THE EFFECTS OF ABHRAKA BHASMA ON SERUM TESTOSTERONE LEVELS AND EPIDIDYMAL SPERM QUALITY IN HEAT-STROKE WISTAR RATS

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ABSTRACT

Context: Abhraka Bhasma is a popular herbo-mineral formulation but there is no documentation of its aphrodisiac properties. Heat causes degenerative changes in the reproductive endpoints.

Aims: To elaborate the possible role of Abhraka Bhasma to alleviate this drawbacks of heat stress in heat stroke rats.

Settings and Design: Thirty two male Wistar rats were divided into four groups. G1 acted as control, G2 comprised dosed-only group, G3 as heat-treated group and G4 were treated with heat-cum-Abhraka Bhasma.

Methods and Material: Sahastraputi Abhraka Bhasma was used as the test drug. G1 were fed with honey, G2 were administered orally with Abhraka Bhasma with honey, G3 were subjected to heat stress at 430°C for 1 h daily for thirty days; were also given honey and Group G4 animals were resorted to heat stress-cum-Abhraka Bhasma administration with honey as vehicle. The rats were euthanized on day 31 and serum was analysed for testosterone levels and cauda semen for sperm count, motility and morphology.

Statistical Analysis Used: Statistix software 9.0 was used to obtain descriptive statistics and one-way ANOVA calculator was used to take out the significance of the data.

Results: Comprehensive analysis showed that there were no significant effects (p=0.393) of heating on testosterone status of hypothermic G3 animals as compared to control rats. However G2 and G4 animals, as compared to G3 and control rats, showed significant increases (P=0.111, P=0.146 respectively) in the serum testosterone levels. Heat stress significantly reduced sperm concentration, sperm motility and resulted in abnormal sperm morphology as compared to dosed animals.

Conclusions: In conclusion, Abhraka Bhasma, due to its androgen increasing property, can be prescribed as aphrodisiac with properties enough to suffice as an anti-impotency fecundity drug for males suffering from sexual insufficiency in tropical and subtropical countries.

KEYWORDS: Aphrodisiac, Sahastraputi, Statistix, ANOVA, Hypothermic, Anti-Impotency, Fecundity

Key Messages: Administration of Abhraka Bhasma following heat treatment could completely compensate the effects of heat on reproductive endpoints encompassing testosterone levels too. Prolonged administration of this formulation will ameliorate fertility in the males, especially those who are exposed to heat. Abhraka Bhasma appears to possess aphrodisiac activity due to its androgen increasing property.

INTRODUCTION

Abhraka Bhasma is a herbo-mineral formulation of Ayurvedic pharmacopoeia constituting mica nanoparticles. It goes through a purification test process that turns them into ash. It is known in the Indian subcontinent since the seventh
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century BC and widely recommended by practitioners for treatment of a variety of chronic ailments. It is subtle, penetrating and spreading. It is a powerful cellular regenerator, treats impotency and has the potency to restore the tissues. It is indicated in the treatment of sexual neurasthenia and spermatorrhoea.

It has affinity for almost all the tissues and its pharmacological actions include aromatic, antiviral, antibacterial, nerve, spermatogenic and hematinic. The therapeutic properties and efficacy of mica Bhasma is that while curing diseases it promotes strength, longevity and seminal power. A person can have sexual intercourse daily with hundred of women and can procreate children with long span of life and great strength like that of a lion. With the advent of nanotechnology, the current belief is that nano-particle size of mica is responsible for the enhanced bioavailability and activity and hence the dose is small which is one ratti to two ratti. [1]

In general, ambient temperatures that surpass upper limits of thermo-neutrality result in reduced fertility, even if limits are only marginally exceeded. The damaging effects of whole body or local heating on the testes of mammals are well known and, although recovery begins about 40 days after a single exposure, even 60 or more days later, there is still a significant reduction in testis mass. The full spectrum of the signs and symptoms occurring during heatstroke in humans can be mimicked by the rodent heatstroke model. Regardless of the evolutionary reason for the location of the testis and epididymis outside the body, a rise in testicular temperature in mammals with external testes leads to reduced sperm output, decreased sperm motility and an increased proportion of morphologically abnormal spermatozoa in the ejaculate. [2] It has also been promoted that testosterone plays a role in the regulation of heat balance in male rats. [3]

