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In-Vivo Evaluation of Vrishya Karma (Spermatogenic & Reproductive Potency) of Kadamba (*Neolamarckia cadamba* (Roxb.) Bosser) in Nicotine-Induced Oligospermia Model

Focusing on Epididymal Sperm Kinetics, Hormonal Regulation, Biochemical Markers, and Testicular Histopathology

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ABSTRACT

Background: Male infertility accounts for 40–50% of global infertility cases, with oligospermia (low sperm count, correlated with Ayurvedic *Ksheena Shukra*) being a predominant cause. Traditional Ayurvedic medicine emphasizes *Vajikarana Chikitsa* using *Vrishya* herbs to revitalize reproductive function. *Kadamba* (*Neolamarckia cadamba* (Roxb.) Bosser) is classically documented as a potent *Shukravardhana* and *Vrishya* agent; however, robust empirical *in-vivo* validation remains scarce.

Objective: To evaluate the *in-vivo* *Vrishya Karma* (spermatogenic and fertility-enhancing efficacy) of Kadamba stem bark (*Twak Churna*) against nicotine-induced oligospermia in male albino rats.

Materials and Methods: Adult male albino rats (150–200 g, n=6/group) were divided into 4 experimental groups: Group A (Normal Control), Group B (Disease Control: Nicotine 1 mg/kg/day, s.c. for 30 days induction + continued), Group C (Test Group: Nicotine 1 mg/kg s.c. + Kadamba Bark *Churna* 90 mg/kg P.O.), and Group D (Reference Test Group: Nicotine 1 mg/kg s.c. + Karpas Seed *Churna* 270 mg/kg P.O.). Following an 8-week treatment protocol, epididymal sperm parameters (count, motility, viability, morphology), serum testosterone, lipid profile, metabolic markers, reproductive organometry, and testicular histopathology were assessed.

Results: Administration of Kadamba (90 mg/kg P.O.) significantly restored epididymal sperm count ($p < 0.001$) and percentage motility compared to the disease control group. Kadamba treatment exhibited marked elevation in serum testosterone levels, reduction in morphological sperm abnormalities, and mitigation of nicotine-induced oxidative damage in seminiferous tubules. Histopathological examination confirmed reversal of germinal epithelial arrest and restoration of Leydig cell density.

Conclusion: *Kadamba* possesses potent *Vrishya* and *Shukra-Janana* activity, effectively mitigating gonadotoxic oligospermia via hormonal amplification, antioxidant defense, and revival of active spermatogenesis.

Keywords: Kadamba, *Neolamarckia cadamba*, *Vrishya Karma*, *Ksheena Shukra*, In-Vivo Study, Oligospermia, Testosterone, Spermatogenesis.

1. INTRODUCTION

Ayurveda, the eternal science of life, considers healthy reproduction as one of the essential pillars of human existence. Conception and healthy progeny depend upon four critical factors: *Ritu* (favorable reproductive season/phase), *Kshetra* (healthy reproductive system/uterus), *Ambu* (nutritive fluids), and *Beeja* (healthy gametes—*Shukra* and *Artava*). Any pathological imbalance in these factors directly leads to reproductive impairment and infertility.

In contemporary medical literature, male infertility contributes to approximately 30–50% of overall infertility cases. A major etiology of male factor subfertility is **Oligospermia**, defined as a sperm concentration of less than 15 million/mL in ejaculated semen. Modern lifestyle stresses, environmental pollutants, excessive tobacco/nicotine consumption, endocrine disruptors, and nutritional deficiencies cause oxidative stress in testicular tissue, disrupting the hypothalamic-pituitary-gonadal (HPG) axis and impairing spermatogenesis.

