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Research Article

TESTICULAR OXIDATIVE STRESS PROTECTIVE EFFECTS OF ABHRAKA BHASMA IN MALE WISTAR RATS AFTER HEAT EXPOSURE

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ABSTRACT

Objective: Testicular hyperthermia leads to excess generation of oxygen radicals, alteration in antioxidant capacity of the stressed tissue and increase in lipid peroxidation resulting in events associated with male infertility. A drug which is capable of mitigating the heat-induced oxidative stress on the testes and restoring its normalcy is called for.

Methods: Thirty two male Wistar rats were divided into four groups. G1 acted as control, G2 comprised Abhraka Bhasma-only group, G3 as heat-treated group and G4 were given combined heat treatment and Abhraka Bhasma administration G1 were fed with honey only, G2 were administered orally with Abhraka Bhasma with honey, G3 were subjected to heat stress at 43^{0}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 testis homogenate was subjected to determination of extent of GSG, GSSG, GSH/GSSG ratio and Malondialdehyde activity.

Results: Abhraka Bhasma administration following heat treatment lowered the TBARS level when compared to heat-treated rats. The GSH activity was statistically higher in G4 animals when compared to heat-treated group. G4 rats showed significantly lower levels of GSSG when compared to G3 rats. Abhraka Bhasma after heat treatment remarkably elevated the GSH/GSSG activity whereas heat lowered glutathione disulphide ratio in G3 animals.

Conclusion: The present results show that Abhraka Bhasma is effective as antioxidants, protects testicular tissue against heat- induced oxidative damage and could be excellent adjuvant support in the therapy of male infertility.

Keywords: Hyperthermia, Glutathione, Lipid peroxidation, Malondialdehyde, Glutathione disulfide ratio, Antioxidant

INTRODUCTION

Abhraka Bhasma is a commonly used effective ayurvedic medicament known to remove the derangement of the *tridoshas* (three basic physical energies) and establishes its equipoise. It is helpful in various types of sexual disorders as impotency, erectile dysfunction and increases sperm count.[1]It benefits in sex debility and various types of autoimmune diseases. With the advent of nanotechnology, the current belief is that during bhasmikaran process the constituents acquire nanoparticles size, which is responsible for its enhanced bioavailibilty and activity and hence the dose is small - one ratti to two ratti . [2]

Heat stress induces upregulation of specific heat shock proteins (HSP70-2), induction of phosphorylation of eIF2α, formation of stress granules (SGs) translocation of DAZL, a germ cell-specific translational regulator, to SGs, and the subsequent blockage of the apoptotic MAPK pathway. Many investigators proposed that one possible mechanism of affecting the function of testis is the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS).[3]Heat exposure results in decreases in glutathione (GSH) levels which cause an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radical ions, leading to increase lipid peroxidation.

Oxidative stress is a major factor in the aetiology of male infertility. Testes remains vulnerable to oxidative stress due to the abundance of highly unsaturated fatty acids (particularly 20:4 and 22:6) and the presence of potential reactive oxygen species (ROS)-generating systems. It is accepted that the intake of antioxidant substances reinforces defenses against free radicals.[4] Animal and human cells express an array of antioxidant enzymes, including Mn^{2+}-dependent superoxide dismutase (MnSOD), copper/zinc (Cu/Zn) SOD, glutathione peroxidase (GPx), glutathione reductase (GR), xanthine oxidase, catalase (CAT), the *bcl-2* protooncogene, peroxiredoxins (Prx), lipocalins like prostaglandin D2 synthase and peroxynitrite(ONOO2). Small-molecular antioxidants include ions and a wide variety of free radical scavengers. The mechanism involved is increase in germ cell is apoptosis partnered with autophagy and subsequent hypospermatogenesis. [5]