According to a new study led by a University of California, San Francisco urologist recreational activities like hot baths, hot tubs or Jacuzzis and modern gadgets like laptops are a real risk factor for male infertility. But does such activities lead to decreased testosterone levels? If yes, how eventually to reverse these baseline testosterone levels to improve reproductive endpoints in heat-stroke individuals? The ASRM estimates that 85 percent to 90 percent of infertility cases can be effectively treated with drug therapy. Isn’t it becomes imperative to develop an anti-impotency fecundity drug for these heat stressed individuals? The answer to that is a little more complex and we have to go to animal studies to get some answers and insight.

In view of the paucity of literature on the action of AB on reproductive functions, the goals of this study was set to prove that this herbo-mineral supplement is highly beneficial in the treatment for infertility especially to individuals who are subjected to heat stress. It brings forth the outcome that Abhraka Bhasma enhances the fertility in heat-stroke rats by altering the testosterone levels and eventually improving the epididymal sperm quality in them.

MATERIALS AND METHODS

Animals: The current experiment was carried out on thirty two healthy adult male albino Wistar rats (150 - 200 gms live body weight) obtained from Haffkine Institute, Parel, Mumbai. Animals were housed in polypropylene cages in an airconditioned laboratory of the Animal House of the research centre and had free access to standard laboratory pellet diet (Amrut rat feed) and water ad libitum. Before conducting the experiment, the animal ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) approved by Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Incubator (heating apparatus): A specially self-designed 8 inch x 8 inch x 24 inch heating apparatus based on modern technology was fabricated and manufactured by Hindustan Apparatus Mfg. Co., Kurla, Mumbai. It was divided into three compartments, the inner chamber being made of stainless steel and outer of mild steel with powder coating (Fig. 1). Inside the chamber a safety thermostat, that cuts off if the incubator overheats to maintain a constant temperature of
430°C, was strategically placed along with a blower and an air ventilator at the top. A state-of-the-art microprocessor that can be programmed to maintain different temperatures for varying intervals was also installed in it. The inner chamber has an electrical heater mounted on an inside wall and covered by a perforated protective panel. Mounted in the chamber wall just above the heater is a fan whose motor extends through the chamber wall into the control area of the case and whose blades face inward.

![Incubator to Maintain Optimal Temperature of 43°C and Humidity of the Atmosphere inside the Compartments](image)

**Figure 1: Incubator to Maintain Optimal Temperature of 43°C and Humidity of the Atmosphere inside the Compartments**

**Abhraka Bhasma (Test Drug)**

Sahastraputi Abhraka Bhasma (subjected to 1000 putas) was procured from a renowned organization, Shree Dhootpapeshwar Ltd, Khetwadi, Mumbai, India. The drug was given to G2 and G4 animals (through oral gavage) and the dose was calculated by extrapolating the therapeutic dose of humans to rat on the basis of BSA ratio (conversion factor 0.018 for rats) by referring to the table of “Paget & Barnes” (Paget & Barnes 1964).

Therapeutic dose of Sahastraputi Abhraka Bhasma: 15-60 mg

Selected Human dose : 60 mg/kg b.w.

i.e. For rats

Human dose  x  0.018  =  X g/200g  of rat

or,  X x 5  =  Y g/kg  of rat

**Experimental Design**

Rats were divided into four groups of eight rats each. After markings the division into groups were done as follows:

- Group G1; served as normal control
- Group G2; treated with Abhraka Bhasma
- Group G3; subjected to heat only
- Group G4; simultaneously given heat and Abhraka Bhasma

G3 and G4 were subjected to heat stress keeping the animals in a self designed incubator at 43°C for 1 hr daily (in the early morning after overnight fast) and then returned to their normal cages (at 33°C) for 4 successive weeks. The
humidity was not less than 50% during this period of time. After heat treatment the animals of G1 and G3 were fed orally with 0.5 ml of honey while animals of G2 and G4 were administered Abhraka Bhasma once daily (by oral gavage) using 0.5ml honey as a vehicle. The rats were fed with basal diet 4 hrs after dosing to get maximum effect of the test drug (OECD guidelines).