In classical Ayurvedic texts, oligospermia is closely correlated with **Ksheena Shukra** (a primary form of *Shukra Dushti*). Acharya Sushruta defines *Ksheena Shukra* as a state characterized by depletion in both the quantitative volume and qualitative integrity of *Shukra Dhatu*, primarily driven by the vitiation of *Vata* and *Pitta Doshas*. To manage this debility, Ayurveda offers a dedicated specialized branch known as **Vajikarana Chikitsa** (Aphrodisiac and Reproductive Therapeutics), utilizing *Vrishya* and *Shukrala Dravyas* to nourish *Shukra Dhatu*, enhance virility, and promote healthy offspring.

कदम्बिस्तिक्स्तिकटुकः कषायो वास्तिनाशनः। शीस्तिलः कफपित्तास्तिक्त्ति-नाशनः शुक्रवर्धनः ॥

— *Raja Nighantu, Prabhadradi Varga 98*

Kadamba (*Neolamarckia cadamba* (Roxb.) Bosser, Family: Rubiaceae) is a renowned Ayurvedic medicinal plant traditionally hailed for its *Shukravardhana* (semen-enhancing), *Veeryavridhikar* (potency-boosting), and *Vrishya* properties. Its Ayurvedic pharmacodynamic profile includes *Tikta*, *Katu*, *Kashaya Rasa*; *Ruksha Guna*; *Sheeta Veerya*; and *Katu Vipaka*. While classical Nighantus emphasize the reproductive properties of Kadamba stem bark (*Twak*), there is a crucial gap in empirical scientific validation through systematic *in-vivo* animal experimentation.

Therefore, the current study was undertaken to scientifically investigate and establish the *in-vivo* *Vrishya Karma* of Kadamba bark in a nicotine-induced oligospermic rat model, evaluating sperm kinetics, endocrine responses, biochemical parameters, and testicular architecture.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Drug Preparation

Authentic stem bark of *Kadamba* (*Neolamarckia cadamba* (Roxb.) Bosser) was collected from clean natural habitats and authenticated at the Department of Dravyaguna Vigyan, M.M.M. Government Ayurveda College, Udaipur. A herbarium voucher specimen was deposited for future reference. The stem bark was shade-dried, pulverized into a fine powder (*Churna*) using an electric grinder, passed through mesh sieve #80, and stored in airtight containers. Pharmacognostical and physicochemical standardization (moisture content, ash values, alcohol/water-soluble extractives, pH, and TLC profiling) were conducted as per Ayurvedic Pharmacopoeia of India (API) standards.

2.2 Experimental Animals and Ethical Clearance

Healthy adult male albino rats (Wistar strain) weighing 150–200 g were procured from a CPCSEA-approved animal facility. Animals were housed in standard polypropylene cages under controlled laboratory conditions (temperature $22 \pm 3^\circ\text{C}$, relative humidity 50–60%, and 12 h light/dark cycle). They were provided with standard pellet feed and purified drinking water *ad libitum*. The experimental protocol received prior ethical approval from the Institutional Animal Ethics Committee (IAEC) as per Form B submission.

2.3 Study Design and Experimental Groups

The animals were acclimatized for 7 days prior to experimentation. A total of 24 rats were randomly assigned to 4 experimental groups (n = 6 per group):

Table 1: Experimental Grouping and Treatment Protocol

Group ID	Group Name	Sample Size (n)	Treatment Protocol & Dosage
Group A	Normal Control	6	Normal saline / vehicle only; no induction.
Group B	Disease Control (Nicotine)	6	Nicotine (1 mg/kg/day, subcutaneous) for 30 days induction + continued.
Group C	Test Item 1 (Kadamba)	6	Nicotine (1 mg/kg s.c.) + Kadamba Bark Churna (90 mg/kg/day P.O.)
Group D	Test Item 2 (Karpas Ref.)	6	Nicotine (1 mg/kg s.c.) + Karpas Seed Churna (270 mg/kg/day P.O.)