The Sertoli cells are the site of action of all hormonal influences modulating testis development. Oxidative stress induced due to heat treatment is manifested in reduced supply of lactate by Sertoli cells to germ cells for nourishment and non-phagocytosis of degenerating germ cells and residual bodies. Sertoli cells transduce the androgenic stimulus co-ordinating this essential step in spermatogenesis.[6]Spermatogenic cells can generate reactive oxygen species either continually or in the form of an "oxidative outburst" – e.g., by activation of NADPH oxidase and NO synthase. Pachytene spermatocytes and early spermatids (steps 1–4) at stages I–IV and pachytene, diplotene, and dividing spermatocytes at stages XII–XIV are the most susceptible cell type to apoptosis. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn Superoxide Dismutase. [7]

Spermatozoa contains polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid, creates an 'electron sink' that renders it highly susceptible to oxidation.[8] ROS may be generated in two ways that is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the level of sperm plasma membrane and (NADH) dependent oxido-reductase (diphorase) at the level of mitochondria that results in a decrease in axonemal protein phosphorylation and sperm immobilization. Leydig cells, after heat exposure showed increase in FasL expression and caspase-3 activity, ROS production by the mitochondrial electron transport chain, steroid hydroxylations by the cytochrome P450 enzymes and decrease in superoxide dismutase-1 and -2, glutathione peroxidase. It has been suggested that reduced glutathione serves as a potent intracellular antioxidant pool of Leydig cells protecting them against oxidative stress. [9]

One of the stable by product of lipid peroxidation is malondialdehyde (MDA) which has been used as an end product in biochemical arrays to monitor degree of lipid peroxidation. Glutathione is a tripeptidyl molecule and present in either the

reduced (GSH) or the oxidized state (GSSG) by forming a disulfide bond between two molecules. GSH via direct reaction with free radicals protects cells against oxidative stress and plays role in biotransformation of drugs.[10] In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress.[11]

There is a paucity of clinical studies that consistently demonstrate, across all data sets, the significance of oxidative stress in the diagnosis of male infertility and the importance of antioxidants in the management of this condition. In the present study the aim was to use this experimental model to obtain information about antioxidant properties of Abhraka Bhasma on testicular oxidative state by investigating GSH, GSSH, GSH/GSSH ratio and MDA levels after inducing hyperthermic condition in rats. The results would facilitate understanding of the potential of Abhraka Bhasma in preventing heat-induced oxidative damage to male reproduction and hence lead to new insight for therapies to protect spermatogenesis and hence fertility in people living in hot regions.

MATERIALS AND METHOD

Animals

The current experiment was carried out on 32 healthy adult male albino Wistar rats (150- 200 gms live body weight) obtained from Haffkine Institute, Parel, Mumbai, India. Animals were housed in polypropylene cages. They had free access to standard laboratory pellet diet (Amrut rat feed) and water *ad libidum.* 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).

Fig. 1: Incubator

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 outer chamber a safety thermostat, that cuts off if the incubator overheats to maintain a constant temperature of 43^0C, was strategically placed along with a blower and an air ventilator at the top. A state-of-the-art digital microprocess-based controller was also installed to maintain different temperatures for varying intervals. The inner chamber had an electrical U-shaped rod heater mounted behind its wall.

Abhraka Bhasma (test drug)

Sahasraputi Abhraka Bhasma (subjected to 1000 putas) was procured from a renowned organization, Shree Dhootpapeshwar Ltd, Khetwadi, Mumbai, India, to study its regenerative potential on heat-damaged testes. 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).[12]

Therapeutic dose of Sahastraputi Abhraka Bhasma: 15-60 mg

For the experiment, selected human dose: 60 mg/kg b.w.

Dose for rats = Human dose x 0.018 = X mg/200g of rat Or, X x 5 = Y g/kg of rat

Therefore, Dose given = 60 x o.018 = 1.08 mg/200g of rat Or, 1.08 x 5 = 5.4 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 43^0C for 1 hr daily (in the early morning after overnight fast) and then returned to their normal cages (at 33^0C) 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 (CCRAS) while animals of G2 and G4 were administered Abhraka Bhasma once daily (by oral gavage) using 0.5ml honey as a vehicle for 30 days. The rats were fed with basal diet 4 hrs after dosing to get maximum effect of the test drug (OECD guidelines).

