Original Article

Assessment of Cinnamaldehyde's Potency on Heat Stress-induced Testicular Impairments in Wistar Rats

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Abstract

Background: Male sterility results from high testicular temperatures, which affect mammalian spermatogenesis. High testicular temperatures affect sperm motility, morphology and fertility according to their magnitude and duration. **Aim:** The aim of the current study is to examine the effects of heat-induced oxidative stress and cinnamaldehyde on Wistar rat testicular structure and function. **Settings and Design:** The rats used in this experiment were Wistar albino rats. **Materials and Methods:** This research has six animals per group. Male Wistar albino rats of 2.5–3 months old and 275–300 g. (I) control, (II) heat stress (HS) in a closed chamber at 41°C for 14 days and (III) HS with cinnamaldehyde (CA) 50 mg/kg body weight for 14 days. (IV) CA alone. After the study, the animals were euthanised, and test samples were taken for sperm count, morphology, haematoxylin and eosin stain for normal cellular morphology, antioxidants and DNA integrity assessments. **Statistical Analysis Used:** The data were analysed statistically using one- and two-way ANOVA tests for comparisons between groups. **Results:** The stress group had significantly lower sperm counts and poor sperm morphology. The stress group's antioxidant capacity is much lower than that of the control group. Animals under stress have fragmented DNA. Treatment with cinnamaldehyde increased overall antioxidant capacity and seminal parameters, and rats behaved most like controls. **Conclusion:** CA restores malondialdehyde levels, total antioxidant capacity, sperm characteristics and mitigates testicular damage in rats exposed to experimental HS.

Keywords: *Cinnamaldehyde, DNA integrity, heat stress, male infertility, sperm analysis*

Introduction

Infertility is a common global health concern. The World Health Organization reports that male factors are responsible for 50% of all reproductive issues and that 9% of all couples in the world experience fertility issues.[1] It is estimated that 8%–12% of couples in the reproductive age range struggle with infertility. One in twenty males in the reproductive age group suffer from male factor infertility,[2] which accounts for approximately half of all cases of infertility. Many of them were diagnosed with idiopathic disease, which can also be caused by improper or suppressive control of genes. Soon after a man reaches puberty, a process known as spermatogenesis begins to take place in his body. The scrotum plays a significant role in ensuring that the testes are kept at the ideal temperature for gametogenesis, which is essential. Testicular thermoregulation is accomplished using a similar counter-current technique.[3] The temperature has been shown to influence the vitality of germ cells and even activate their death response, and it has been observed that even small variations in physiological or

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ambient temperatures can initiate numerous signalling pathways.

Germ cell and Leydig cell apoptosis,[4,5] which in turn reduces sperm production, is accelerated by elevated ambient temperatures.[6] Heat stress (HS) caused oxidative stress modifications and an assault on sperm production by eliminating spermatogenic cells and causing Sertoli and Leydig cells to degenerate.[7,8] Sperm dysfunction can be the result of testicular hyperthermia in humans,[9] which can cause sperm cells to undergo apoptosis, interfere with sperm production and lower sperm concentration.[10] The HS that people experience is one of the key factors contributing to infertility around the world. People who labour in regions that are prone to heat are more likely to have abnormal sperm counts, motilities and viabilities, in addition to other metrics. The condition known as HS occurs when an animal is subjected to temperatures that are outside of its physiological range and exceed its capacity for compensation. Although it typically affects the entire body, HS can sometimes be confined to a particular organ or anatomical region.[11] Numerous studies conducted over the course of the last few decades have demonstrated that high temperatures have a detrimental effect on the process of spermatogenesis in a wide range of animal species.[12] When the testes warm up, it is because the animal is in a hot environment and the scrotal skin cannot stay cool enough through perspiration.[13] Several lines of evidence are beginning to point towards reactive oxygen species (ROS) as a possible cause of HS-induced infertility and impairment of the testicular antioxidant defence system,[14,15] increased production and lipid peroxidation.[16] There is minimal scientific evidence to support the use of herbal plants for treating similar conditions despite their long history of usage in traditional Indian medicine. As a consequence of this, they demonstrate a wide range of pharmacological activities that are important from a therapeutic perspective. Cinnamon, a familiar spice as well as a kind of traditional herbal medicine,[17] has been put to wide use throughout the countries of Asia to treat ailments and improve health.[18,19] Cinnamaldehyde (CA) is the principal bioactive component of the essential oil that is extracted from the stem bark of the cinnamon tree. The presence of a cinnamoyl moiety within the structure of CA analogues. As a result of the presence of a highly reactive unsaturated carbonyl pharmacophore (Michael acceptor) in their structures, these compounds have a propensity to react as electrophiles with certain enzymes and/or receptors. Because of their bioactivities, particularly the anti-inflammatory and anti-cancer effects, trans-CA, 2-benzoyloxycinnam-aldehyde (2-BCA) and 2-hydroxycinnamaldehyde (2-HCA), three naturally occurring compounds that belong to this class, have generated a great deal of attention. These compounds are trans-CA, 2-BCA and 2-HCA, respectively. Researchers have discovered a correlation between CA and the prevention of a wide variety of diseases, including endotoxaemia, sepsis, diabetes, ulcerative colitis and arthritis.[20,21] CA has been shown to provide considerable protection against joint disease in animal models of arthritis.[22] CA not only significantly decreased the amount of the inflammatory mediator tumor necrosis factor-α, interleukin (IL)-6 content in RA patients peripheral monocytes, but it also prevented arthritis patients synovial fibroblasts from releasing IL-1 and matrix metalloproteinase -13.[23] CA has the potential to boost testosterone levels as well as sperm quality indicators such as population, viability and motility,[22] in addition to healthy spermatogenesis and fertility.[23,24] In addition, it has been observed to maintain sexual and androgenic activities in mature male rats while simultaneously increasing their muscular mass.[25] Therefore, the purpose of this study is to evaluate the efficacy of CA on the male Wistar rat and sperm parameters, as well as histological change and related gene expression in long-term HS-induced rats.