2.4 Induction of Oligospermia and Drug Administration

Oligospermia was induced by subcutaneous administration of Nicotine hydrogen tartrate at a dose of 1 mg/kg body weight once daily for 30 consecutive days in Groups B, C, and D. Following induction, nicotine administration was maintained alongside oral administration of test formulations (Kadamba bark *Churna* 90 mg/kg in Group C; Karpas seed *Churna* 270 mg/kg in Group D) suspended in distilled water for an additional 30 days (total experimental duration: 60 days / 8–10 weeks).

2.5 Semen Analysis and Epididymal Sperm Evaluation

At the end of the 60-day treatment period, animals were euthanized under light ether anesthesia. The testes and cauda epididymis were carefully dissected and weighed. The cauda epididymis was placed in 2 mL of pre-warmed (37°C) Phosphate Buffered Saline (PBS, pH 7.4) and gently minced to release spermatozoa.

- **Epididymal Sperm Count:** Sperm concentration was calculated using a Neubauer Hemocytometer following Trypan Blue staining under light microscopy (400x magnification) and expressed as $\times 10^6$ cells/mL.
- **Sperm Motility:** A drop of sperm suspension was placed on a pre-warmed glass slide, covered with a coverslip, and percentage progressive motility was determined visually across 10 random microscopic fields.
- **Sperm Viability & Morphology:** Viability was assessed via Eosin-Nigrosin dye exclusion test. Morphological abnormalities (head defects, coiled tails, bent midpieces) were quantified by counting 200 sperm per slide.

2.6 Biochemical Assays and Hormonal Profiling

Blood samples were collected via cardiac puncture, allowed to clot at room temperature, and centrifuged at 3000 rpm for 15 minutes to separate serum. Serum levels of **Testosterone** were measured using Enzyme-Linked Immunosorbent Assay (ELISA) kits. Metabolic markers including serum glucose, total cholesterol, triglycerides, and total protein were analyzed spectrophotometrically using standard diagnostic kits.

2.7 Histopathological Examination

Testicular tissues were fixed in 10% neutral buffered formalin, dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections of 5 μ m thickness were cut using a microtome and stained with Hematoxylin and Eosin (H&E). Tubular diameter, germinal epithelial height, and Leydig cell architecture were qualitatively and quantitatively examined under optical light microscopy.

2.8 Statistical Analysis

Data were expressed as Mean $\pm$ Standard Deviation (SD). Statistical significance between groups was analyzed using One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Effect on Body Weight and Reproductive Organ Weights

Nicotine administration (Group B) resulted in a significant decrease in testicular weight (1.12 ± 0.08 g) and epididymal weight (0.24 ± 0.03 g) compared to Normal Control Group A (1.68 ± 0.11 g and 0.42 ± 0.04 g, respectively; $p < 0.001$). Treatment with **Kadamba bark Churna (90 mg/kg)** significantly prevented testicular atrophy and restored relative testicular weight to 1.52 ± 0.09 g ($p < 0.01$ vs Group B).

Table 2: Effect of Kadamba on Testicular and Epididymal Weights in Rats

Experimental Group	Initial Body Wt (g)	Final Body Wt (g)	Testes Weight (g)	Epididymis Weight (g)
Group A (Normal Control)	178.5 $\pm$ 8.2	224.3 $\pm$ 10.5	1.68 $\pm$ 0.11	0.42 $\pm$ 0.04
Group B (Disease Control)	181.2 $\pm$ 7.8	189.6 $\pm$ 9.2####	1.12 $\pm$ 0.08####	0.24 $\pm$ 0.03####
Group C (Kadamba 90 mg/kg)	179.8 $\pm$ 8.5	215.4 $\pm$ 11.2**	1.52 $\pm$ 0.09**	0.37 $\pm$ 0.03**
Group D (Karpas 270 mg/kg)	180.4 $\pm$ 8.0	212.1 $\pm$ 10.8**	1.48 $\pm$ 0.10*	0.35 $\pm$ 0.04*

Values are Mean $\pm$ SD (n=6). ####p < 0.001 vs Group A; **p < 0.01, *p < 0.05 vs Group B.

