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Profertility effects of Shilajit on cadmium-induced infertility in male mice

Raghav Kumar Mishra[1] | Ashish Jain[2] | Shio Kumar Singh[1]

[1]Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India

[2]Department of Microbiology, Smt. Chandibai Himathmal Mansukhani College, University of Mumbai, Ulhasnagar, Thane, India

Correspondence
Raghav Kumar Mishra, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi-221005 (U.P.), India.
Email: raghavmishra@rediffmail.com

Abstract

Shilajit is claimed as a Vajikarak (aphrodisiac) and used for the treatment of male infertility by traditional healers of the Indian subcontinent. Therefore, the present investigation was designed to assess the effectiveness of Shilajit for treatment of male infertility resulting from exposure to perilous chemicals. Effect of daily oral administration (p.o.) of Shilajit (50 mg, 100 mg and 200 mg/Kg BW) was investigated for a single spermatogenic cycle (35 days) in cadmium-induced (2 mg/Kg BW, p.o. for 35 days) infertile adult (12–14 week) swiss male mice. Shilajit treatment increased weights of reproductive organs, testicular daily sperm production, activities of testicular Δ^5 3β-HSD and 17 β-HSD enzymes and serum level of testosterone. Histopathological evaluation of testis revealed that Shilajit restored spermatogenesis as reflected by a gradual augmentation in germ cell layers with increased doses of Shilajit compared to cadmium-treated mice. Further, Shilajit treatment reverted back the adverse effects of cadmium on motility and concentration of spermatozoa. Secretory activities of the epididymis and seminal vesicle and libido, fertility and the number of litters per female were also improved by Shilajit in cadmium-treated mice. Results thus suggest the potent androgenic nature of Shilajit and its role in fertility improvement against cadmium-induced infertility.

KEYWORDS

aphrodisiac, fertility, Shilajit, sperm dynamics, spermatogenesis

1 | INTRODUCTION

Since the inception of human civilisation, reproductive health has been considered as one of the important components of the well-being because of its religious and psychological impact on social life (Sheoran & Sarin, 2015). Males are responsible for 50% of all infertility cases among 15%–20% of infertile couples of the world (Agarwal, Mulgund, Hamada, & Chyatte, 2015). Major causes of male infertility are environmental pollution, stress and changes in lifestyle (Jenardhanan, Panneerselvam, & Mathur, 2016; Yao & Mills, 2016). Male infertility treatment is often related to issues of efficacy, cost, ease of application and the side effects. The use of natural products are as ancient as the human civilisation, and several herbs such as *Withania somnifera*, *Asparagus racemosus*, *Curculigo orchioides and Chlorophytum borivilianum* have been used for treatment of male infertility in India (Lohiya, Balasubramanian, & Ansari, 2016; Thakur et al., 2011). However, clinicians have been hesitant to support herbal rehabilitation, owing to a lack of quality assurance for the safety and efficacy of these supplements (Muneer, Kalsi, Nazareth, & Arya, 2014).

Shilajit (Asphaltum) is a thick, sticky and yellowish to dark brown exudation in the Himalayan ranges at altitudes between 1000 to 5000 m. Shilajit is a compact humic substance (60%–80%) containing fulvic acid, benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3, 4-benzocoumarins, amino acids and phenolic lipids (Carrasco-Gallardo, Guzman, & Maccioni, 2012). In Indian medicinal system (ayurveda), Shilajit is an established rejuvenator and prescribed

to treat genitourinary disorders, digestive disorders and diabetes (Agarwal, Khanna, Karmarkar, Anwer, & Khar, 2007). Modern systematic research has scientifically validated a number of medicinal properties such as antioxidant, anti-inflammatory, chemoprotectant and immunomodulator, thereby advocating Shilajit as a truly panacea in traditional medicine (Stohs, 2014).