**Sampling and Analysis**

The effect of heat can be observed after short time exposure but this case does not hold true for Abhraka Bhasma. To explore the possible effect of slowly affecting trial drug on blood profile, groups were continuously treated with the test drug for 30 days. On day 31, following an overnight fast, blood samples of six animals from each group were collected by retro-orbital plexus method after which they were euthanized by rapid decapitation method for sperm quality determination.

**Serum Testosterone Assay**

For serum testosterone, 2-3 ml blood sample was collected via retro-orbital plexus from each animal just prior to necropsy and stored in Gel Vacutainer assay tube. Samples were left at room temperature for 30 - 60 min. After complete hemolysis, the serum in the vacutainer tube was obtained through centrifugation at 5000 rpm for 5 min to isolate the sera. The isolated sera of all the animals were aspirated with Pasteur pipette and assayed for the testosterone values at Metropolis Healthcare Ltd., Worli, Mumbai. Testosterone estimation was done by Chemiluminescence Immunoassay (CLIA) method (the ADVIA Centaur Testosterone assay) using ADVIA Centaur system with sensitivity and assay range of 10 – 1500 ng/dL (0.35 – 52.1 nmol/L)

**Epididymal Sperm Analysis**

Although many factors which are likely to influence or at least indicate the potential for fertility are routinely assessed (including semen pH, viscosity, colour and odour), sperm concentration, motility and morphology are generally considered the three most important and informative parameters. Hence these three parameters were considered for the study.

In order to obtain semen specimens for evaluation, the testes from each rat were carefully exposed, caudal part of the epididymis was excised and trimmed free of adjoining fatty tissues. The sperms were released from the cauda epididymis by mincing in 2 ml of M199 medium containing 0.5% bovine serum albumin and allowed to stand for few minutes to allow spermatozoa to swim out into the medium. 20 microlitre of the filtrate were used for assessing the sperm count (number of spermatozoa/mL), Subsequently, 30 microliters of the filtrate were counted to calculate the percentage of motile spermatozoa. For sperm morphology 10 microlitre of the diluted sample was used to form the smear. The sperm parameters were analyzed using a microscope Olympus with a 10 x field.

**Sperm Count**

To evaluate sperm concentration, epididymal cauda was minced with scissors to release sperms in 2 ml of Medium 199 containing 0.5% Bovine serum albumin at 370C. An aliquot of it was diluted 1:4 with sperm diluents fluid, further homogenized at room temperature; the sample thus obtained was used for counting sperms using the manual method. The sample was stained with trypan blue and spread on hemocytometer and sperm heads were counted manually under the optical microscope. The data is expressed as the total number of sperms per one cauda epididymal tissue.
The cauda epididymis sperm reserves were determined using the standard hemocytometric method using the improved Neubauer’s counting chamber. The sperm concentration was then calculated by putting a drop of immersion oil and placed on a microscope at a magnification of X 100. The sperm count was recorded in million and expressed as \((X) \times 10^6\) million/ml, where \(X\) is the number of sperm in a 16-celled square.

**Quantitative Sperm Motility (%)**

Percentage sperm motility and levels were reported as the mean of motile sperm, according to the WHO method. The sperm motility were determined as described by Saalu et al. (2008).[6] Briefly, 30 microlitre of diluted semen were placed on a slide, two drops of warm 2.9% sodium citrate were added, slide covered with cover slip and examined under the microscope using 40 x objective for sperm motility. The proportion of motile spermatozoa was determined by counting 100 cells in randomly selected fields.