Sampling and Analysis

After 30 days of treatment six animals from each group were sacrificed by rapid decapitation method to dissect out testes for antioxidant and lipid peroxidation studies. Standard laboratory techniques were followed for the same. For, oxidative parameters analyses, right testes was excised, rinsed with phosphate buffered saline solution, pH 7.4 to remove red blood cells and clots and then weighed. Tissue samples were minced with scissors and homogenized in 4 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4). The homogenate was centrifuged at 10,000 x g for 15 minutes at 4^0C to obtain the supernatant. Supernatant was diluted 5 times and used for estimating the following biochemical parameters. [13]

Assay of lipid peroxidation (Malondialdehyde (MDA)) concentrations

2-ThioBarbituric Acid Reactive Substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress. TBARS assay values are usually reported in malondialdehyde (MDA) equivalents, a compound that results from the decomposition of polyunsaturated fatty acid lipid peroxides.[13] The TBARS assay is a well-recognized, established method for quantifying these lipid peroxides. Lipid peroxidation assay was determined by estimating TBARS by the method suggested by Ohkawa *et al* (1989). The extent of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances formed in testes homogenate. The reaction mixture contained 1.5 ml of trichloroacetic acid (20%) and 1.5 ml of thiobarbituric acid (1%) to this 0.5 ml of tissue homogenate was added. The reaction mixture was heated at 100°C for 15 minutes it was then centrifuged and the absorbance was read at 535 nm. The % inhibition of lipid per oxidation was calculated by comparing the results of the test with those of blank. Quercetin was used as a reference standard.

Assay of testicular reduced glutathione (GSH) concentration

GSH was determined by the method of Ellman (1959). The reaction mixture contained 0.2 ml of 0.4 M tris HCl buffer (pH =7.0),0.1 ml of 10mM Sodium azide,0.2 ml of plasma,0.2ml standard glutathione (GSH),0.1ml of 0.2mM H_2O_2.The contents were incubated at 37°C for 10 minutes. The reaction was arrested by adding 0.4 ml of 10 % TCA and centrifuged. The supernatant was assayed for glutathione content by using Ellman's reagent (19.8 mg of 5, 5'-dithiobis nitrobenzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate). Reduced glutathione was expressed as microgram/mg tissue.

Assay of testicular oxidised glutathione (GSSH) concentration

0.5 ml portion of the tissue extract was incubated at room temperature with 200 µl of 0.04 M *N*-ethyl maleimide (NEM) for 30 min to interact with GSH present in the tissue. To this mixture, 4.3 ml of 0.1 N NaOH was added. 100 µl of this mixture was taken and added to 1.8 ml of 0.1 N NaOH and 100 µl of the 0.1% OPT solution. After thorough mixing and incubation at room temperature for 15 min, the solution was transferred to a quartz cuvette. The fluorescence at 420 nm was determined with the activation at 350 nm. [14]

Statistical analysis: All datas 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. [15] The significance of difference was set up at ($p<0.05$). The histograms were plotted with Excel Programme.

RESULTS

Assessment of body weight and testis wt

Alterations in mean of body weight, testes weight and relative testis weight (testis weight/body weight ratio) recorded are provided in Table 1.

Table 1: Changes in gross anatomical parameters

Treatment groups	Initial body wt. (g) [I]	Final body wt. (g) [F]	Body wt. difference (g) [D=F-I]	Testis wt. (g) [T]	Relative testis wt (%) [T/F x100]
Control	188+/- 10.06	199.83+/- 10.63	11.83	0.78 +/- 0.11	0.39 +/- 0.04
Abhraka Bhasma alone	184.17 +/- 11.10	191.5 +/-17.04	7.5	0.79 +/- 0.09	0.41 +/- 0.02
Heat alone	178.13 +/- 8.8	169.83+/-9.99	- 8.3	0.65 +/- 0.08	0.38 +/- 0.03
Heat + Abhraka Bhasma	182.33 +/- 10.5	192.17 +/- 17.12	9.84	0.77 +/- 0.1	0.40 +/- 0.03

