research Institute (NVRI), Jos, Nigeria. Animals were housed under standard laboratory conditions (12 h light–dark cycle) with free access to feed and water. Animals were acclimatized before experimentation. Only proven fertile males were used. All procedures were approved by the Faculty of Basic Medical Sciences Ethical Committee, University of Cross River State (UNICROSS/FBMSEC/2026-MB006), in accordance with international guidelines for laboratory animal care.

Experimental design

Seventy-two rats were randomly divided into three groups (n = 24 per group). Group I served as control and received corn oil (0.4 mL/kg/day). Groups II and III received benzene fractions of *C. papaya* seed extract orally at 100 mg/kg and 200 mg/kg body weight per day, respectively, for 21 days. Animals were sacrificed on Days 7, 14, and 21 (n = 4 per group per time point). For recovery studies, treatment was withdrawn after Day 21, and animals were sacrificed on Days 28, 35, and 42 (n = 4 per group per time point). Animals were anaesthetized with ketamine (90 mg/kg, i.p.) and sacrificed.

Sperm Quality Assessment

The cauda epididymis was carefully excised and immediately immersed in 0.9% normal saline at room temperature. The tissue was gently teased apart to facilitate the release of spermatozoa into the medium, producing a sperm suspension. The suspension was subsequently filtered through a fine metallic mesh to remove tissue debris and ensure a homogeneous sample. An aliquot of the filtrate was diluted (1:20, v/v) with 0.9% normal saline to obtain an appropriate sperm concentration for analysis. Sperm parameters, including concentration, motility, viability, and morphology, were evaluated using standard laboratory procedures in accordance with the World Health Organization guidelines [19], with minor modifications adapted for rodent samples.

Estimation of acrosin and hyaluronidase activities

Acrosin activity was determined spectrophotometrically at 25°C following the method of [20], with slight modifications for rat caudal epididymal sperm. Spermatozoa were obtained from the caudal epididymis, dispersed in phosphate-buffered saline (PBS, pH 7.2 - 7.4), and purified using Ficoll solution (11% Ficoll in 0.12 M NaCl and 0.025 M HEPES buffer, pH 7.4 - 7.6). The sperm pellet was washed, lysed, and proacrosin was activated to acrosin by incubation in Tris–HCl buffer (50 mM, pH 8.0) at 37°C under detergent-assisted conditions. The assay was performed in a total volume of 1.0 mL containing Tris–HCl buffer (50 mM, pH 8.0), $CaCl_2$ (50 mM), and N-α-benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA·HCl) prepared in dimethyl sulfoxide and diluted to working concentration with extender containing 0.01% Triton X-100 in HEPES-NaCl buffer. Benzamidine was added to control and test tubes to terminate the reaction and prevent non-specific proteolysis. The reaction mixture was incubated at 37°C, centrifuged at 1000 × g for 15 minutes, and the absorbance of the supernatant was measured at 410 nm against a reagent blank. Acrosin activity was calculated using the molar extinction coefficient of p-nitroaniline and expressed as micro–international units per 10^6 spermatozoa (μIU/10^6 sperm).

Hyaluronidase activity was determined spectrophotometrically based on the method of [21], which measures the release of N-acetyl-D-glucosamine end groups from hyaluronic acid. Spermatozoa obtained from the caudal epididymis were dispersed in phosphate-buffered saline (PBS, pH 7.2–7.4), washed, and lysed to obtain the enzyme extract. The assay was carried out using hyaluronic acid (1 mg/mL) as substrate in 0.1 M sodium citrate buffer (pH 4.5) containing 0.2 M NaCl. The enzyme extract (expressed as units/mL) was incubated with the substrate at 37°C for 15 minutes in a defined reaction volume. The reaction was terminated by the addition of potassium tetraborate ($K_2B_4O_7$, pH 9.1) and potassium carbonate (K_2CO_3), followed by heating at 100°C for 8 minutes and subsequent cooling on ice. p-Dimethylaminobenzaldehyde reagent was then added, and the mixture was incubated at 37°C for 10 minutes to allow color development. Absorbance was measured at 585 nm against appropriate blanks. Hyaluronidase activity was calculated using an N-acetylglucosamine standard curve. One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of N-acetylglucosamine per minute under the assay conditions. Results were expressed as units/mL and further normalized per 10^6 spermatozoa.