3.2 Effect on Epididymal Sperm Parameters

Subcutaneous nicotine exposure induced profound oligospermia in Group B, causing a drastic decline in epididymal sperm count ($22.4 \pm 3.1 \times 10^6/mL$) compared to normal control Group A ($68.5 \pm 5.2 \times 10^6/mL$; $p < 0.001$). Furthermore, progressive sperm motility dropped to 28.5% and abnormal sperm morphology surged to 34.2%.

Co-administration of **Kadamba bark Churna (90 mg/kg P.O.)** remarkably reversed nicotine-induced gonadotoxicity, increasing sperm count to $56.8 \pm 4.2 \times 10^6/mL$ (a 153.5% increase over Group B; $p < 0.001$). Active progressive motility significantly rose to 68.4%, sperm viability reached 78.2%, and morphological defects dropped significantly to 12.1%.

Table 3: Epididymal Sperm Profile Following Treatment with Kadamba

Group & Treatment	Sperm Count ()	Sperm Motility (%)	Viability (%)	Abnormal Morphology (%)
Group A (Normal)	68.5 $\pm$ 5.2	82.4 $\pm$ 4.1	88.6 $\pm$ 3.8	6.8 $\pm$ 1.2
Group B (Nicotine Control)	22.4 $\pm$ 3.1####	28.5 $\pm$ 3.5####	41.2 $\pm$ 4.2####	34.2 $\pm$ 3.1####
Group C (Kadamba 90 mg/kg)	56.8 $\pm$ 4.2***	68.4 $\pm$ 4.8***	78.2 $\pm$ 3.9***	12.1 $\pm$ 1.8***
Group D (Karpas 270 mg/kg)	52.1 $\pm$ 3.8***	64.2 $\pm$ 4.2***	73.5 $\pm$ 4.1***	14.5 $\pm$ 2.0***

Values are Mean $\pm$ SD (n=6). ###p < 0.001 vs Group A; ***p < 0.001 vs Group B.

Key Finding — In-Vivo Spermatogenic Efficacy:

Kadamba bark treatment (90 mg/kg) achieved a **2.53-fold increase in epididymal sperm count** and a **2.40-fold increase in sperm motility** compared to the untreated oligospermic group, demonstrating direct *Shukra-Janana* and *Vrishya* activity.

3.3 Serum Testosterone and Biochemical Profile

Serum testosterone levels were markedly suppressed in nicotine-exposed Group B (1.15 ± 0.18 ng/mL vs 4.25 ± 0.32 ng/mL in Group A; $p < 0.001$). Kadamba administration significantly elevated serum testosterone levels to 3.48 ± 0.28 ng/mL ($p < 0.001$), indicating restoration of Leydig cell endocrine output and HPG axis stability.

Table 4: Biochemical and Serum Testosterone Markers Across Groups

Parameters	Group A (Normal)	Group B (Nicotine)	Group C (Kadamba)	Group D (Karpas)
Serum Testosterone (ng/mL)	4.25 ± 0.32	$1.15 \pm 0.18^{###}$	$3.48 \pm 0.28^{***}$	$3.12 \pm 0.25^{***}$
Serum Total Protein (g/dL)	7.2 ± 0.4	$5.1 \pm 0.3^{##}$	$6.8 \pm 0.4^{**}$	$6.5 \pm 0.3^*$
Total Cholesterol (mg/dL)	88.4 ± 5.2	$124.6 \pm 8.1^{###}$	$96.2 \pm 6.0^{**}$	$102.4 \pm 7.2^*$
Triglycerides (mg/dL)	72.1 ± 4.5	$112.5 \pm 7.8^{###}$	$82.4 \pm 5.1^{**}$	$88.1 \pm 6.2^{**}$

3.4 Histopathological Observations

- **Group A (Normal Control):** Intact seminiferous tubules lined with regular germinal epithelium showing all stages of active spermatogenesis (spermatogonia, primary spermatocytes, spermatids, and abundant luminal spermatozoa) with healthy interstitial Leydig cells.
- **Group B (Disease Control):** Severe testicular disorganization, marked degeneration and vacuolation of germinal epithelium, arrest of spermatogenesis, empty tubular lumens devoid of spermatozoa, and Leydig cell atrophy.
- **Group C (Kadamba Treated):** Substantial histological recovery with well-organized seminiferous tubules, densely packed germ cells, prominent restoration of spermatogenesis up to mature spermatozoa in lumens, and regenerated interstitial Leydig cells.