*Values are Means ± SD; ***n* = 6 in each group; ***P<0.05

Table 2: P values of body weight, testes weight and relative testis weight %

	P values					
	G1 - G2	G1 - G3	G1 - G4	G2 - G3	G2 - G4	G3 - G4
Final body wt	0.334	0.001	0.374	0.023	0.947	0.02
Testis wt.	0.867	0.041	0.872	0.017	0.723	0.45
Testis relative weight	0.299	0.635	0.635	0.069	0.512	0.275

*Values are Mean +/- SD; ***n* = 6 in each group; *** (P<0.05) is considered as significant

Table 1 depicts that there was increase in weight of control, Abhraka Bhasma alone and combined Abhraka Bhasma and heat-treated group 30 days post heating. However, heat-treated rats lost their weights when compared with their initial weights in the same period. [16] The weight gained at the end of the experiment was maximum in case of controls (11.83 g) followed by Abhraka Bhasma-cum-heat treated rats (9.83 g). The weight gain was least in the Abhraka Bhasma alone administered group on day 31 after the start of the experiment. However, it was seen that there was minor variations in the mean weight of testes in control, Abhraka Bhasma alone and combined heat-Abhraka Bhasma administered animals. Table 2 shows that there is no significant difference ($P>0.05$) in body weight between control, Abhraka alone and heat-cum-Abhraka Bhasma administered rats. However, there is significant difference between heat-treated animals as compared to the rest of animals ($P<0.05$). In G3 animals, the difference in final weight amounting to 4.5 % decrease from their initial mean live weight were observed.

Concerning testicular weights, significant decreases ($p<0.05$) were observed in heat-treated animals compared to that of Abhraka Bhasma alone group but not significantly ($p>0.05$) lowered than those of the control and heat-cum-Abhraka Bhasma administered group. [22] However, there was no significant difference ($P<0.05$) in testes weight between control, Abhraka Bhasma alone and heat cum Abhraka Bhasma administered animals.

Upon calculation of relative testes weight %, no significant difference was found between the experimental groups. However, on comparison it was seen that the P value was highest for G2-G3 and lowest for G3-G4 (Table 2). The mean relative testis weight was comparatively highest in case of G2 animals and lowest in G3 rats. The decreasing sequence of glutathione disulfide ratio amongst the four groups were found to be in the sequence G2>G4>G1>G3 (Table 3).

Assessment of testicular LPO and Glutathione levels

The testicular content of Glutathione and LPO of the experimental Wistar rats are represented in Fig. 2. It show the levels of MDA, GSH, GSSG and GSH/GSSG in the testes of control and experimental rats while the Table 4 denotes the comparative P values of all the four experimental groups

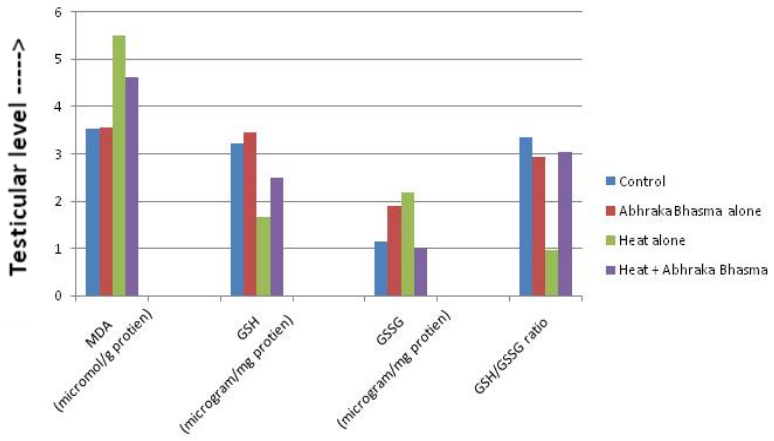

Fig. 2: Bar graph of LPO (Malondialdehyde) and Glutathione (GSH and GSSG) levels

Table 3: P values of LPO and Glutathione parameters

Parameters	P values					
	G1-G2	G1-G3	G1-G4	G2-G3	G2-G4	G3-G4
Malanodialdehyde	0.981	0.005	0.937	0.002	0.945	0.027
Reduced glutathione	0.477	0.001	0.035	0.001	0.014	0.038
Oxidised glutathione	0.176	0.011	0.63	0.616	0.134	0.01
GSH/GSSG	0.753	0.036	0.781	0.073	0.923	0.012