Seminal and biochemical markers

Acid phosphatase activity was measured in epididymal supernatant obtained after centrifugation of PBS-homogenized cauda epididymis (3000 rpm, 15 min). Analysis was performed using a BioVision kit (USA) according to manufacturer's instructions and expressed in U/L.

Seminal plasma was obtained from cauda epididymal suspension by centrifugation (3000 rpm, 15 min). Sialic acid concentration was measured using a BioAssay Systems kit (USA) and expressed as mg/mL.

Blood was collected via cardiac puncture, allowed to clot for 1 h, and centrifuged at 2000 rpm for 15 min to obtain serum. Testosterone was measured using a Diagnostic Automation kit (USA) according to manufacturer's instructions and expressed as ng/mL.

Combined Fertility Index (CFI) and Normalization

The Composite Fertility Index (CFI) was calculated as an integrative measure of sperm functional competence by incorporating sperm motility, morphology, concentration, and fertilization-related enzyme activities [22]. Percentage values for sperm motility and normal morphology were first converted to fractional form prior to computation. The CFI was derived using the expression: $CFI = (M/100) \times (NM/100) \times (SC) \times (AA) \times (HA) \div 10^4$ where M = sperm motility (%), representing the proportion of progressively motile spermatozoa; NM = normal morphology (%), representing the proportion of structurally normal spermatozoa; SC = sperm concentration ($\times 10^6$/mL); AA = acrosin activity (μIU per 10^6 spermatozoa); and HA = hyaluronidase activity (IU per 10^6 spermatozoa). A scaling factor (10^4) was applied to adjust the magnitude of computed values for ease of interpretation and graphical representation without altering relative differences between groups. This approach minimizes inter-parameter variability while preserving proportional contributions of individual components. The CFI is a dimensionless index.

To further enhance biological interpretability, CFI values were normalized relative to the control group to obtain the Rescaled Composite Fertility Index (RCFI). The mean control CFI across experimental time points (e.g. 0.075) was used as the reference baseline, and individual CFI values were divided by this value, yielding a dimensionless

index in which control values approximate unity (≈1.0). This normalization enables standardized comparison of fertility status across experimental groups. For interpretative purposes, RCFI values ≈1.0 indicate normal fertility, whereas values <1.0 indicate impaired fertility.

Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple comparisons. Statistical significance was set at $p < 0.05$. Graphical analyses were performed using GraphPad Prism (version 9.0).

RESULTS

The phytochemical analysis of the benzene-eluted fraction of *Carica papaya* seed chloroform extract confirmed the presence of all tested secondary metabolites (Table 1), including alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, and flavonoids. Quantitatively, alkaloids were the most abundant (0.20 ± 0.001 mg/100 g), followed by flavonoids (0.09 ± 0.002 mg/100 g) and anthraquinones (0.08 ± 0.002 mg/100 g). Lower levels were observed for tannins (0.06 ± 0.001 mg/100 g) and cardiac glycosides (0.06 ± 0.001 mg/100 g), while saponins recorded the least concentration (0.04 ± 0.003 mg/100 g). Overall, the fraction was dominated by alkaloids, with moderate contributions from flavonoids and anthraquinones, suggesting these as the major bioactive constituents.

