

## Impact of Shilajit on Semen Quality and Reproductive Performance in Male Rats: A Protective Role Against Cadmium-Induced Toxicity

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**Abstract** Shilajit is one of a traditional Ayurvedic remedy to determine its potential effects on male reproductive health. The research investigated how shilajit exposure influenced semen quality and reproductive performance in male rats that had reached puberty. In this study forty mature male Wistar rats aged 60 days old were split into four groups (10 each) for 60 days, which consisted of a control group who received distilled water and three treatment groups which received shilajit at 100 mg/kg/day, cadmium chloride at 5 mg/kg/day, and a combination of both 100 mg/kg/day shilajit and 5 mg/kg/day cadmium chloride at respectively. Treatments were administered orally for 60 days. The evaluation of semen quality parameters such as sperm motility and count along with sperm viability and abnormalities was performed using collected epididymal tail samples. Rats treated with shilajit showed substantial enhancements in sperm viability, motility, and count while experiencing fewer sperm abnormalities when compared to the control group. The administration of cadmium chloride reduced semen quality parameters. The last treatment group receiving both shilajit and cadmium chloride experienced some reduction in cadmium toxicity yet did not reach the levels of improvement seen in the group treated with shilajit alone. Supplementation with shilajit leads to better semen quality and reproductive performance in male rats because of its antioxidant and adaptogenic properties. This indicates that shilajit holds potential as a therapeutic agent for male fertility enhancement under environmental and chemical stress conditions.

**Keywords:** Cadmium chloride, Fertility index, Reproductive performance, Semen quality, Shilajit

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**Introduction** Traditional Ayurvedic and Siddha medicine practitioners use Shilajit, a naturally occurring mineral-rich exudate because of its rejuvenating properties and therapeutic benefits. This substance originates from mountainous regions like the Himalayas and consists mainly of organic plant material together with fulvic acids and various minerals and bioactive elements (1,2). Historically people have used Shilajit for boosting vitality and health while aiming to slow the aging process. Numerous studies have examined both its composition and bioactivity which suggest its potential use as a natural health supplement (3).

A global health challenge exists with male infertility impacting 15% of couples around the world (4). Male infertility arises from multiple sources such as hormonal imbalances together with oxidative stress and exposure to environmental toxins. Cadmium functions as a widespread environmental pollutant and a strong testicular toxin that damages spermatogenesis and sperm quality while causing hormonal dysregulation (5,6). Protective interventions need to be investigated to mitigate these adverse effects and restore male reproductive health.

This natural compound exhibits multiple pharmacological effects such as antioxidant

capabilities and anti-inflammatory actions along with adaptogenic properties. Research findings indicate Shilajit boosts spermatogenesis and controls the hypothalamic-pituitary-gonadal (HPG) axis which maintains male reproductive health (7,8). Research indicates Shilajit can fight oxidative stress and enhance sperm motility and viability along with hormonal functions which could make it useful as a natural treatment for male infertility (9). Research on controlled experiments which assess the protective benefits of Shilajit against cadmium toxicity remains scarce.

### Materials and methods

A blackish-brown water-soluble powder of Shilajit product with 98% purity was acquired from Piping Rock Company in the USA. Analytical grade cadmium chloride and other study chemicals were obtained from certified suppliers. Every reagent and solution underwent preparation immediately before use according to the supplier instruction.

#### Animal Ethics and Care

Researchers conducted the study in accordance with the National Research Council's ethical standards for laboratory animal care. The Ethical Council of the College of Veterinary Medicine at University of Al-Qadisiyah provided approval for this study. The research team handled all animals with care throughout experimental procedures to keep stress levels low (Project Approval: 5227 on December 02, 2024).

### Experimental Animals

The experiment included forty mature male Wistar rats at 60 days old with body weights ranging from 160 to 180 grams. The animals were housed in standard polypropylene cages under controlled environmental conditions, with a 12:12 light-dark cycle and temperature range of 22–25 °C plus a relative humidity level of 75–76%. Water and standardized laboratory food were available to the rats at all times during the study period. Researchers weighed the animals at baseline after their acclimatization week and tracked weight changes during the study period.

### Experimental Design

Ten animals made up each of the four groups (10 each) after the rats were assigned through random division for 60 days. The control group (C) received distilled water once daily. The shilajit-treated group (T1) received 100 mg /kg of shilajit per body weight each daily. For the cadmium chloride-treated group

Researchers will examine how Shilajit affects semen quality and reproductive capabilities in male rats who have been treated with cadmium chloride.