* ($P<0.05$) is considered as significant

The effect of lipid peroxides on the testicular content of MDA, denoted as TBARS level, is shown in Fig. 2. Heat treatment for 1 hr daily at 43^0C for four consecutive weeks induced a potent increase in MDA levels i.e. the TBARS level in heat treated group as compared to rest of the animals ($P<0.05$). Abhraka Bhasma successively lowered the mean levels of MDA in G4 group of animals (Fig.2). G2 animals showed approximately comparable MDA levels to that of control Fig. 2. However, there was no significant difference between control, Abhraka Bhasma alone and Abhraka Bhasma administered following heat treatment ($P>0.05$; Table 3). Indeed, Abhraka Bhasma administration following heat treatment decreased the lipid peroxidation (as indicated by the TBARS level) in the testicular cells.

Reduced Glutathione activity showed that there was significant differences ($P<0.05$) in all the animals except G1 and G2 which showed no significant difference between them ($P>0.05$; Table 3). The mean level of GSH activity was notably lower in case of heat alone group of animals as compared to G1, G2 and G4 rats. The P values of G1-G4 and G3-G4 were comparable, the values being $P=0.35$ and $P=0.38$ respectively. A notable elevation ($p<0.05$) in GSH content was, however, observed after Abhraka Bhasma challenge following heat treatment as compared to G3 animals, the mean value being 2.49 +/- 0.51 in G4 and 1.67 +/- 0.67 in G3 rats. Highest GSH activity was found in Abhraka Bhasma alone rats indicating the antioxidant effect of Abhraka Bhasma in testicular cells.

The testicular activities of GSSG after heat treatment were notably elevated in G3 rats comparable to that of the control ones resulting in a statistically significant ($p<0.05$) difference between them (Table 3). However, post administration of Abhraka Bhasma after heat significantly decreased the testicular GSSG activity in G4 animals by 54% as compared to heat challenged animals. However, the GSSG level of G4 rats showed no significant difference ($P>0.05$) with G1 and G2 animals (Table 3). The GSSG activities (mean value) of G4 animals were the lowest, the value being 1.01+/- 0.532 and the highest recorded in heat alone rats as 2.19 +/- 0.74 (Fig. 2).

The relative testis weight (GSH: GSSG ratio) remarkably decreased from a basal value of 3.3 +/- 2.31 to 0.96 +/- 0.74 in the heat stroke rats. The glutathione disulphide ratio of G3 animals was significantly lowered ($p<0.05$) as compared to G2 and G4 rats as well. It didn't significantly differ amongst G1, G2 and G4 groups of animals (Table 3). The GSH:GSSH ratio in different experimental groups ranged from mean value of 0.96 to 3.36 in the testicular tissues with the following rank order: G1 > G4 > G2 > G3.

DISCUSSION

Abhraka Bhasma increases masculine strength, power of retention of semen, nutrition, longevity, growth of semen, retentive faculties and the power of begetting children. Mica subjected to *putam* for more than one hundred times is entitled to the name of *bijam* (seed). Such a mica increases semen, vitality, complexion and strength of body. Cellular internalization of the test drug is due to mica nanoparticles which constitute them. Hence, as an output, efficacy of the test drug can be enhanced inspite of administering in lesser doses. As an expectation, quicker is their cellular internalization and consequent effects. Possible mechanism for rapid onset of their actions is action upon RNA/DNA molecules or by protein synthesis within the cell. To the best of our knowledge, the effect of Abhraka Bhasma on male reproductive system and function and the related mechanism of action are not well understood.