Oral administration of the benzene-eluted fraction of *Carica papaya* seed chloroform extract significantly ($p < 0.05$) reduced sperm functional parameters in a dose- and time-dependent manner (Table 2). During treatment (Days 7 – 21), sperm count decreased from 46.15 ± 0.30 × 10^6/mL (control) to 28.04 ± 0.51 × 10^6/mL and 22.41 ± 0.45 × 10^6/mL in the 100 and 200 mg/kg groups, respectively (~ 39 – 51% reduction). Corresponding declines were observed in viability (75.51 ± 1.11% to 48.40 ± 1.54% and 35.22 ± 1.45%), motility (65.50 ± 1.10% to 40.00 ± 1.40% and 30.00 ± 1.34%), and normal morphology (85.50 ± 1.05% to 60.20 ± 1.42% and 50.40 ± 1.35%). Following withdrawal (Days 28 – 42), partial recovery occurred but values remained below control. By Day 42, sperm count improved to 40.00 ± 0.35 × 10^6/mL and 38.00 ± 0.32 × 10^6/mL (~ 13 – 17% residual reduction), with similar improvements in viability (68.00 ± 1.20% and 63.00 ± 1.31%), motility (58.00 ± 1.20% and 54.00 ± 1.24%), and morphology (78.00 ± 1.11% and 74.00 ± 1.26%). Overall, effects were consistently more pronounced in the 200 mg/kg group, confirming dose dependence.

The benzene-eluted fraction of *Carica papaya* seed chloroform extract significantly ($p < 0.05$) modulated sperm acrosomal enzyme activities in a dose- and time-dependent manner (Table 3). During treatment (Days 7 – 21), acrosin activity declined from 11.72 ± 0.38 μIU/10^6 sperm (control) to 8.50 ± 0.39 μIU/10^6 sperm and 7.00 ± 0.40 μIU/10^6 sperm in the 100 and 200 mg/kg groups, respectively. A similar reduction was observed for hyaluronidase (2.49 ± 0.04 to 1.90 ± 0.05 and 1.50 ± 0.03 U/10^6 sperm). Following withdrawal (Days 28 – 42), partial recovery was recorded, though values remained below control. By Day 42, acrosin activity increased to 11.20 ± 0.40 and 10.80 ± 0.42 μIU/10^6 sperm, compared with 12.00 ± 0.32 μIU/10^6 sperm in control, while hyaluronidase rose to 2.35 ± 0.05 and 2.20 ± 0.05 U/10^6 sperm. Across all time points, the 200 mg/kg group consistently showed greater suppression and slower recovery, confirming a dose-dependent inhibitory effect on acrosomal enzyme function.

The effect of the benzene-eluted fraction of *Carica papaya* seed chloroform extract on selected reproductive biochemical parameters was significant ($p < 0.05$), exhibiting dose- and time-dependent alterations in serum testosterone, serum acid phosphatase, and seminal sialic acid levels (Table 4). During treatment (Days 7 – 21), serum testosterone declined from 4.28 ± 0.05 ng/mL (control) to 2.70 ± 0.03 and 1.80 ± 0.02 ng/mL in the 100 and 200 mg/kg groups, respectively. Similar reductions were observed in serum acid phosphatase (4.18 ± 0.03 to 3.20 ± 0.02 and 2.70 ± 0.02 U/L) and seminal sialic acid (50.40 ± 0.50 to 30.50 ± 0.56 and 26.10 ± 0.47 mg/dL). Following withdrawal (Days 28 – 42), partial recovery occurred across all parameters, though values remained below control. By Day 42, testosterone increased to 3.80 ± 0.04 and 3.20 ± 0.03 ng/mL (vs 4.30 ± 0.05 ng/mL control), acid phosphatase to 3.90 ± 0.03 and 3.50 ± 0.03 U/L, and seminal sialic acid to 44.00 ± 0.49 and 40.00 ± 0.52 mg/dL in the 100 and 200 mg/kg groups, respectively. Overall, the 200 mg/kg group consistently exhibited greater suppression and slower recovery, confirming a dose-dependent effect.