In Indian medicinal system (ayurveda), Shilajit has also been ascribed as a potent vajikarak (aphrodisiac) and is used to treat male sexual dysfunction (Stohs, 2014; Wilson et al., 2011). Few scientific studies also seem to support, in part, the androgenic effect of Shilajit on male reproductive health (Biswas et al., 2010; Kaur, Kumar, Kumar, Kharya, & Singh, 2012; Mishra, Verma, Singh, & Singh, 2012; Park, Kim, & Hana, 2006). The vajikarak (aphrodisiac) potential of Shilajit is highly acclaimed in ancient texts (Agarwal et al., 2007; Park et al., 2006). However, the notion that whether Shilajit could also be used as a natural protectant against exposure to harmful reproductive toxicants remains unclear. Therefore, there is a need for a mechanistic approach to validate the efficacy of Shilajit as natural aphrodisiac, which can protect the male reproductive organs from toxic chemicals and can also serve as a medicine for male infertility treatment.

The objective of this study, therefore, was to find out whether Shilajit could improve the male fertility after exposure to perilous chemicals. For this purpose, we have selected a well-known male reproductive toxicant, viz. cadmium which enters the body via a number of routes including food, water and air (WHO, 2010; Manfo, Nantia & Mathur, 2014; Yari et al., 2016). We have evaluated the efficacy of Shilajit, following cadmium exposure on daily sperm production, serum testosterone level, testicular steroidogenic enzymes (Δ^5 3β-HSD and 17β-HSD), epididymal spermatozoa, secretory functions of the epididymis and seminal vesicle, and on fertility to ascertain its efficacy as a natural medicine for male infertility management.

2 | MATERIALS AND METHODS

2.1 | Test material and chemicals

Shilajit as natural supplement was purchased from Dabur India Ltd. (Batch No. JK0065). To ensure homogenous solution for animal treatment, content of Shilajit capsule was suspended in Milli-Q water at 20% concentration (w/v) for 5 hr at 35°C and filtered through 40-micron filter. The filtrate was then evaporated to dryness in a hot air oven at 35°C and stored at room temperature in an airtight container. Cadmium ($CdCl_2$, Merck, CAS No. 35658-65-2) was dissolved in distilled water and used.

All other chemicals used in this study were of analytical grade and were purchased from HiMedia Laboratories, Mumbai or Merck India Ltd., Mumbai, unless stated otherwise.

2.2 | Animals and treatment

Forty adult (32–34 g) Swiss albino male mice of 12–14 weeks of age were procured from the colony of the Central Drug Research Institute. The animals were maintained under uniform husbandry conditions (temperature 25 ± 2°C, humidity 55%–60% and photoperiod 12L: 12D). Animals were provided standard pellet diet and drinking water ad libitum. Mice were randomly divided into five groups (eight mice /group) and housed in separate polypropylene cages (430 × 270 × 150 mm) with stainless steel grill-top and dry rice husk as bedding material. The study was approved by the Institutional Animal Ethics Committee. The doses of Shilajit (50, 100 and 200 mg/kg BW) and cadmium (2 mg/kg) were chosen as per Trivedi, Mazumdar, Bhatt, and Hemavathi (2004) and Ola-Mudathir, Suru, Fafunso, Obioha, and Faremi (2008), respectively. Doses were administered orally as follows:

Group	Treatment	Duration (days)	Autopsy (days after last treatment)
I	Control (distilled water; 200 μl/mouse/day)	70	1
II	$CdCl_2$ (2.0 mg/kg BW)	35	36
III	$CdCl_2$ (2.0 mg/kg BW) for 35 days followed by Shilajit (50 mg/kg BW) for 35 days	70	1
IV	$CdCl_2$ (2.0 mg/kg BW) for 35 days followed by Shilajit (100 mg/kg BW) for 35 days	70	1
V	$CdCl_2$ (2.0 mg/kg BW) for 35 days followed by Shilajit (200 mg/kg BW) for 35 days	70	1

2.3 | Autopsy

Animals were autopsied (except group II) after 24 hr of the last treatment by decapitation, whereas animals treated with cadmium alone (group II) were autopsied 35 days after the last treatment. At the time of autopsy, body weight of all animals was recorded and the trunk blood was collected. The testes, epididymis and seminal vesicle were excised, blotted free of blood and secretions, and weighed to nearest 0.10 mg.