Biological cells react to the asymmetric distribution of interfacial forces across their structures. Biological system change important properties in response to environmental signals. Several studies have suggested that heat exposure could result in oxidative stress, which in turn lead to cytotoxicity.[17]Heat-stress increased oxygen radicals, possibly by the disruption of the electron transport assemblies of the membrane. Heat-induced reactive oxygen species (ROS) formation may be an additional factor that provides molecular changes in DNA, proteins, lipids and other biological molecules that may contribute to low fertility.[18]Hypoxia probably increases production of ROS through the ischemia reperfusion mechanism, thus it can be considered as a higher index of testicular oxidative stress.[19]

Peroxidative damage is currently regarded as the single most important cause of impaired testicular function and heat has been regarded as causing pathological consequences in rats resulting in oxidative stress. This study sets out the extent of oxidative stress precipitated by heat and levels of antioxidant glutathione to protect this effect in testicular tissues of heat-stroke rats. Some of the antioxidant formulations combine a large number of antioxidants with aggressive doses, raising the possibility of 'reductive stress'by potentially depleting the physiological levels of ROS known to be

critical for normal sperm function.[20] Thus, under the current status quo, the majority of infertile men are not diagnosed or adequately treated for sperm oxidative stress and a significant number take such formulations arbitrarily. To address this question this study was undertaken wherein the authors examine the underlying causes of testicular oxidative stress with respect to three markers viz., MDA, GSH and GSSG and provide data regarding the effectiveness of AB therapeutic strategy in order to assess the role of this tripeptide metabolism in understanding of the mechanism of heat.

A study by Lue Y et al., 2000 has reported that testis weight and testicular sperm counts of the male rats in the heat exposure group (43 °C, 15 min) were decreased to 65.4% and 28.9% of control levels, respectively. In this study, heat decreased the body weight by 5% from its initial body weight in G3 animals. The testes weight of heat alone rats differed by 17 % when compared with G4 rats. Thus it can be inferred that Abhraka Bhasma could clearly increase the weights of testes but maintained the body weight (Table 1). A second experiment reported that testis weight was still only 70% of control at 105 days after a single exposure of the testes to heat.[21]Making one rat testis cryptorchid for 48 hours reduces testis weight to about 60% of control after 21, compared with 35% for a single 30 minute 43^{o}C exposure, but the testes after both treatments remained at about the same size for up to 185 days. [22], [23]Testes weight were measured to evaluate the response of testes of rats to hyperthermia and potential protective effects of Abhraka Bhasma treatments on testicular cells We demonstrate here that testes-weight and body-weight reduction seen in G3 rats is due to the effect of heat. This is in agreement with previous results obtained in both mice. [24] The decrease in testis weight is related to spermatogenic failure, as suggested by the concomitant decrease in sperm concentration. The relative testes weight was less in the heat alone groups than rest of the animals but there was no significant differences in the relative testes weight of all the four groups compared.

MDA is considered an index of lipid peroxidation is a reactive end product of lipid peroxidation. MDA level can reflect the degree of damage of testicular tissues induced by ROS (reactive oxygen species, e.g., O2•−and OH•). [25] The ROS-induced injury normally causes increase of MDA level in testicular tissues. This study showed that after negative control was exposed to heat (43 °C, 15 min), the MDA level significantly increased in the testicular tissues ($P< 0.05$) (Table 3). This indicated that the antioxidant system of the testicular tissues in negative control rats had already been damaged by heat exposure. However, the results in combined heat-Abhraka group showed lower levels of MDA as compared to negative controls. This indicated that Abhraka Bhasma could effectively scavenge free radicals and suppressed the lipid peroxidation, as shown by lower TBARS levels, and alleviate the damage to testes induced by heat exposure. The mechanism behind this might be that Abhraka Bhasma directly removed H2O2, scavenged OH • induced by H2O2 or indirectly scavenged the free radicals by activating antioxidant enzyme systems in the testicular tissues. Indeed, heat treatment with *Abhraka Bhasma* could demote the TBARS parameters to the normal range..