The Composite Fertility Index (CFI) and its rescaled form (RCFI) showed significant ($p < 0.05$), dose-dependent reductions in overall sperm fertilizing capacity during the treatment phase (Day 21), with partial recovery observed following withdrawal (Day 42), as presented in Table 5 and Figure 1. At Day 21, the control group recorded a CFI of 0.074 ± 0.001 (RCFI: 1.00 ± 0.04), while marked declines were observed in the treated groups. The 100 mg/kg group showed a significant reduction to 0.011 ± 0.002 (RCFI: 0.15 ± 0.05), representing an approximate 85% decrease relative to control. A more pronounced suppression was evident in the 200 mg/kg group, with CFI reduced to 0.004 ± 0.001 (RCFI: 0.05 ± 0.04), corresponding to an approximate 95% decline. These reductions indicate severe impairment of overall sperm functional competence at peak exposure. Following withdrawal (Day 42), both indices exhibited significant recovery across treated groups. The control group remained stable (CFI: 0.076 ± 0.001; RCFI: 1.01 ± 0.03), while the 100 mg/kg group improved to 0.048 ± 0.002 (RCFI: 0.64 ± 0.04), reflecting substantial restoration of fertility potential. Similarly, the 200 mg/kg group increased to 0.036 ± 0.001 (RCFI: 0.48 ± 0.05),

indicating partial but incomplete recovery. Despite this improvement, both treated groups remained significantly lower than control values ($p < 0.05$), with the 200 mg/kg group consistently exhibiting greater suppression and slower recovery. Overall, the pattern of decline and recovery in CFI and RCFI closely mirrors changes observed in individual sperm parameters and biochemical indices, confirming a dose-dependent and partially reversible impairment of male reproductive function.

Table 1: Qualitative and Quantitative Phytochemical Analysis of the Benzene-Eluted Fraction of *Carica papaya* Seed Chloroform Extract

Parameters	Qualitative	Quantitative (mg/100g)
Alkaloids	+	0.20 ± 0.001
Saponins	+	0.04 ± 0.003
Tannins	+	0.06 ± 0.001
Anthraquinones	+	0.08 ± 0.002
Cardiac Glycosides	+	0.06 ± 0.001
Flavonoids	+	0.09 ± 0.002

Values are expressed as mean ± standard deviation (SD). Each determination was performed in triplicate, and "+" indicates presence

Table 2: Sperm Functional Changes During Treatment and Withdrawal Phases in Adult Male Rats Following Administration of the Benzene-Eluted Fraction of *Carica papaya* Seed Chloroform Extract

Parameters	Groups	Treatment Phase			Withdrawal (Post-treatment) Phase		
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Sperm Count ($\times 10^6$/mL)	Control	46.00 ± 0.35^a	46.20 ± 0.30^a	46.15 ± 0.30^a	46.10 ± 0.20^a	46.24 ± 0.34^a	46.00 ± 0.32^a
	100 mg/kg	42.10 ± 0.30^b	35.02 ± 0.40^b	28.04 ± 0.51^b	32.00 ± 0.42^b	36.52 ± 0.30^b	40.00 ± 0.35^b
	200 mg/kg	40.05 ± 0.40^c	30.20 ± 0.52^c	22.41 ± 0.45^c	27.06 ± 0.55^c	33.00 ± 0.42^c	38.00 ± 0.32^c
Sperm Viability (%)	Control	75.02 ± 1.02^a	76.01 ± 1.20^a	75.51 ± 1.11^a	76.05 ± 1.05^a	75.80 ± 1.10^a	75.05 ± 1.10^a
	100 mg/kg	70.10 ± 1.20^b	60.03 ± 1.35^b	48.40 ± 1.54^b	55.05 ± 1.42^b	62.12 ± 1.31^b	68.00 ± 1.20^b
	200 mg/kg	66.12 ± 1.34^c	50.00 ± 1.54^c	35.22 ± 1.45^c	45.20 ± 1.50^c	55.20 ± 1.45^c	63.00 ± 1.31^c
Sperm Motility (%)	Control	65.04 ± 1.10^a	66.00 ± 1.20^a	65.50 ± 1.10^a	66.20 ± 1.00^a	65.80 ± 1.10^a	65.00 ± 1.10^a
	100 mg/kg	60.12 ± 1.20^b	50.20 ± 1.35^b	40.00 ± 1.40^b	45.00 ± 1.32^b	52.00 ± 1.20^b	58.00 ± 1.20^b
	200 mg/kg	57.00 ± 1.30^c	42.04 ± 1.42^c	30.00 ± 1.34^c	38.00 ± 1.45^c	46.00 ± 1.30^c	54.00 ± 1.24^c
Sperm Morphology (%)	Control	85.00 ± 1.04^a	86.00 ± 1.10^a	85.50 ± 1.05^a	86.20 ± 1.13^a	85.80 ± 1.08^a	85.00 ± 1.02^a
	100 mg/kg	80.10 ± 1.20^b	71.00 ± 1.31^b	60.20 ± 1.42^b	65.00 ± 1.30^b	72.00 ± 1.22^b	78.00 ± 1.11^b
	200 mg/kg	76.50 ± 1.04^c	65.00 ± 1.42^c	50.40 ± 1.35^c	58.00 ± 1.40^c	66.00 ± 1.30^c	74.00 ± 1.26^c