2.4 | Daily sperm production

To evaluate the effect of different treatments on daily sperm production in testis, elongated spermatids (step 14–16 spermatids) resistant to sonication were counted according to Meistrich and van Beek (1993). In brief, one testis was placed in 1 ml ice-cold distilled water, homogenised and sonicated for 1.5 min. The sample was counted in a haemocytometer under a phase contrast microscope at 40X. In mice spermatogenesis, developing spermatids spend 4.84 days

TABLE 1 Effects of Shilajit on body weight, and absolute and relative weight (per 100 g of body weight) of the reproductive organs of male mice rendered infertile by cadmium treatment

Group	Body weight		Testis		Epididymis		Seminal vesicle	
	Initial (g)	Final (g)	Absolute (mg)	Relative (mg/100 gBW)	Absolute (mg)	Relative (mg/100 gBW)	Absolute (mg)	Relative (mg/100 gBW)
I DW	32.00 ± 0.00	32.87 ± 0.99	116.03 ± 6.74	358.46 ± 15.69	59.49 ± 3.01	181.41 ± 7.41	126.52 ± 7.71	386.55 ± 34.29
II Cd	32.00 ± 0.00	31.12 ± 0.99	54.47* ± 10.57	176.48* ± 37.88	32.77* ± 5.93	105.68* ± 18.36	73.36* ± 6.16	236.88* ± 21.87
III Cd+S50	34.00 ± 0.00	33.5 ± 0.70	62.08* ± 4.53	187.49* ± 38.74	39.98* ± 3.89	120.40* ± 10.78	88.38* ± 5.33	266.10* ± 10.08
IV Cd+S100	34.00 ± 0.00	34.12 ± 0.83	86.34* ± 7.06	253.73* ± 16.03	46.36* ± 4.83	136.40* ± 14.33	103.24* ± 5.68	303.62* ± 21.12
V Cd+S200	34.00 ± 0.00	34.5 ± 0.53	116.72 ± 8.30	337.45 ± 25.06	59.42 ± 3.09	172.72 ± 8.09	126.86 ± 6.61	366.68 ± 18.96

Note. Values are mean ± S.D. for five animals.
*Significantly different from controls (Group-I) ($p < 0.05$).

in step 14–16. Therefore, to calculate daily sperm production, the number of steps 14–16 spermatids was divided by 4.84 (Mishra & Singh, 2016).

2.5 | Sperm analyses

At the time of euthanasia, cauda epididymal spermatozoa from five mice in each group were obtained in Dulbecco's modified eagle medium maintained at 37°C. Motility and concentration were assessed according to WHO (1999) laboratory manual. The tissue was minced carefully in the medium with fine forceps and scissors to ensure the extrusion of spermatozoa. The tissue fraction was removed with the help of fine forceps and a needle, and the suspension was used for the assessment of motility and concentration of spermatozoa (Mishra & Singh, 2016; Patel, Singh, Singh, & Singh, 2017).

2.6 | Testis histopathology

For histopathological studies, testes were excised, blotted free of blood and fixed in freshly prepared Bouin's fluid. Testes were dehydrated in graded ethanol series, cleared in benzene and embedded in paraffin wax. For histological examination, 6-micron sections were stained with periodic acid–Schiff (PAS) and counter-stained with Harris haematoxylin and observed under a light microscope (Mishra & Singh, 2009a).

2.7 | Steroidogenic enzyme assay

To evaluate the effect of treatment on testicular steroidogenic pathway, activities of Δ^5 3β hydroxysteroid dehydrogenase (3β-HSD) and 17β hydroxysteroid dehydrogenase (17β- HSD) were measured according to methods of Talalay (1962) and Jarabak, Adams, Williams-Ashaman, and Talalay (1962), respectively, with minor modifications (Mishra & Singh, 2008). Briefly, testes were homogenised in ice-cold 20% spectroscopic-grade glycerol having 5 mM of potassium phosphate and 1 mM EDTA. The homogenate (tissue concentration 100 mg/ml) was centrifuged for 30 min at 10,000 g at 4°C, and the supernatant was used for enzyme assay. The enzyme activities were measured at 340 nm against a blank without NAD. One unit of enzyme activity is equivalent to a change in absorbance of 1.0 μmole NAD min^{-1} mg $protein^{-1}$, using the molar extinction coefficient of NADH as 6,220 M^{-1} cm^{-1}.