**Sperm Morphology**

For morphological abnormalities counting, 10 microlitre of washed spermatozoa was used to make a smear. The smear was allowed to air dry after which it was fixed in 70% ethanol for one minute, ready to be stained with Harris’ Hematoxylin for 10 seconds. It was then washed under tap water for 10 minutes and in distilled water for 1 minute and then stained in aqueous 1% eosin for 5 minutes. Finally, the slides were washed with distilled water, and observed under a light microscope at 100 x magnification to evaluate sperm morphology.[7] Both normal and abnormal sperm cells were observed and 500 sperms/slide were counted and the sperm morphology was estimated in percentage.

**Statistical Analysis**

All data were expressed as means ± standard deviation of mean of six rats per group and are also rounded off to nearest digit. Statistix 0.9, version 3, Beta was used for descriptive statistics and then differences between groups were analyzed by Analysis Of Variance (ANOVA) calculator.[8] The significance of difference was set up at \((p<0.05)\). The histograms were plotted with Excel Programme while the bar chart was developed using Statistix programme.

**RESULTS**

**Serum Testosterone Levels**

Figure 1 summarizes the mean free sera levels of testosterone (ng/dl) for different groups of rats monitored at day 31 of heating and drug administration. The testosterone level of G2 was and G4 animals varied significantly \((P=0.007; P=0.042\) respectively) from the respective controls. However, no significant \((P=0.555)\) change in G3 animals when compared to control in accordance with previous findings.[9]

The test drug increased mean serum testosterone level in G2 and G4 animals than control (G1) and heat-treated (G3) animals at 31 day post-treatment (Figure 2). G4 rats produced the largest increase in mean serum total testosterone. The testosterone level was assayed highest in one of the rats of G4 group (as high as 1049.74 ng/dl) and as low as 70.34 ng/dl in the G3 group (Figure 3).

| Table 1: P Values of Serum Testosterone Levels of Male Wistar Rats Post 30 Days of Heating and Administration of Abhraka Bhasma; \((P<0.05)\) is Considered as Significant; Datas are Expressed as Means ± SD |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                | G1-G2          | G1-G3          | G1-G4          | G2-G3          | G2-G4          | G3-G4          |
| P values       | 0.007          | 0.555          | 0.042          | 0.006          | 0.966          | 0.033          |
Epididymal Sperm Quality

Sperm Counts

A one-way ANOVA conducted across the sperm count of the epididymides for the four groups of rats showed no significant difference between G1, G2 and G4 animals (Table 2). However, a visible reduction in sperm count in the heat-treated group G3 was there when compared with that of the others (Table 2). Post hoc analysis revealed that the highest sperm numbers were found to be in G2, it being $59 \times 10^6$ ml and the lowest being in heat-treated animals, it being $10 \times 10^6$ ml. Sperm count of G3 rats were 42%, 54% and 48% lower than the G1, G2 and G4 animals respectively.

Table 2: P Values of Sperm Parameters of Male Wistar Rats Post 30 Days of Heating and Administration of Abhraka Bhasma; (P<0.05) is Considered as Significant; Datas are Expressed as means ± SD
Table 3: Epididymal Sperm Cell Parameters at Day 31 of the Experiment in Wistar Rats Exposed to 1 H Heat at 43°C Heat Followed by Oral Administration of Abhraka Bhasma Dats are Expressed as Mean ± SD; Mean with the Same Superscription on the Same Row are Significant at the Level P<0.05; N = 6

<table>
<thead>
<tr>
<th></th>
<th>GROUP G1 (CONTROL)</th>
<th>GROUP G2 (DOSED)</th>
<th>GROUP G3 (HEAT-TREATED)</th>
<th>GROUP G4 (HEAT-TREATED + DOSED)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPERM COUNT (x 10^6 cell/ml)</td>
<td>40.17 ± 11.91^a</td>
<td>51.17± 12.66^b</td>
<td>23.33 ± 10.65^abc</td>
<td>44.83 ± 7.49^c</td>
</tr>
<tr>
<td>SPERM MOTILITY (%) (mean±SD)</td>
<td>63.00 ± 15.21</td>
<td>75.17 ± 16.29</td>
<td>44.67 ± 12.21</td>
<td>60.17 ± 9.20</td>
</tr>
<tr>
<td>SPERM ABNORMALITY (%) (mean±SD)</td>
<td>2.00 ± 3.16^a</td>
<td>0.50 ± 0.84^b</td>
<td>20.83 ± 13.01^abc</td>
<td>1.33 ± 1.97^c</td>
</tr>
</tbody>
</table>