4. DISCUSSION

The findings of this *in-vivo* investigation provide convincing scientific evidence supporting the classical Ayurvedic claims regarding the **Vrishya Karma** (spermatogenic and fertility-enhancing potential) of *Kadamba* (*Neolamarckia cadamba* (Roxb.) Bosser).

4.1 Pharmacodynamic Rationale (Ayurvedic Perspective)

According to Ayurvedic pharmacology, Kadamba bark possesses *Tikta*, *Katu*, *Kashaya Rasa*, *Sheeta Veerya*, and *Katu Vipaka*. While *Tikta-Kashaya Rasa* acts as *Kaphapittahara* and cleanses the *Srotas* (microchannels), its overall systemic action promotes *Shukradhatu Agni*. Classical literature specifically assigns **Shukravardhana** and **Veeryavridhikar** properties to Kadamba. In *Ksheena Shukra* (oligospermia), chronic exposure to toxins like nicotine causes *Srotorodha* (channel blockage) and vitiates *Vata-Pitta Dosha*, leading to premature depletion of *Shukra Dhatu*. Kadamba restores *Srotas* patency, alleviates *Pitta-Vata* imbalances, and provides metabolic nourishment (*Brimhana*) to the reproductive apparatus.

4.2 Mechanism of Action in Nicotine-Induced Gonadotoxicity

Nicotine exerts its gonadotoxic effects through lipid peroxidation, free radical generation (reactive oxygen species—ROS), and disruption of the Leydig cell membrane, which suppresses the LH-stimulated synthesis of testosterone. The resulting hypoandrogenic environment causes germ cell apoptosis and arrests spermatogenesis.

The therapeutic efficacy of Kadamba bark (90 mg/kg P.O.) operates through a multi-target mechanism:

1. **Androgenic Stimulation & HPG Axis Revival:** Kadamba treatment led to a significant elevation in serum testosterone levels (3.48 ± 0.28 ng/mL). Active phytoconstituents (such as cadambine, cadambagenic acid, indole alkaloids, and flavonoids) in Kadamba stimulate Leydig cell steroidogenesis, providing the required intratesticular testosterone threshold necessary to maintain quantitative spermatogenesis.
2. **Antioxidant Defense & Cytoprotection:** Polyphenols and flavonoids in Kadamba act as powerful free radical scavengers, mitigating nicotine-induced oxidative stress in testicular cell membranes. This protects sperm cell membrane integrity, leading to a marked decrease in sperm head/tail morphological abnormalities (from 34.2% down to 12.1%).
3. **Revival of Germinal Epithelium & Sperm Kinetics:** By protecting Sertoli cell junctional complexes and enhancing metabolic enzyme activity, Kadamba promotes germ cell proliferation and differentiation, directly explaining the observed 153.5% increase in epididymal sperm density and enhanced progressive motility.

5. CONCLUSION

The present study systematically validates the classical Ayurvedic therapeutic profile of **Kadamba** (*Neolamarckia cadamba* (Roxb.) Bosser) as a potent *Vrishya* and *Shukrajana* agent. Oral administration of Kadamba bark *Churna* at a dose of 90 mg/kg effectively ameliorates nicotine-induced oligospermia in male albino rats by elevating serum testosterone, restoring epididymal sperm count, enhancing sperm motility and viability, and preserving testicular histoarchitecture. These results provide strong empirical justification for integrating Kadamba as a safe, natural, and cost-effective phytotherapeutic option for managing male infertility and oligospermia.

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