Data are expressed as mean ± SD. Each group consisted of 24 rats, with 4 animals sacrificed at each time point (7-day intervals). Statistical differences were determined using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$. Different superscripts (a, b, c) indicate significant differences within the same time point. The study comprised treatment (Days 7-21) and reversal (Days 28-42) phases.

Table 3: Changes in Sperm Acrosomal Enzyme Activity During Treatment and Withdrawal Phases in Adult Male Rats Following Administration of the Benzene-Eluted Fraction of *Carica papaya* Seed Chloroform Extract

Parameters	Groups	Treatment Phase			Withdrawal (Post-treatment) Phase		
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Acrosin ($\mu IU/10^6$ sperm)	Control	12.05 ± 0.41^a	11.80 ± 0.36^a	11.72 ± 0.38^a	11.70 ± 0.40^a	11.90 ± 0.38^a	12.00 ± 0.32^a
	100 mg/kg	11.10 ± 0.45^b	9.50 ± 0.42^b	8.50 ± 0.39^b	9.80 ± 0.42^b	10.80 ± 0.40^b	11.20 ± 0.40^b
	200 mg/kg	9.50 ± 0.46^c	8.00 ± 0.40^c	7.00 ± 0.40^c	8.50 ± 0.41^c	10.00 ± 0.42^c	10.80 ± 0.42^c
Hyaluronidase ($U/10^6$ sperm)	Control	2.50 ± 0.05^a	2.48 ± 0.04^a	2.49 ± 0.04^a	2.50 ± 0.04^a	2.49 ± 0.05^a	2.50 ± 0.04^a
	100 mg/kg	2.20 ± 0.04^b	2.05 ± 0.03^b	1.90 ± 0.05^b	2.00 ± 0.03^b	2.25 ± 0.05^b	2.35 ± 0.05^b
	200 mg/kg	1.95 ± 0.05^c	1.75 ± 0.04^c	1.50 ± 0.03^c	1.70 ± 0.04^c	1.95 ± 0.04^c	2.20 ± 0.05^c

Data are expressed as mean ± SD. Each group consisted of 24 rats, with 4 animals sacrificed at each time point (7-day intervals). Statistical differences were determined using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$. Different superscripts (a, b, c) indicate significant differences within the same time point. The study comprised treatment (Days 7-21) and reversal (Days 28-42) phases.