Figure 4: Comparitive Column Graph of Means of Epididymal Sperm Parameters of the Four Experimental Groups of Wistar Rats Post Administration of Abhraka Bhasma at 31 Day of Experiment

Sperm Motility

G3 group exhibited low significance (P<0.05) in comparison to the animals of other three groups (Table 2). However, there was no significant difference (P>0.05) between G1, G2 and G4 animals (Figure 3)

Sperm Abnormality

The degree of abnormality of spermatozoa of rats in the heat-treated groups was significantly (P<0.05) higher than what was observed in rats in each of the other groups

(60). 20 % of sperms in the heat-treated group (G3) were expressed as a percentage of morphologically abnormal sperms (Table 3). The mean value of abnormal sperms of rats in group G1, G2 and G4 were significantly (P<0.05) lower compared with that of the heat-treated rats (Table 1). The abnormal sperms comprised of double-head, headless and double-tailed sperms (Figure 4).
DISCUSSIONS

In the present study the response of the serum testosterone levels to administration of Abhraka Bhasma after whole body heating in rats for thirty days was examined as testes does not make a full recovery in that time. Abhraka Bhasma is an aphrodisiac and treats impotency. Not much is revealed through literature as to how it cures impotency and sexual debility. This study reveals that in rats administration of Abhraka Bhasma treatment results in increase of the mean hormone concentration and subsequently sperm quality. Abhraka Bhasma supposedly elevates the testosterone levels by, either, affecting its secretion or its metabolism.

Heat stress virtually results in direct and indirect losses in reproduction ability of animals. The temperature when exceeds the thermal comfort zone results in their suffering heat stress. In severe cases, when the core temperature rises along with rise in humidity, spermatogenic cells degenerate and reproduction performance is reduced. Others reported that heat stress reduces steroidogenic efficiency in Leydig cells, but that LH secretion in these heat-stressed animals is
increased proportionately, resulting in little or no net change in circulating testosterone as can be seen by this study.\textsuperscript{[10]}

Additionally, Lue et al. (2000) observed reduced effectiveness in the ability of circulating testosterone to stimulate other cells, which could lead to further increases in steroidogenesis.

A possible hypothesis to explain this pattern of changes in hormone levels could be that Abhraka Bhasma probably contain some androgenic analogue, which elevates the LH levels sufficiently, consequently increasing intratesticular production of testosterone by Leydig cells. Changes in testosterone concentrations alters the secretion of Leydig cells’ estrogens, estradiol and estrone. Heat-stress results marked decrease in their levels in heat-treated rats. Abhraka Bhasma appears to exert steroidal effect similar to 1,4,6-androstatriene-3,17-dione, at multiple sites within putative HPA (Hypothalamic–pituitary–adrenal axis) axis control pathways. This results in increase in hormonal level inspite of heat-stress in G4 animals.

Abhraka Bhasma seems to suppress the activities of stressors, pro-opiomelanocortin-derived (opioid) peptides secreted by the Leydig cells and/or corticosteroids, either by triggering the release of GnRH by accelerating hypothalamic gonadotropin-releasing hormone pulse frequency or by exciting Leydig-cell LH-receptors.\textsuperscript{[11]} Hence substantial amount of secretion of LH and FSH from the pituitary is maintained causing an increase in testosterone level and spermatogenesis.