Materials and Methods

Source of chemicals

CA, ethidium bromide and all the other chemicals were purchased from Sigma (St. Louis, MO, USA). Total Antioxidant Capacity Kit (ELabScience, United States; Cat no. E-BC-K219-M) and Total Hormone Assay Kit were purchased from CUSA Biotechnology.

Animals

A 12-week-old Wistar rats weighing 275–300 g were used in this study. Animals were housed in cages and maintained the standard conditions (with 12 h dark and light cycle) with rat feed and water *ad libitum*. This study was conducted after the approval of the institutional animal ethical committee and the approval number (IAEC No. 01/04/22). Both the Canadian Council on Animal Care Guide and the Committee for the Purpose of Control and Supervision of Experiments on Animals CPCSEA (India) Guidelines[26] for appropriate laboratory animal care. The animals were grouped into four categories. The animals in Group I, which served as the control, were housed in conventional cages throughout the experiment. Animals in the HS group (Group II) were exposed to 41°C for 1 h, which lead them to experience HS. The animals in Group III were subjected to HS after being treated with CA Orally (50 mg/kg of CA in Dimethyl Sulphoxide) for 14 days. Group IV animals received CA alone throughout the experimental period.

Sampling

Blood sampling

Blood samples were collected by a retro-orbital sinus puncture technique using a microhaematocrit tube with appropriate labelling in the time frame of 8:00 a.m. to 10:00 a.m. Blood samples were centrifuged at 2500 rpm for 10 min for serum separation and stored at −80°C for further analysis.

Tissue sampling

Testis excised after sacrificing the rats by cervical dislocation and rinsed thoroughly with a saline solution. Dissecting out the caudal epididymis (which is free of lipids, vas deferens and other tissue) from either side of the testis of rats and immersing it in 10 mL of 0.87% warmed normal saline for the sperm cells to release.[27,28]

Sperm analysis

Sperm count

The sperm was collected from the epididymis by slicing with anatomical scissors, immersed in 5 ml of physiological saline, rocked for 10 min and incubated at room temperature for 2 min. The supernatant fluid was 100-fold diluted using a solution of 5 g sodium bicarbonate, 1 mL (35%) formalin and 25 mg eosin in 100 mL distilled water. The total number of sperm was determined using a haemocytometer. The diluted sperm suspension was added to each counting chamber in an amount of 10 µL, and it was let stand for 5 min before being counted using a ×400 light microscope[29] (BX53-F2, Olympus Optical USA Ltd.).