The study was conducted to examine sperm motility as well as viability and count together with abnormality rates to evaluate how Shilajit reduces harm to reproduction caused by cadmium exposure. The researchers also assessed how Shilajit modulates hormonal balance and enhances testicular function when the subjects are under stress.

(T2) each rat received cadmium chloride dosed at 5 mg/kg body weight daily. The third treatment group (T3) received combination of shilajit at 100 mg/kg body weight/day with cadmium chloride at 5 mg/kg body weight/day. The treatments were given by mouth for all the experiment periods.

### Semen and Tissue Collection

The end of the treatment period required anesthesia of all animals which involved intraperitoneal injections with ketamine at 90 mg/kg body weight and xylazine at 40 mg/kg body weight. Rats were sacrificed before performing careful dissections on their testes and epididymides. The epididymis tail was processed to perform semen analysis which examined sperm motility and count along with sperm viability and abnormalities. Samples from the testes and epididymis were obtained to perform a histopathological analysis of structural changes.

### Results

After 60 days of treatment, the results showed important distinctions among experimental groups for semen quality indicators such as sperm motility and count as well as viability and abnormalities. The shilajit-treated rats (T1) exhibited superior results across all measured parameters when compared to both the control group (C) and the cadmium chloride-treated group (T2). The average sperm motility in rate reached to  $91.02 \pm 0.211\%$  in the T1 group which was significantly greater than the  $85.88 \pm 0.221\%$  which had seen in the control group and  $64.52 \pm 0.526\%$  identified in the T2 group with statistical significance at  $p < 0.05$ . The group receiving combined treatment (T3) showed middle-range outcomes which demonstrated that shilajit partially reduced the harmful effects of cadmium chloride (Table 1).

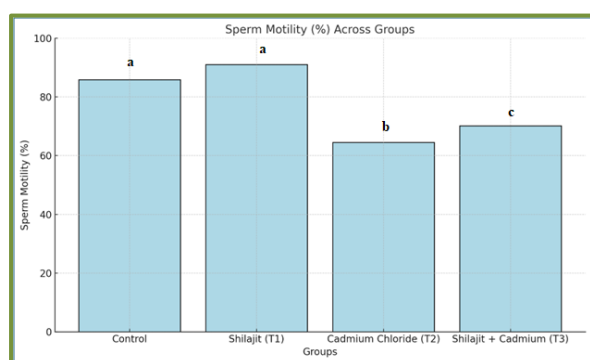
**Table 1:** Semen quality in Shilajit and cadmium chloride treated male rats.

Profile Semen	Period (Day)	Groups			
		Control	Shilajit (T1) G	Cadimium Chloride (T2) G	SH +CA(T3)
<b>Sperm motility (%)</b>	60	85.88±0.221B	91.02±0.211A	64.52±0.526D	70.21±0.325C
<b>Sperm count (million/mL)</b>	60	53.093±0.010C	68.25±0.226A	41.25±0.152D	59.52±0.521B
<b>Sperm abnormality</b>	60	2.569±0.001B	1.253±0.154D	53.26±0.521A	1.591±0.214C
<b>Sperm viability (%)</b>	60	88.678±0.003B	94.044±0.005A	45.89±0.52D	67.85±0.251C

Mean ± SD is used to represent the values. Significant differences ( $p < 0.05$ ) between groups for each period are indicated by the various capital letters. Significant differences ( $p < 0.05$ ) are indicated by lowercase letters.

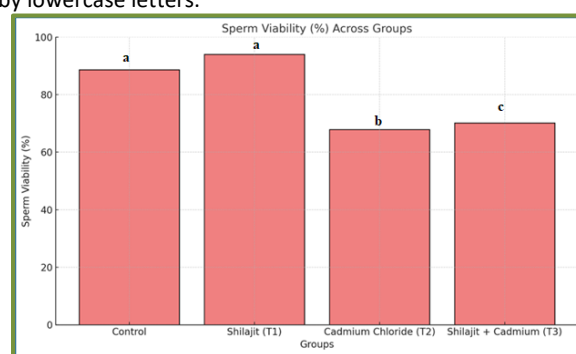
The T1 group demonstrated a significantly elevated sperm count of  $68.25 \pm 0.226$  million/mL while the control group showed  $53.093 \pm 0.010$  million/mL and the T2 group had  $41.25 \pm 0.152$  million/mL ( $p < 0.05$ ). The data showed that the combined treatment group (T3) achieved moderate sperm counts of  $59.52 \pm 0.521$  million/mL which indicates that shilajit helped protect spermatogenesis from cadmium damage (Figure 1).