Abhraka Bhasma may contain steroidal components which blocks glucocorticoid receptors in serum overcoming deactivation of glucocorticoids by heat which are now are able to act on testicular interstitial cells to ameliorate the testicular response to gonadotropins. Abhraka Bhasma might be directly enhancing testicular functions leading to activation of the gonadotropins either by decreasing the corticosteroid levels or by overcoming the suppressive effect of exogenous CORT on testicular 11β-HSD activity and testosterone concentrations.

Testosterone are anabolic steroids synthesized from cholesterols. There is a possibility that Abhraka Bhasma triggers CYP11A, a mitochondrial cytochrome P450 oxidase, which causes the oxidative cleavage of cholesterol to give pregnenolone.

Since suppression might be occurring partly in heat-treated animals in the first step, the C\textsubscript{19} steroids are produced in less quantities in them as compared to dosed animals. In addition, lesser oxidation of the 3-hydroxyl group by 3-β-HSD (hydroxysteroid dehydrogenase) to produce androstenedione might occur in G3 animals but not in G4 animals. In the final and rate limiting step, it can be postulated that the C-17 keto group androstenedione is reduced in greater amounts by 17-β hydroxysteroid dehydrogenase to yield testosterone in G4 animals but not sufficiently in G3 animals.

Probable heat through some mechanism disrupts SHBG (sex hormone binding globulin) protein synthesis because of which testosterone though produced is found in lesser amounts in the blood of heat-treated rats. Abhraka Bhasma acts as an excitator of SHBG synthesis.\textsuperscript{[12]} As a result its transportation, and hence its availability, is increased in the latter case than in the former.

On the metabolic level, it can also be hypothesized that Abhraka Bhasma dosage inhibits cytochrome P\textsubscript{450} enzyme 5α-reductase and aromatase (CYP19A1). As a result lesser amounts of testosterone is reduced to 5α-dihydrotestosterone (DHT) and estradiol respectively. Abhraka Bhasma might be prohibiting CYP3A, which clears testosterone and DHT, induction or would be producing some metabolites which have endocrine disrupting function. Thus it can be hypothesised that the level of testosterone increases through either the effect on steroidogenesis enzymes in testes, or its activation properties on adrenergic systems involved in steroidogenesis. This effect can be explained by the stimulation of the hypothalamus–pituitary-axis (HPA) which resulted in increasing the concentration of serum testosterone.
The andrological parameters - sperm cell count, motility, morphology- were used in this study to evaluate the effect of prolonged administration of Abhraka Bhasma on epididymal sperms of Wistar rats. The clinical findings of this study suggests that heat decreases sperm count but the number instead of just being restored is increased by administering Abhraka Bhasma.

The process of spermatogenesis is highly sensitive to fluctuations in the environment, particularly hormones and temperature. Maintainence of this process is achieved via the binding of testosterone by androgen binding protein present in the seminiferous tubules. Abhraka Bhasma might be ameliorating sperm production through increase in testosterone levels causing pathological changes in sperm cells probably by activating the genes in Sertoli cells. The impaired transportation of sperms from the testes to the vas deferens might be the potential mechanism of low sperm number dysfunction in rats after treatment with heat. Also the administration of Abhraka Bhasma (5.4 g/kg bw) might have caused significant increase in the sperm number by evoking electrical pulses in the epididymal portion.

It can be hypothesized that significantly lower testosterone responses in heat-treated rats indicated a defect in Leydig cell secretory function which detrimentally influenced the spermatogenesis and epididymal sperm maturation process in this study. This hypothesis is supported by the reduced epididymal caudal sperm content and motility profiles. It is possible that AB treats hypoxia in the germinal epithelium by unblocking potassium channels after heat inactivation of the testes resulting in polarization of the tubules and consequently improving sperm numbers.

Sperm motility can be affected by low testosterone levels, depletion of seminal fructose (hypoglycemia), reduced ATP production and/or reduced ATP levels via ATPase hydrolysis which results in insufficient energy and poor sperm motility as observed by Adenubi et al., 2010.