Motility

To examine the progressive motility of sperm, the seminal fluid that was taken from the cauda epididymis by using a pipette was diluted with tris buffer solution (Cat. No. 648315, MERK India) and made up to 2 mL. The appropriate volume of sample was placed on a slide and then viewed under a phase contrast microscope. The percentage of motility (×400) on the slide was visually examined for each sample at 37°C. The data obtained from three distinct fields was analysed, and the final score was determined.[30]

Morphology

The morphology of the sperm was analysed by adding 900 µL of sperm sample and 100 µL of 1% eosin 1% aqueous eosin Y (10:1) to the sperm solution and preparing a smear on glass slides. The smear was allowed to dry naturally in the air and placed in the glass slides for mounting (DPX mounting medium). Under a light microscope, the magnification was adjusted to ×400, and visually examined the samples (200 cells from each sample) for morphological abnormalities.[31]

Viability

The percentage of viable (membrane-intact) spermatozoa was examined as reported.[32] The sperm suspension was diluted with nigrosine-eosin to achieve a final volume of 20 µl. Smeared the sample on a glass slide and allowed it to dry. Then, the slide was examined using light microscopy with the magnification adjusted to ×400, and the samples were visually examined (BX53-F2, Olympus Optical USA Ltd.). A differential count of 200 spermatozoa was carried out by comparing the number of unstained sperm (with an undamaged membrane) to the number of stained sperm.

Hypo–osmotic swelling test

The HOST was performed for determining the membrane integrity as it measures the sperm membrane's capacity to maintain the sperm cell and its surroundings in a state of relative equilibrium. Fluid infiltration causes the sperm tail to 'swell' and coil. A higher number of enlarged sperm implies that they have a healthy plasma membrane. By adding 0.1 mL of semen with 1 mL of a hypo-osmotic solution-1% sodium citrate solution (100 mOsm/kg) and incubated for 30 min at 37°C.[18] After fixing the samples in a buffered 2% glutaraldehyde solution at 37°C and examining 200 spermatozoa under a microscope, the percentage of spermatozoa with coiled mid-pieces and tail segments were calculated.

DNA fragmentation and agarose gel electrophoresis

The phenol–chloroform method was used for DNA fragmentation as described.[33] Washed the 60 mg of minced testis tissue thrice with ×1 Phosphate buffer solution and added ×1 tissue lysis buffer and homogenised. After homogenisation, the samples were transferred to the appropriately labelled tubes and centrifuged at 12,000 rpm for 10 min. The supernatants were discarded and resuspended the pellet using 150 µL of lysing buffer and incubated at 80°C for 20 min. The tubes were kept in a water bath at 37°C for 30 min after adding proteinase K. After incubation, an equal volume of (phenol–chloroform–isoamyl alcohol) PCI was added to the samples and centrifuged tubes at 10,000 rpm for 20 min. Chloroform: isoamyl alcohol (24:1) was added to the aqueous layer and centrifuged at 12,000 rpm for 20 min at 25°C. After centrifugation, the samples were transferred to fresh tubes, and 2.5 volumes of 70% alcohol were added to the tube and centrifuged for 4 min at 12,000 rpm at 4°C. Then, DNA samples were loaded into dry wells of a 1.5% agarose gel in TAE Buffer (Tris-acetate-ethylenediaminetetraacetic acid) with 0.5 µg/mL ethidium bromide using 5 µL of 6X DNA loading buffer.

Biochemical parameters

Total antioxidant capacity and malondialdehyde levels

The total antioxidant capacity was analysed using the Elabscience total antioxidant kit. The tissues were washed and homogenised using homogenising medium at 2°C–8°C at the ratio of 9:1 and centrifuge homogenate at 10,000 g for 10 min at 4°C. To 10 µL of the supernatant, add 20 µL of the peroxidase solution and 170 µL of ABTS working solution. Mix well and stand for 6 min at room temperature. The colour intensity was measured spectrometrically at 414 nm. The malondialdehyde (MDA) levels in the seminal fluid were measured using thiobarbituric acid reactive substances assay.[34] To the 50 µl of seminal fluid from the epididymal tail, add 1.0 mL of 15% trichloroacetic acid and 0.2% butylated hydroxytoluene (dissolved in ethanol), and the mixture was centrifuged at 4000 g for 15 min at 4°C. Then, 1.0 mL thiobarbituric acid (0.375% in 0.25 M) hydrochloric acid was added to the 500 µL of supernatant and kept in a boiling water bath for 20 min and cooled. The colour intensity was recorded spectrometrically at 532 nm.

Hormone analysis

Testosterone (T) – (Cat Log. No CSB-E05100r CUSABIO, USA), follicular stimulating hormone (FSH) (Cat Log. No CSB-E06869r CUSABIO, USA) and luteinising hormone (LH) (Cat Log. No CSB-E12654r CUSABIO, USA), in serum samples were analysed using T, FSH, LH ELISA kits procured from CUSA Biotechnology and followed manufacturer's protocol.