2.8 | Biochemical assay in epididymis and seminal vesicle

Sialic acid concentration in the epididymis was determined according to Aminoff's thiobarbituric acid method (Aminoff, 1961), while that of fructose in the seminal vesicle was estimated by the method of Lindner and Mann (1960) as performed by us in an earlier study (Verma & Singh, 2017).

TABLE 2 Effects of Shilajit on seminiferous tubules, daily sperm production, motility and concentration of spermatozoa in the cauda epididymidis, concentrations of sialic acid in the epididymis and fructose content in the seminal vesicles of male mice rendered infertile by cadmium treatment

Group	Daily sperm production ($X10^6$)	Affected seminiferous tubules (%)	Sperm parameters		Biochemical estimations	
			Motility (%)	Concentration ($X10^6$)	Sialic acid concentration in epididymis (μmoles/100 g tissue)	Fructose concentration in seminal vesicle (μg/100 g tissue)
I DW	2.17 ± 0.40	10.24 ± 1.55	79.2 ± 2.07	11.84 ± 0.93	129.25 ± 5.29	228.85 ± 6.25
II Cd	0.76* ± 0.18	74.89* ± 5.18	21.5* ± 7.28	2.56* ± 1.04	74.44* ± 10.04	132.95* ± 15.17
III Cd+S50	1.31* ± 0.23	56.26* ± 6.66	40.5* ± 3.58	5.16* ± 0.96	84.52* ± 8.92	157.72* ± 12.88
IV Cd+S100	2.00 ± 0.12	31.29* ± 7.03	73.11 ± 5.09	8.23* ± 0.73	104.82* ± 8.06	198.16 ± 11.47
V Cd+S200	2.74 ± 0.39	11.68 ± 2.46	80.5 ± 3.10	11.00 ± 1.30	131.08 ± 7.34	224.66 ± 13.95

Note. Values are mean ± S.D. for five animals.
*Significantly different from controls ($p < 0.05$).

2.9 | Serum testosterone level

After decapitation, blood was collected and serum was separated by centrifugation (3981 g, 20 min, 4°C) and frozen at −20°C. The level of testosterone in the serum (100 μl) was measured by ELISA kit (DiaMetra, Segrate, Italy) as per manufacturer's instructions. The sensitivity of the assay was 5 pg/ml with intra- and interassay coefficient of variations being 5.1% and 7.5% respectively.

2.10 | Fertility studies

Twelve hours before the autopsy, fertility of treated males was evaluated through natural mating. Briefly, males of all the groups were allowed to mate with proestrus females (1:1) for overnight. The mating was confirmed by the presence of vaginal plug or the presence of spermatozoa in vaginal smears, and the mated animals were kept separately. The mated females were allowed to complete the term for litters, and following fertility indices were calculated (Mishra & Singh, 2013).

$$\text{Index of libido} = \frac{\text{Number of males inseminated females}}{\text{Number of males paired}} \times 100$$

$$\text{Male fertility index} = \frac{\text{Number of females pregnant}}{\text{Number of males paired}} \times 100$$

2.11 | Statistical analyses

All data, except body weight and fertility test results, were analysed by one-way analyses of variance (ANOVA) followed by Newman–Keuls' multiple range test. The chi-square test was used to analyse data on male fertility. Body weight and number of litters per female were analysed by Student's *t* test. *p* values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Body and reproductive organ weights

Body weight of animals treated with cadmium alone or with cadmium followed by Shilajit remained unaltered in comparison with distilled water-treated animals. In contrast, absolute and relative weights of the testis, epididymis and seminal vesicles were significantly decreased in animals treated with cadmium alone compared to control animals. However, weights of reproductive organs showed an increase in animals treated with cadmium followed by Shilajit as compared to cadmium-alone-treated animals (Table 1).