Table 4: Changes in Selected Biochemical Parameters During Treatment and Withdrawal Phases in Adult Male Rats Following Administration of the Benzene-Eluted Fraction of *Carica papaya* Seed Chloroform Extract

Parameters	Groups	Treatment Phase			Withdrawal (Post-treatment) Phase		
		Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Serum Testosterone (ng/mL)	Control	4.25 ± 0.05^a	4.30 ± 0.06^a	4.28 ± 0.05^a	4.31 ± 0.06^a	4.26 ± 0.05^a	4.30 ± 0.05^a
	100 mg/kg	3.40 ± 0.04^b	3.00 ± 0.03^b	2.70 ± 0.03^b	2.50 ± 0.03^b	3.20 ± 0.05^b	3.80 ± 0.04^b
	200 mg/kg	2.80 ± 0.03^c	2.20 ± 0.03^c	1.80 ± 0.02^c	1.50 ± 0.04^c	2.50 ± 0.03^c	3.20 ± 0.03^c
Serum Acid Phosphatase (U/L)	Control	4.15 ± 0.05^a	4.20 ± 0.05^a	4.18 ± 0.03^a	4.20 ± 0.05^a	4.16 ± 0.04^a	4.20 ± 0.05^a
	100 mg/kg	3.60 ± 0.03^b	3.40 ± 0.03^b	3.20 ± 0.02^b	3.00 ± 0.02^b	3.60 ± 0.03^b	3.90 ± 0.03^b
	200 mg/kg	3.10 ± 0.02^c	2.90 ± 0.02^c	2.70 ± 0.02^c	2.50 ± 0.03^c	3.10 ± 0.05^c	3.50 ± 0.03^c
Seminal Sialic Acid (mg/dL)	Control	50.30 ± 0.60^a	50.10 ± 0.52^a	50.40 ± 0.50^a	50.50 ± 0.60^a	50.30 ± 0.54^a	50.15 ± 0.45^a
	100 mg/kg	45.20 ± 0.54^b	38.10 ± 0.48^b	30.50 ± 0.56^b	35.10 ± 0.52^b	40.20 ± 0.51^b	44.00 ± 0.49^b
	200 mg/kg	42.00 ± 0.20^c	34.50 ± 0.50^c	26.10 ± 0.47^c	30.25 ± 0.54^c	35.30 ± 0.50^c	40.00 ± 0.52^c

Data are expressed as mean ± SD. Each group consisted of 24 rats, with 4 animals sacrificed at each time point (7-day intervals). Statistical differences were determined using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$. Different superscripts (a, b, c) indicate significant differences within the same time point. The study comprised treatment (Days 7-21) and reversal (Days 28-42) phases.

Table 5: Composite Fertility Index (CFI) and Rescaled Composite Fertility Index (RCFI)

Phase	Group	CFI	RCFI
Day 21 (Treatment)	Control	0.074 ± 0.001^{c}	1.00 ± 0.04^{c}
	100 mg/kg	0.011 ± 0.002^{b}	0.15 ± 0.05^{b}
	200 mg/kg	0.004 ± 0.001^{a}	0.05 ± 0.04^{a}
Day 42 (Withdrawal)	Control	0.076 ± 0.001^{c}	1.01 ± 0.03^{c}
	100 mg/kg	0.048 ± 0.002^{b}	0.64 ± 0.04^{b}
	200 mg/kg	0.036 ± 0.001^{a}	0.48 ± 0.05^{a}

Values are expressed as mean ± SD (n=4). Different superscripts (a, b, c) indicate significant differences down the group with significance set at $p < 0.05$. CFI and RCFI are dimensionless.