Histopathological studies

The histological examination of the testis was done using haematoxylin and eosin. The tissues were fixed using a neutral-buffered formalin solution for 10 h. After washing the testis sample with 70% alcohol, it was processed for dehydration by descending grades of paraffin embedding, paraffin sections 5 µ thick and then it was stained with haematoxylin and eosin.[35]

Statistical analysis

The data were analysed statistically using GraphPad Prism version 8.3 (GraphPad Software, San Diego, CA, USA) and expressed as the mean ± standard deviation (SD), one- and two-way ANOVA tests for comparisons between groups and analysis of variance for multiple comparisons. Statistical significance was set at a value of $P < 0.05$.

Results

Body and tissue weight

Table 1 shows body weight and tissue weight in (g). Values are expressed in mean ±SD.

Sperm analysis

Efficacy of cinnamaldehyde in sperm count

The Group II animals (HS-induced) showed a declining sperm count when compared to that of the control animals (Group I). On treatment with CA, we observed an increase in the quantity of mature sperm, their motility and sperm count compared to HS-induced animals, while the control group exhibited higher sperm counts [Figure 1a and b]. Throughout the investigation, no experimental animal groups showed any signs of mortality or morbidity.

Motility

The HS-induced rats (Group II) showed a significant increase in non-progressive motile (PM) and non-motile sperm compared to the control animals (Group I). In contrast, giving CA to animals that were under a lot of HS (Group III) increased the number of PM sperm [Figure 2].

Morphology

A sperm was considered to have an aberrant head with a typical hook on it rather than a banana-shaped, triangular or enormous head. There were also reports of the animals having an abnormally curved neck or tail, as well as various deformities in their pathological condition. A similar trend in sperm was documented in the HS group (Group II). After 14 days of CA treatment, the sperm morphology returned to normal (Group III) [Figure 3a-c].

Viability

A viability test was performed for counting the viable sperm, and it was observed that dark-stained sperm were higher in number as they were depleted in the HS-induced group (Group II), as this HS damages membrane integrity and induces DNA fragmentation compared to control animals (Group I). After the CA induction for 14 days, the animals showed a decrease in

Table 1: Body weight and tissue weight in (g)

Parameter	Group I	Group II	Group III	Group IV
Body weight	315±26.07681	297±14.8324	306.5±1781853	299.1667±11.58303
Absolute tissue weight testis	3.331667±0.256944	3.008333±0.090646	3.196667±0.206753	3.185±0.112027
Epididymis	1.07±0.053572	1.028±0.020736	1.072±0.04274	1.066±0.053572
Caudal epididymis	0.523333±0.040825	0.508333±0.034303	0.566667±0.04761	0.573333±0.044121

Values are expressed in mean±SD. SD=Standard deviation

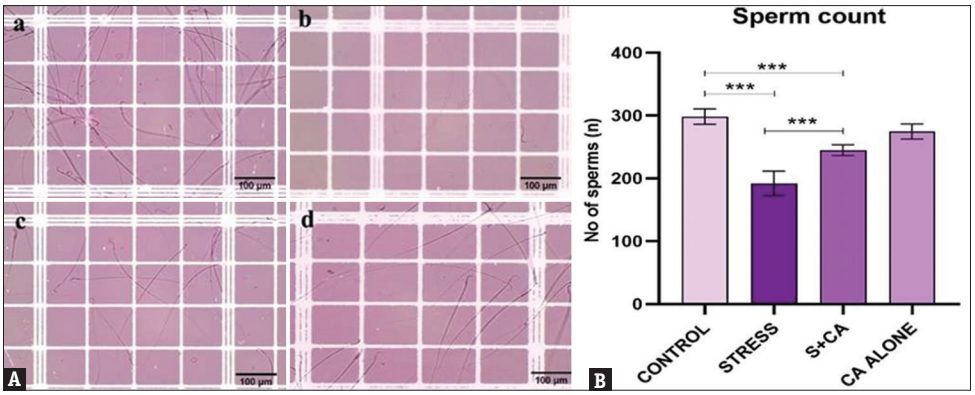

Figure 1: (A and B) Sperm count. (A) Light microscopic image of sperm count (a) control rat (b) heat stress (c) stress + cinnamaldehyde (CA) (d) CA alone treated group (B) sperm count in control and different experimental groups *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde

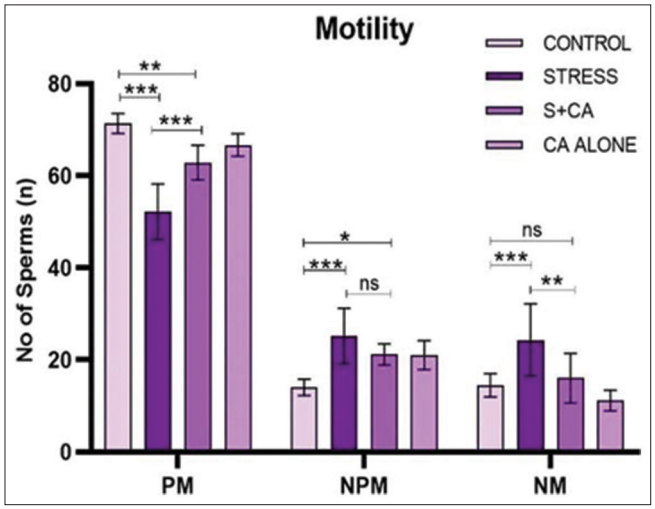

Figure 2: Sperm Motility in control and different experimental groups *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde, PM: Progressive motile, NPM: Non-progressive motile, NM: Non-motile

the number of damaged sperm and retained membrane integrity as the DNA fragmentation was noticeably decreased in Group III [Figure 4a and b].

Hypo–osmotic swelling test

The HOST results revealed that the HS-induced animals (Group II) showed an increase in the number of busted-tailed sperm as the integrity of the membrane was lost, which indicates an abnormality. Furthermore, there was an increase in the quantity of fragile sperm and the presence of cytoplasmic droplets, both of which are signs of a damaged sperm membrane, but a rise in the size of the sperm's inflated tail in the cinnamaldehyde-treated group. On CA exposure (Group III), the animals showed a significant decrease in change in the number of busted-tailed sperm and observed retaining of the intact membrane [Figure 5a and b].

DNA fragmentation

Fragmented DNA was observed through electrophoresis in the stress group, and after 14 days of treatment with CA, intact DNA was observed in stress + CA (Group II; Lane II) and a degraded band (Group III; Lane 3) was observed in the stress group [Figure 6].

Biochemical parameters

Total antioxidant capacity and malondialdehyde levels

The extent of the tissue damage was assessed by analysing lipid peroxide levels and total antioxidant capacity in the experimental rats. Assessment of MDA levels in the experimental groups dictated that the HS group had inclined levels of MDA [Figure 7] and a decrease in the levels of the total antioxidant capacity [Figure 8], indicating a severe oxidative imbalance. On CA treatment, the levels of MDA declined and the total antioxidant capacity was markedly inclined, showing that CA is efficient in defending against the oxidative damage caused by free radicals.

Hormone analysis

Serum levels of testosterone, LH and FSH were shown to be significantly lowered in the stress group compared to the control group. Serum hormone levels were higher in CA-treated groups when compared to the stress group, indicating that CA increases serum hormone levels [Figure 9].

Histopathological studies

The histopathological examination of the seminiferous tubules in the control rats depicted a distinctive cellular organisation. In the basement membrane, the spermatogonia had dark nuclei, whereas primary spermatocytes with large and round nuclei were arranged in a coiling pattern. Moreover, the immature spermatids were in multiple layers, while the mature ones were found embedded in Sertoli cells, with their tail in the lumen. In contrast, heat-stress-exposed rats displayed abnormal cellular organisation with deeply stained, shrunken pyknotic nuclei, extensively degenerated seminiferous tubules and reduced germinal

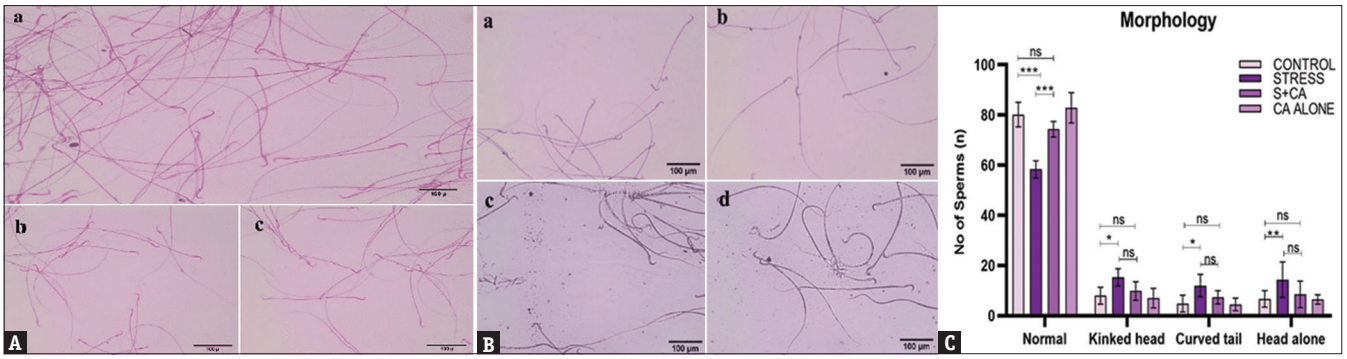

Figure 3: (A and B) Morphology. (A) Morphology analysis (a) control group; (b) stress + treated group; (c) cinnamaldehyde treated alone (B) Morphology analysis in stress group (a) shows head alone; (b) shows body alone; (c and d) cytoplasmic burst and curved tails and body. (C) Morphology of the sperm in control and different experimental groups *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde

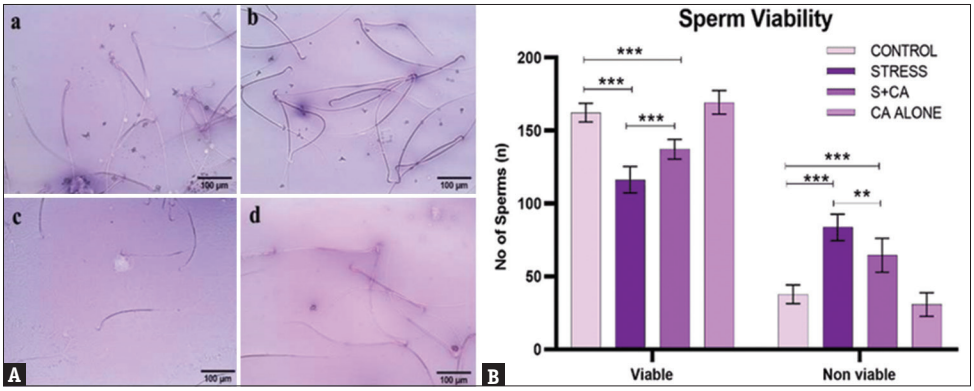

Figure 4: (A and B) Viability. (A) Microscopic image of sperm viability. (a) Control rat sperm count (b) represents heat stress (c) stress + CA: cinnamaldehyde (CA) (d) CA alone (B) Viable and non-viable sperm cells in control and groups. Sperm viability in control and different experimental groups *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde

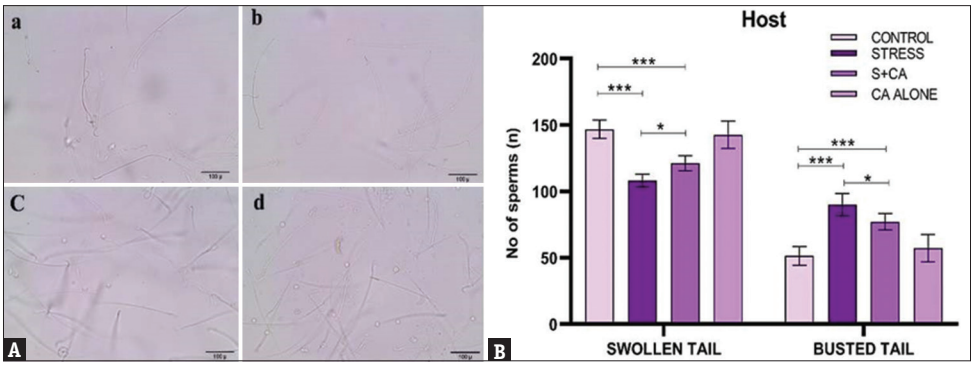

Figure 5: (A and B) Hypo–osmotic swelling test (HOST). (A) represents microscopic image of HOST of rat sperm (a) control rat sperm (b) represents heat stress (41°C) induced rat sperm (c) stress + cinnamaldehyde (CA) treated rat sperm (d) CA alone given rat sperm. (B) Number of sperms with Swollen and busted tails in control and different experimental groups *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde

epithelial and spermatid cells. Spermatocytes discovered in the lumen have irregular morphology [Figure 10]. However, treatment with CA showed halting heat-induced deterioration, and fewer nuclei shrank as a result of the preservation of the germinal epithelium and seminiferous tubules. Furthermore, a rise in the number of spermatocytes was observed in the lumen when compared to the HS group.