3.2 | Daily sperm production

In comparison with distilled water-treated controls, testicular daily sperm production was significantly decreased in animals treated with cadmium alone. However, in animals treated with cadmium

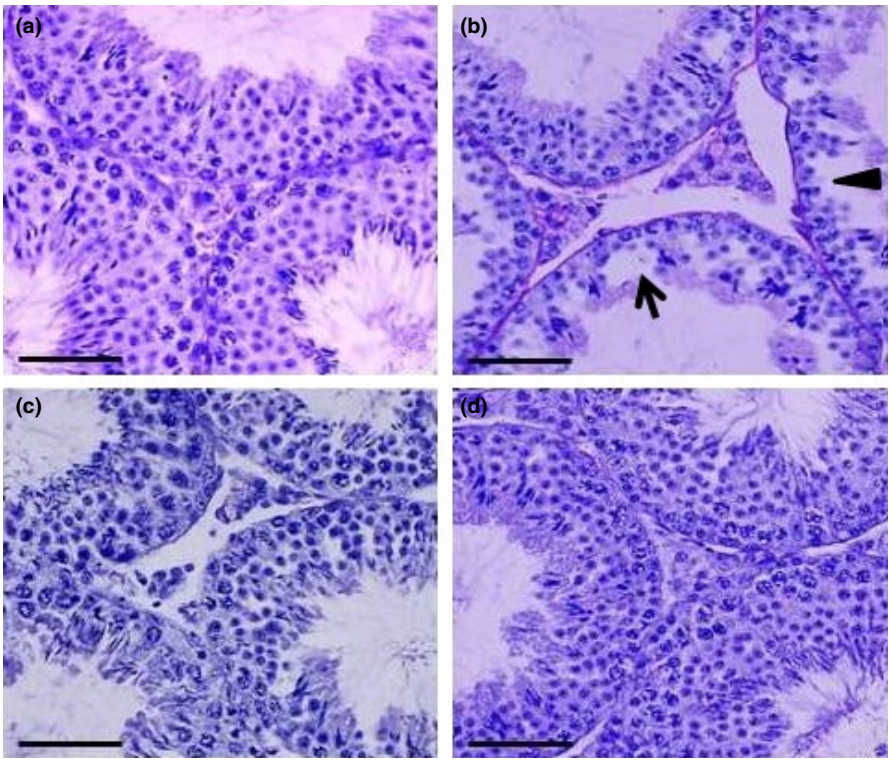

FIGURE 1 a-d is PAS- Haematoxylin stained sections of mouse testis. Barr(-) = 50 μm. (a), Testis of a mouse treated with DW for 70 days having normal appearance of seminiferous tubules. (b), Testis of a mouse treated with cadmium (2 mg/kg BW/day for 35 days) and sacrificed after 35 days of last treatment to show the atrophic appearance of seminiferous tubules. Note the appearance of intraepithelial vacuoles (arrow) and loss of germ cells (arrowhead). (c), Testis of a mouse treated with Shilajit (100 mg/kg BW/day for 35 days) after cadmium treatment (2 mg/kg BW/day for 35 days). Note the regeneration in seminiferous tubules. (d) Testis of a mouse treated with Shilajit (200 mg/kg BW/day for 35 days) after cadmium treatment (2 mg/kg BW/day for 35 days). Note the appearance of normal semniferous tubules

followed by Shilajit, testicular daily sperm production increased with increasing doses of Shilajit compared to cadmium-alone-treated animals, and this was comparable to distilled water-treated control in 200 mg dose group of Shilajit (Table 2).

3.3 | Testicular histopathology

On histological examination, no changes in the testes were observed in the controls (Figure 1a). By contrast, marked alterations were noticed in testes of mice treated with cadmium with significant increase in affected seminiferous tubules (Table 2). Some of the seminiferous tubules were lined by Sertoli cells and a few germ cells or by a single layer of germ cells (Figure 1b). Histological examination of testes from animals treated with Shilajit after cadmium exposure revealed that Shilajit could restore spermatogenesis by gradual increase in germ cell layers (Figure 1c,d), with a decrease in percentage of affected seminiferous tubules in a dose-dependent manner (Table 2).

3.4 | Testicular enzyme assay

As compared to controls, cadmium treatment significantly decreased the activities of testicular Δ^5 3β-HSD and 17β-HSD. In contrast, supplementation of Shilajit after cadmium treatment increased the activities of testicular Δ^5 3β-HSD and 17β-HSD in cadmium-treated animals. Further, there was a gradual recovery in the activities of these enzymes in Shilajit-treated groups and activities of these enzymes recovered to control level in animals supplemented with 200 mg dose of Shilajit (Figure 2).