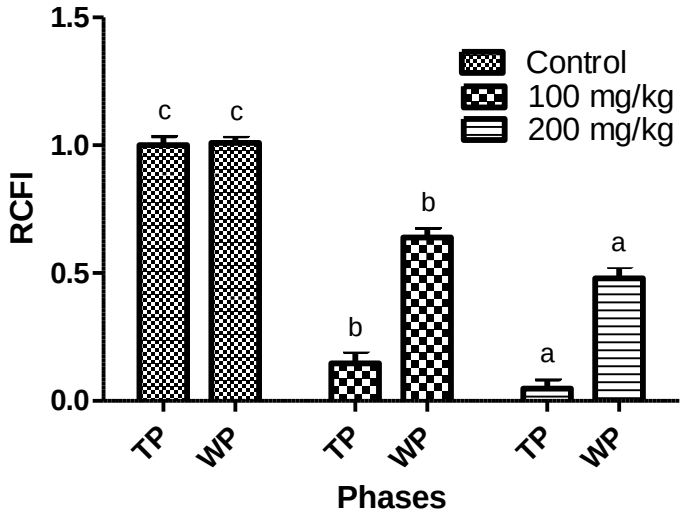

Figure 1: Effect of the Benzene-Eluted Fraction of *Carica papaya* Seed Chloroform Extract on the Rescaled Composite Fertility Index (RCFI) During Treatment and Withdrawal Phases (TP & WP)

DISCUSSION

The present study demonstrates that the benzene-eluted fraction of the chloroform extract of *Carica papaya* seeds exerts a significant, dose- and time-dependent impairment of male reproductive function, with partial reversibility following withdrawal. The coordinated alterations in sperm quality, acrosomal enzyme activity, hormonal profile, and Composite Fertility Index (CFI) indicate a broad disruption of reproductive physiology involving both testicular and post-testicular processes.

The observed reductions in sperm count, motility, viability, and normal morphology suggest interference with spermatogenic activity and epididymal sperm maturation. The simultaneous impairment of these parameters indicates disruption across multiple stages of sperm development and functional maturation. The partial recovery observed after cessation of treatment further suggests that the effect is largely reversible within the study period, although recovery was incomplete, particularly at the higher dose [7], [2].

The significant inhibition of acrosin and hyaluronidase activities indicates compromised fertilizing capacity, as these enzymes are essential for cumulus dispersion and zona pellucida penetration. This functional deficit suggests that exposure affects not only sperm production but also fertilization competence, likely through disruption of acrosomal stability and enzymatic functionality [23], [8].

Endocrine disturbance appears to be a key underlying mechanism, as reflected by the reduction in serum testosterone [24], [25]. This suggests impaired Leydig cell steroidogenesis and/or disruption of hypothalamic–pituitary–gonadal axis regulation. The accompanying decreases in acid phosphatase and seminal sialic acid further indicate dysfunction of accessory sex glands and epididymal secretory activity, with reduced seminal sialic acid contributing to decreased sperm membrane stability and functional resilience [26].

Importantly, the phytochemical profile of the fraction dominated by alkaloids with contributions from flavonoids and anthraquinones, provides a plausible biochemical basis for these effects. Alkaloids have been widely associated with anti-fertility activity through suppression of steroidogenesis and germ cell proliferation, while flavonoid and anthraquinone constituents may contribute to oxidative stress–mediated impairment of sperm membrane integrity and enzyme activity [11], [6], [18].

The Composite Fertility Index (CFI) and its rescaled form (RCFI) showed a marked, dose-dependent decline following exposure to the benzene-eluted fraction of *Carica papaya* seed chloroform extract, indicating a significant reduction in overall male fertility potential through integrated disruption of sperm quality, acrosomal enzyme activity, and endocrine function [22]. The pronounced suppression, especially at the higher dose, reflects a systemic impairment of reproductive competence consistent with individual parameter changes [28]. Following withdrawal, both indices exhibited partial recovery, suggesting reversibility of the effect [24]; however, values remained below control, particularly in the high-dose group, indicating incomplete restoration of full reproductive function within the study period.

In conclusion, the benzene-eluted fraction of *Carica papaya* seed chloroform extract induces a reversible but significant suppression of male reproductive function through combined endocrine, spermatogenic, and enzymatic disruption. The phytochemical composition, particularly the predominance of alkaloids, likely contributes to these effects. These findings underscore the reproductive sensitivity to concentrated plant fractions and highlight the need for caution in their potential use. Further studies are warranted to elucidate specific molecular pathways and confirm long-term reversibility beyond the current experimental window.

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Conflicts of Interest

The author declares no conflict of interest.

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