Discussion

Germ cell destruction due to HS is the most noticeable effect on the testis.[12,36] The ratio of pro-oxidative molecules that overlap antioxidants in response to elevated ROS is the measure of oxidative stress.[37,38] Motility is influenced by oxidative stress because of the way it disrupts axoneme structure,[7] which ultimately causes abnormalities in sperm tails and a decline in the

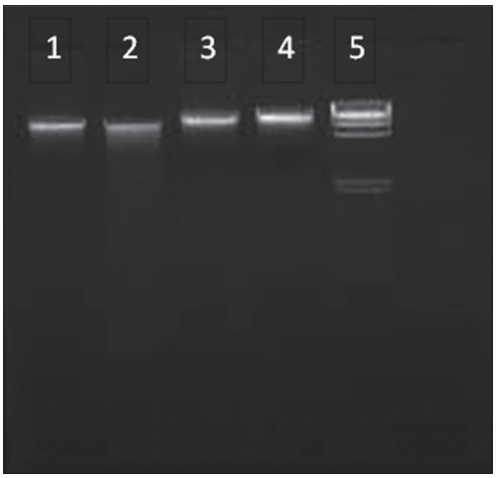

Figure 6: DNA integrity. DNA integrity of testis in control and different experimental groups. (1) Control, (2) heat stress, (3) stress+ CA treatment, (4) CA treatment alone, (5) lambda phage-restricted DNA

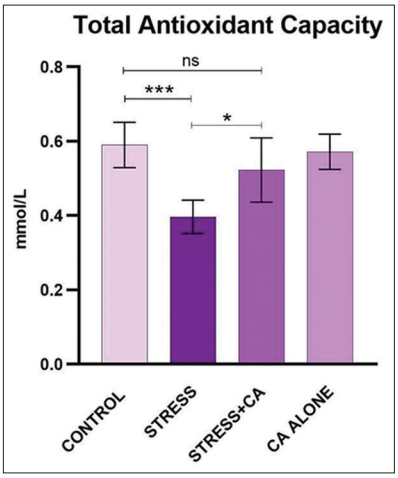

Figure 7: Total antioxidant capacity. CA: Cinnamaldehyde

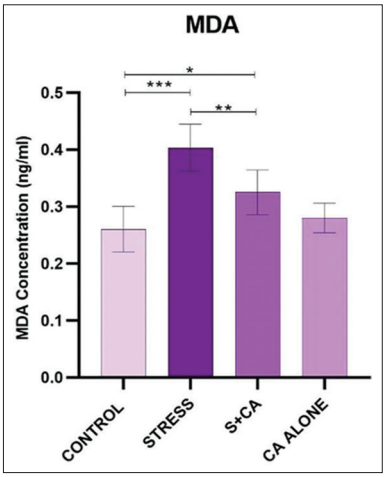

Figure 8: Malondialdehyde levels. Values are expressed as mean ± standard deviation for three experiments in each group. Values are statistically significant at the levels $*P < 0.033$, $**P < 0.002$ and $***P < 0.001$, respectively. CA: Cinnamaldehyde, MDA: Malondialdehyde

motility of the sperms.[29] Sperm cells are, especially vulnerable to peroxidation by ROS because of their high polyunsaturated fatty acid content.[39] After 14 days of therapy, researchers found a statistically significant improvement in the baseline morphology and motility of the sperm. Reduced testicular space of the Leydig cells, which could release steroid hormones and other substances involved in the control of the hypothalamic–pituitary–gonadal axis, is a direct outcome of oxidative stress's negative impact on testicular function.[40] The effects of scrotal HS on sperm survival, sperm DNA integrity and offspring sex ratio in mice, they discovered that HS reduced sperm concentration, viability and motility, which is consistent with the present findings.[41] We found that the findings were consistent with our own.[42] Our study results showed that the rats that were subjected to HS showed the following abnormal conditions: increased immotility of sperm, a decline in sperm count, motility, viability, morphology, progressive motility, non-progressive motility, DNA integrity and fragility, which were observed in the study was done by Abdollahi *et al*. and Ngoula *et al*.[43,44] This study discovered increased levels of ROS during HS, which can decrease sperm quality by reducing the phosphorylation of axonemal proteins, disrupting the respiratory chain and ATP synthesis and increasing mitochondrial membrane permeability.[44] Histological architectural alteration in the HS group, as seen in the study.[45] In treatment with CA, the testicular structural integrity was preserved, and their functionality was improved.

Furthermore, the mean values of the sperm parameter groups that underwent HS were substantially different from the control group. Group III (CA-treated group) in our study had a considerably higher sperm count compared to the stress group. In Group III, the sperm count was higher than in Group II, but it was not significantly different from Group I (the control group) in Group IV (the treated-alone group).