3.5 | Serum testosterone level

Cadmium treatment significantly decreased the serum level of testosterone as compared to distilled water-treated controls. On the other hand, supplementation of Shilajit after cadmium treatment increased the serum testosterone level in cadmium-treated animals. The increase in serum testosterone level was directly proportional to the dose of Shilajit, and serum testosterone levels of animals supplemented with 200 mg Shilajit after cadmium exposure were comparable with distilled water-treated controls (Figure 2).

3.6 | Sperm parameters

Oral exposure of mice to cadmium significantly decreased the motility and the concentration of spermatozoa in the cauda epididymidis as compared to distilled water-treated controls. These parameters improved with increasing doses of Shilajit after cadmium treatment and were comparable to distilled water-treated controls in 200 mg dose group of Shilajit (Table 2).

3.7 | Biochemical assay in epididymis and seminal vesicle

Cadmium treatment significantly decreased the concentrations of sialic acid in epididymis and fructose in seminal vesicles as compared to controls. Treatment of Shilajit significantly increased the concentrations of sialic acid and fructose in 200 mg dose group, compared to cadmium-treated animals (Table 2).

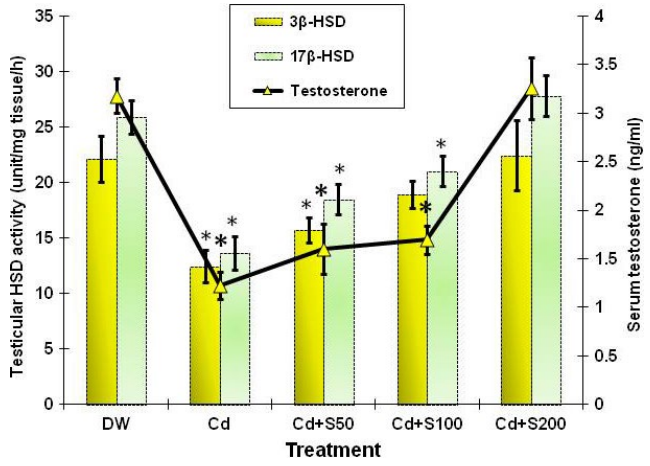

FIGURE 2 Effect of Shilajit on testicular steroidogenic enzymes, ($\Delta^5$3β-hydroxysteroiddehydrogenase and 17β-hydroxysteroiddehydrogenase) and serum testosterone level of male mice rendered infertile by cadmium treatment. Mean ± SD; n = 5

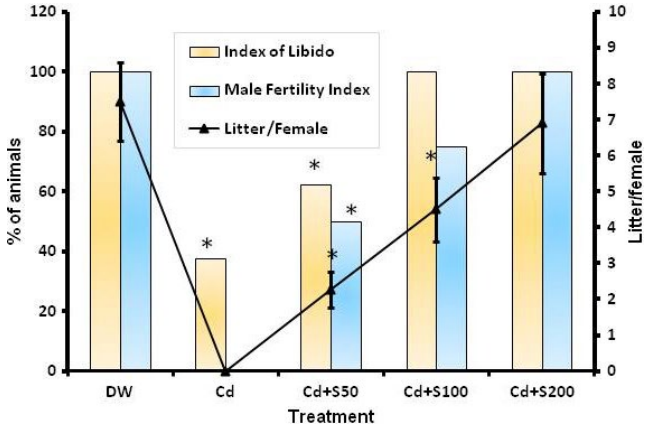

FIGURE 3 Effects of Shilajit on index of libido, male fertility index and on litter from female sired by male mice rendered infertile by cadmium treatment. Mean ± SD; n = 8

3.8 | Fertility

Libido, fertility and number of litters in females inseminated by cadmium-treated males were significantly decreased as compared to distilled water-treated controls. On the other hand, treatment with Shilajit after cadmium exposure reverted back the adverse effects of cadmium on libido, fertility and number of litters (Figure 3).

4 | DISCUSSION

Shilajit is a well-known natural rejuvenator in Indian subcontinent. In spite of its wide use in curing male infertility by the traditional healers of the Indian subcontinent, Shilajit has not been scientifically evaluated for its potential in the management of male fertility after exposure to environmental and occupational toxicants. Therefore, the present study was designed to evaluate whether oral exposure of Shilajit can recover the reproductive impairment in male mice after exposure to well-known reproductive toxicant, viz. cadmium.