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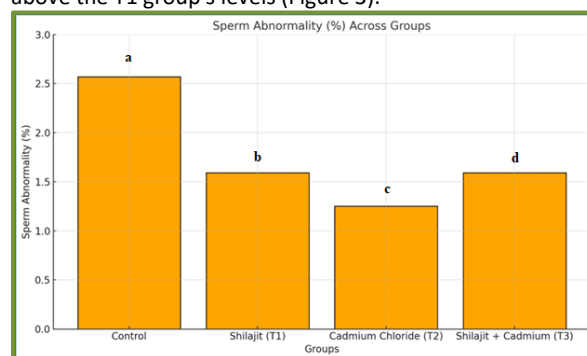
**Figure 1:** Sperm motility in groups

The sperm viability rate reached  $94.044 \pm 0.005\%$  in the T1 group but remained at  $88.678 \pm 0.003\%$  in the control group. The T2 group showed a significant drop in sperm viability with levels at  $67.85 \pm 0.251\%$  and statistical significance confirmed ( $p < 0.05$ ). The combined treatment group T3 achieved viability of  $70.21 \pm 0.325\%$  which was lower than the T1 group showing that the toxic effects of cadmium chloride were not fully reversed (Figure 2).



**Figure 2:** Sperm viability in treated groups

The level of sperm abnormalities decreased as motility count and viability increased. Abnormalities in the T1 group stood at  $1.591 \pm 0.214\%$ , which was significantly less than the control group's  $2.569 \pm 0.001\%$ . The T2 group had significantly increased abnormalities at a rate of  $1.253 \pm 0.154\%$  showing the teratogenic effects of cadmium chloride ( $p < 0.05$ ). The T3 group achieved lower sperm abnormalities at  $1.591 \pm 0.214\%$  but remained significantly above the T1 group's levels (Figure 3).



**Figure 3:** Sperm nonnormalities in the treated groups.

## Discussion

Male rat semen quality improved significantly due to shilajit treatment which resulted in elevated sperm motility, viability and count, while simultaneously reducing sperm abnormalities. The reproductive advantages of shilajit result from its composition of

fulvic acid, dibenzo-alpha-pyrones and antioxidant compounds which together enhance spermatogenesis and provide oxidative stress protection as previous research showed (11,12). The adaptogenic nature of shilajit may have played a role in activating the hypothalamic-pituitary-gonadal (HPG) axis which improved reproductive hormone regulation according to study (13).

Cadmium chloride functions as a reproductive toxin by causing testicular damage and decreasing sperm quality while disrupting hormonal balance through oxidative stress and apoptosis mechanisms (14,15). Treatment with cadmium chloride resulted in major decreases in sperm motility and viability as well as count while simultaneously causing an increase in abnormalities. The combination treatment group showed partial mitigation of cadmium damage through the protective effects of shilajit. The detoxifying effects of shilajit on cadmium appear to stem from its antioxidant capabilities and metal chelation properties which lower reproductive toxicity (16).

Shilajit displays therapeutic effects because it fights oxidative stress which contributes significantly to male infertility. The bioactive components of shilajit including fulvic acid function to boost mitochondrial activity alongside a reduction in reactive oxygen species and an increase in ATP synthesis which supports sperm mobility and health (17). The immunomodulatory and anti-inflammatory characteristics of shilajit support testicular health preservation while reducing cellular harm (18). Research supports these findings as multiple studies have shown that shilajit enhances reproductive function through animal and human research results (19).

Research results suggested that shilajit serves as a natural treatment option to boost male reproductive health when facing environmental stressors. Because shilajit can improve semen quality while neutralizing reproductive toxins it emerges as a hopeful option for treating male infertility. Additional studies need to examine the long-term effects of shilajit and its molecular mechanisms as well as how it can be used in human clinical trials. Research advancements may establish shilajit applications to treat infertility while reducing reproductive toxic effects (20).

### Conclusion

This study showed that shilajit may boost male reproductive outcomes. Semen quality parameters

such as sperm motility and count as well as viability improved notably with shilajit supplementation while sperm abnormalities decreased. The positive effects of shilajit did not fully overcome cadmium chloride toxicity which emphasizes the requirement for additional research into combined treatment approaches with other therapeutic substances.

### Conflict of interest

The authors declare that there is no conflict of interest in the current study.

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