In the current investigation, testosterone, LH and FSH levels were significantly higher in the treated group (Group III) than in the control group. This result implies that CA can alter the secretion of these hormones. Khaki's research showed that treatment with *Cinnamomum Verum* increased serum testosterone total antioxidant levels while decreasing MDA level, and the results of our study confirmed that the MDA level decreased after treatment with CA and that the total antioxidant level rose after treatment in the stress group (Group II) compared to the control group (Group I). Following treatment with CA, the level of the total antioxidant capacity increased. This conclusion likewise resembles the outcomes of the study.[46]

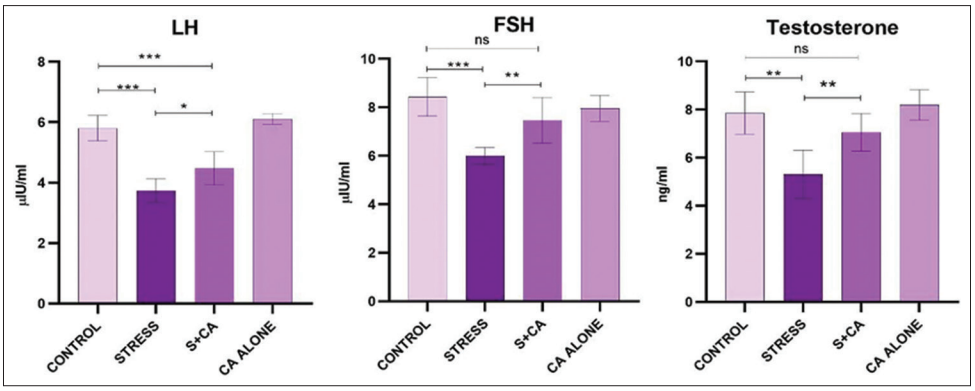

Figure 9: Hormone analysis. luteinising hormone, follicular stimulating hormone and testosterone levels in serum. Values are expressed as mean ± standard deviation for three experiments in each group. Values are statistically significant at the levels. *$P < 0.033$, **$P < 0.002$ and ***$P < 0.001$, respectively. CA: Cinnamaldehyde, LH: Luteinising hormone, FSH: Follicular-stimulating hormone

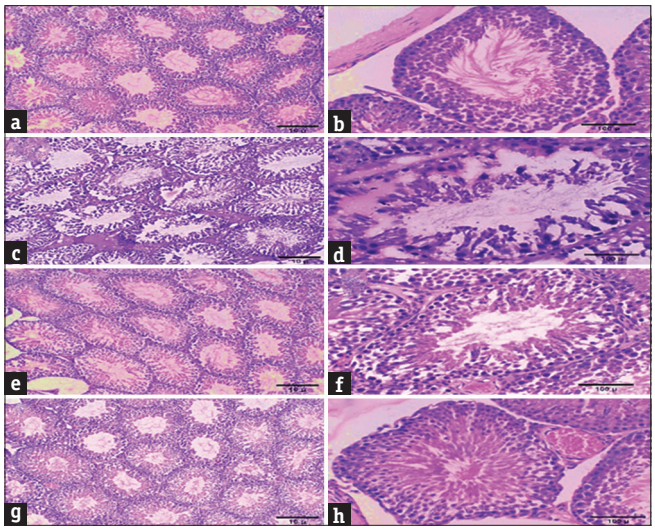

Figure 10: Histology. Testis sections from control and other experimental groups were photographed under a microscope. Seminiferous tubules germinal epithelia, testicular interstitial tissue and control groups ×10 and ×40, respectively, are shown in (a) and (b), which represent the control groups, (c and d) represent Heat stress group (41°C) abnormal dis integration and destruction seen decreased germline epithelium in ×10 and ×40, respectively (e and f) represents Stress + cinnamaldehyde (CA) (50 mg/kg body weight) treated group shows significant improvement in the testicular microscopic architecture respectively with ×10 and ×40. (g and h) group represents CA alone treated animals shows structurally similar to the control group in ×10 and ×40 microscopic images

Conclusion

Our study highlights the limited but promising evidence supporting the preventive effects of CA (50 mg/kg body weight) against HS-induced damage to sperm parameters and testicular tissue. Our findings demonstrate that CA has the capacity to elevate spermatogenesis-promoting hormones, improve sperm parameters including count, viability and morphology, and mitigate testicular damage in response to HS. However, further investigations are warranted to elucidate the precise molecular mechanisms underlying these effects. Further studies are needed to unravel the intricate pathways by which CA influences men's reproductive systems. This deeper understanding will enable researchers to stratify individuals based on the severity of pathological conditions and tailor CA treatment accordingly. Overall, our study underscores the importance of ongoing research to optimise CA-based interventions for improving male reproductive health outcomes.

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Author's contribution

PM: Conceptualisation, methodology, data curation, writing-original draft preparation. RR: Visualisation, investigation and supervision. DW, JP and NR: Software, validation and editing. All authors read and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

This study's data sets are accessible upon reasonable request from the corresponding author.

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