Cadmium is a nonessential element which enters the body via a number of routes including food, water and air, and exerts toxic effects on the male reproductive organs (Acharya, Mishra, Patro, & Panda, 2008; Manfo et al., 2014; Yari et al., 2016). In the present study, chronic exposure to cadmium had no effect on body weight of animals. However, absolute and relative weights of testis, epididymis and seminal vesicle were adversely affected, indicating its targeted action on the male reproductive system. Treatment with Shilajit restored the weights of reproductive organs affected by cadmium to control levels in animals treated with the higher (200 mg) dose of Shilajit after cadmium exposure. In evaluation of reproductive toxicity, absolute and relative weights of reproductive organs provide basic information on functional environment of reproductive organs (Fernandes et al., 2007). Therefore, the dose-dependent increase in absolute and relative weights of reproductive organs in animals treated with Shilajit suggests that it restores the functional status of reproductive organs damaged by cadmium. In the present study, chronic exposure to cadmium had an adverse effect on the histoarchitecture of testis. Histological examination of testis revealed that treatment with Shilajit restored the spermatogenic activity by gradually increasing the germ cell layers in testis of cadmium-treated infertile mice. Shilajit has been reported to increase the rate of spermatogenesis in normal rat (Park et al., 2006). In the present study, Shilajit supplementation reverted back the decrease in testicular daily sperm production by cadmium exposure. Testicular daily sperm production is a quantitative index of spermatogenesis (Amann, 2008). Hence, the effect of Shilajit on cadmium-induced testicular toxicity suggests that Shilajit may offer protection against testicular toxicity and may restore normal spermatogenesis in infertile males.

Cadmium is a known endocrine disruptor with the ability to inhibit the testicular steroidogenesis by inhibiting the activities of steroidogenic enzymes (Jiménez-Ortega, Cano Barquilla, Fernández-Mateos, Cardinali, & Esquifino, 2012). In testicular steroidogenic events, Δ^5 3β-HSD and 17β-HSD are the prime enzymes that play key regulatory roles in testicular androgenesis (O'Donell, Meachem, Stanton, & McLachlan, 2006). In the present study, cadmium exposure significantly reduced the serum testosterone level by inhibiting the activities of Δ^5 3β-HSD and 17β-HSD. In contrast, Shilajit reverted back the inhibitory effects of cadmium on steroidogenesis by stimulating the activities of Δ^5 3β-HSD and 17β-HSD and increased serum testosterone level. This suggests that Shilajit stimulates the steroidogenesis in males and the stimulatory effect of Shilajit seems to be more prominent at high doses.

Testicular spermatozoa entering the epididymis are immature and immotile, and the epididymis plays a significant role in maturation of spermatozoa (de Souza, Schorr-Lenz, Lucca, & Bustamante-Filho, 2017). Cadmium has been reported to induce morphological abnormalities and decrease sperm concentration in the cauda epididymidis of rat (Acharya et al., 2008). In the present study, cadmium

exposure adversely affected the cauda epididymal spermatozoa and decreased the sialic acid concentration in the epididymis. Treatment with Shilajit reverted back the adverse effects of cadmium on motility and on the concentration of spermatozoa in the cauda epididymidis. Sialic acid is a true secretory product of the epididymis, and an optimum level of sialic acid is essential for the maintenance of functional integrity of the spermatozoa (Prasad & Rajalakshmi, 1976; Rajalakshmi, 1992). The possibility exists that the dose-dependent increase in motility of spermatozoa in Shilajit-exposed animals might be due to an improvement in functional environment of the epididymis as there was an increase in sialic acid concentration in these animals.

Seminal fructose is a good marker of seminal vesicular function (Mishra & Singh, 2009b). Decreased fructose concentration in cadmium-exposed animals suggests the adverse effects of cadmium on seminal vesicle. On the other hand, the gradual increase in fructose concentration in Shilajit-treated animals suggests an improvement in functional environment of seminal vesicular function (Gonzales, 2001). This was reflected by Shilajit's undoing the adverse effects of cadmium on fertility, which resulted in a gradual increase in number of litters in females treated with Shilajit in comparison with cadmium-treated animals.