Glutathione has pleiotropic roles, which include the maintenance of cells in a reduced state, serving as an electron donor for certain antioxidative enzymes. Reduced glutathione, a uiquitous sulfhydryl-containing tripeptide, levels were decreased and Oxidised glutathione levels were increased in heat-treated rat testis. These findings are probably related to the inhibition observed in GR and GST activities, since the interconversion GSH/GSSG/GSH is maintained by GR. [26]In this context, the decline observed in GSH levels highlight a relationship between heat and oxidative stress in rat testis. The enzymatic and non-enzymatic antioxidant defense dysregulation could be either the cause or the consequence of the oxidative stress due to poor ROS scavenging and, consequently, resulting in damage to testes cells. Zamoner et al., 2008 proposed that irrespective of the levels of ROS generation, oxidative stress occurs if the antioxidants are lowered. Abhraka Bhasma administration to rats orally, daily for 30 days was found to increase the reduced glutathione (GSH) and decrease oxidized glutathione (GSSG) concentrations in testicular tissue. These alterations resulted into a significant elevation in GSH/ GSSG ratio in G4 tissues. The GSH/GSSG ratio is particularly important for testis, which has a high mitotic index, in view of GSH involvement in the DNA, RNA and protein synthesis The observed higher magnitude of changes in testicular tissue are of significance as testis has been reported to be a highly sensitive organ towards heat exposure. A simultaneous inhibition in the activities of glutathione reductase and glucose-6-phosphate dehydrogenase in these tissues may be responsible for an altered GSH/GSSG ratio as these enzymes take part in GSSG to GSH conversion. Under hyperthermic states, the decrease in GSH/GSSG ratio and also a decrease in GSH contents in testicular cells of hyperthermic rats suggest severely hindered GSH metabolism resulting in disturbed testicular redox pool in heat-stroke rats.

The present work was designed to investigate the potential testiculo-protective effect of Abhraka Bhasma as antioxidant-rich pharmaceutical. Possibly antioxidant activities of Abhraka Bhasma were due to the presence of nanoparticles constituting them which penetrated deep into the tissues and efficiently produce protective effects in the heat-dmaged testicular cells. Thus it can be inferred that protecting potential of Abhraka Bhasma could be at least in part due to its free radicals scavenging capability. The mechanism might include the binding of metal ions, scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS) or their precursors, up-regulation of endogenous antioxidant enzymes or the repair of oxidative damage to biomolecules. Abhraka Bhasma ameliorates heat-induced oxidative stress in the testes suppressing lipid peroxidation and restoring glutathione content of the testicular cells to their normal physiological levels.

It warrants a further approach for its testiculo-protective potential to act as anti-impotency fecundity drug. The fact that Abhraka Bhasma increases sexual debility ameliorating effects on heat-induced testicular damage in laboratory can be beneficial for treatment of male infertility in people residing in tropical and sub-tropical countries. These observations suggest that Abhraka Bhasma could be excellent adjuvant support in the therapy of male infertility. However, further studies are required in order to confirm these effects in humans. There is a need for further investigation with randomised controlled studies to confirm the efficacy and safety of Abhraka Bhasma supplementation in the medical treatment of male infertility as well as the need to determine the ideal dose of each compound to improve semen parameters, fertilisation rates and pregnancy outcomes.

In conclusion, this work provides the novel evidence that Abhraka Bhasma has a protective effect in heat-induced oxidative stress in rat testicular cells by elevating glutathione activities and decreasing TBARS levels. This is attained by attenuating free radical scavenging enzymes and reducing lipid peroxidation in testes of hyperthermic rats. Our results indicate that the adverse effect of the generated heat had a negative impact on testicular enzymatic activity and positive effect on malondialdehyde values. This finding also indicated the possible role of Abhraka Bhasma in mitigating the oxidative stress imposed on the testes and restoring normalcy to the testes. Abhraka Bhasma ameliorates heat-induced oxidative stress in the testes suppressing lipid peroxidation and restoring glutathione content of the testicular cells to their normal physiological levels. Oral supplementation with Abhraka Bhasma may go some way to improve a couple's chance of conception by improving fertility in males residing in hot regions.

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