Inorganic ions viz. Potassium, Sodium, Hydrogen and Ca2+ deficiency affects testosterone production and thus affect the sperm motility. Thus it can be implicated that Abhraka Bhasma triggers the formation of internal inorganic ions, cAMP (which act as messengers) and release of acids which initiate motility in sperms. It can be further postulated that Abhraka Bhasma initiates the copious secretion of the accessory glands and mediation of intracellular alkalization triggered by a sodium-proton exchange. Normal sperm motility is possible that administering Abhraka Bhasma produces positive effects on sperm activity by altering the two antagonistic intracellular messengers- increasing intracellular [Ca2+] but decreasing intracellular [H+] to stimulate sperm motility.

Probably Abhraka Bhasma initiates sperm motility by the direct stimulation of axonemal proteins, indirectly stimulated by intracellular cAMP too, by higher pH and ion pumps of the sperm plasma membrane that can shuttle protons across the cell membrane. This was possible due activation of protein kinase (PKA) which is thought to cause phosphorylation of the axoneme or elevating cAMP due to the activation of sperm soluble adenylyl cyclase (SACY).

Hypo-function of seminal vesicle may affect the fertility parameters as sperm motility, sperm chromatin stability, and immune-protection which may get changed due to heat. It can be hypothesized that Abhraka Bhasma normalizes these defects by stimulating prostatic secretion which makes spermatozoa motile. Possibly heat-stress results in disruption of “countercurrent heat exchange system” between the pampiniform plexus and the testicular artery which causes problems with spermatogenesis.

Spermatozoa are particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) and their cytoplasm contains low concentrations of scavenging enzymes. Heat causes oxidative stress which causes thermal damage of spermatogenic cells and leads to apoptosis and DNA strand breaks. It can be hypothesized that probably Abhraka Bhasma effectively
reduces adverse effects on fertility by oxidative stress elicitation due to their radical scavenging and metal chelating functions.

Heat disturbs the transcriptionally silent spermatozoa to preserve their molecular and functional integrity. Lenzi et al., (1998) demonstrated that, altered sperm morphology might reflect disturbances during spermiogenesis, spermiation and sperm passage through epididymis due to changes in testosterone levels. Abhraka Bhasma reverses these changes resulting in normal sperms.

The sperm head defects may in part be due to reduced nuclear compaction in teratozoospermic patients, as observed by Zini et al. (2009), and harmful temperature fluctuations, ultimately leading to fertilization failure and subfertility. High level of DNA traced in testicular tissue of Abhraka Bhasma administered animals will ascertain this theory and a study on it is called for.

Since other treatments applied are becoming more expensive and often carry serious side effects, there should be scientific dissemination of information on the therapeutic efficacy of Abhraka Bhasma on reproductive endpoints. Ayurvedic pharmacopoeias have implicated Abhraka Bhasma having aphrodisiac properties which is proved by this study. The lack of preliminary data on these herbo-mineral formulations leads to indiscriminate use of these medicines which might lead to health hazards. Mica nanoparticles, constituting Abhraka Bhasma, is hereby implicated as possible bioactive agents which can penetrate the blood-testis barriers, reach deep within the tissue and are responsible for its aphrodisiac effect due to their nanosize. The physiological relevance of these nanoparticles is as yet uncertain, although their potential importance is clear by this study in relation to maintenance of the high levels of sera testosterone level required for quantitatively normal spermatogenesis. More research work is recommended to prove that Abhraka Bhasma can be used to treat erectile dysfunction, early ejaculation, increase libido and treat decreased sex drive.

The above-cited research demonstrates that Abhraka Bhasma raises testosterone levels and consequently augments sperm health. Further, it demonstrates adverse effects of heat stress on reproductive traits in male rats and postulates that administration of Abhraka Bhasma following heat treatment could completely compensate the effects of heat on reproductive endpoints encompassing testosterone levels too. It indicates that the prolonged administration of this formulation will ameliorate fertility in the males, especially those who are exposed to heat. It is concluded that Abhraka Bhasma appears to possess aphrodisiac activity due to its androgen increasing property.

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