Generally, the causes of male infertility include semen or sperm abnormalities, sperm transport disorders and aspermia. Among these causes, more than half results from disorders of the testicular function in producing normal spermatozoa (Park et al., 2006). Oxidative stress plays a critical role in defective sperm formation, sperm count profile and in male infertility (Esteves & Agarwal, 2011). Cadmium increases the production of free radicals and oxidative stress, thereby inducing male infertility (Acharya et al., 2008). Although the present study did not investigate the effect of Shilajit on free radicals or on oxidative stress caused by cadmium, it is pertinent to mention here that Shilajit is known to possess significant antioxidant activity with the ability to regenerate (recycle) ascorbic acid (Vitamin C) after neutralising the free radicals (Agarwal et al., 2007). Further, the present study indicates that Shilajit may stimulate testosterone biosynthesis by increasing the activity of testicular hydroxysteroid dehydrogenases. Pituitary gonadotropins are prime regulators of testicular androgenic enzymes activities; therefore, the increase in the activities of testicular Δ^5 3β-HSD and 17β-HSD may be due to the effect of Shilajit on pituitary gonadotropins secretion (Mishra & Singh, 2016; Verma & Singh, 2017). It is, therefore, reasonable to assume that Shilajit improves the functional status of male reproductive organs and the related endocrine pathways through pituitary–testicular axis, presumably by acting as an antioxidant; however, this needs further investigations. A hypothetical diagram describing proposed mechanism for profertility effect of Shilajit in cadmium-treated mice is shown in Figure 4.

In conclusion, the present study indicates that Shilajit stimulates testosterone biosynthesis and spermatogenesis in mice and restores the functional status of male reproductive organs in cadmium-induced infertile mice. Results also suggest the profertility nature of Shilajit in males. However, further studies are warranted to elucidate the role of Shilajit as a safe and effective chemoprotectant and natural medicine for male infertility management.

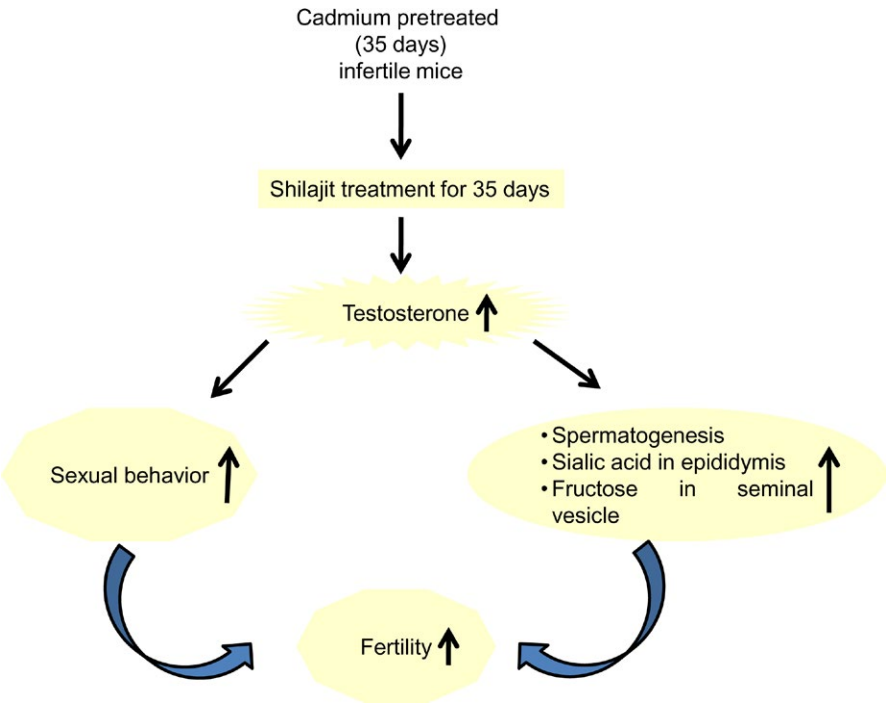

FIGURE 4 A hypothetical diagram describing proposed mechanism for profertility effect of Shilajit in cadmium-treated mice

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ORCID

Raghav Kumar Mishra http://orcid.org/0000-0001-9346-5074
Shio Kumar Singh http://orcid.org/0000-0002-8222-2429

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