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Original Article

Low dose of melatonin ameliorates cryptorchidism-induced spermatotoxicity in rats

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ABSTRACT

Introduction: Cryptorchidism has been associated with spermatotoxicity and oxidative stress while melatonin is a well-known anti-oxidant. This study investigated the possible ameliorative effect of melatonin on cryptorchidism-induced spermatotoxicity and oxidative stress.
Methods: Thirty six male Wistar rats were randomised into sham-operated (n = 18) and bilaterally cryptorchid (n = 18) groups, each of which were subdivided into 3 oral treatment groups (n = 6 rats each) that received normal saline, low dose (4 mg/kg) and high dose (10 mg/kg) melatonin.
Results: Cryptorchidism reduced sperm parameters, oestradiol, luteinising hormone, follicle stimulating hormone and glutathione peroxidase activity, but increased testosterone and lactate dehydrogenase activity. The cryptorchidism-induced spermatotoxicity and oxidative stress were ameliorated by low dose of melatonin but exacerbated by its high dose.
Discussion: Melatonin's effect on cryptorchidism-induced spermatotoxicity is dose-dependent.

1. Introduction

Cryptorchidism, a congenital abnormality found in 2%–5% of newborn males, is defined as failure of descent of one (unilateral) or both (bilateral) testes into the scrotum, leaving it in the intra-abdominal position (2%), external ring (9%), ectopic (11%) and commonly the inguinal canal (63%).[1] It can be congenital or acquired, and can be caused by environmental and genetic factors.[2] It can occur as an isolated event or as part of a variety of syndromes.[3] It impairs spermatogenesis (which is more severe in bilateral cryptorchidism than in unilateral cryptorchidism)[1] and increases incidence of testicular cancer.[4]

Melatonin (N-acetyl-5-methoxytryptamine), referred to as chemical expression of darkness because its peak in the blood of vertebrates always coincides with the dark phase of light/dark cycle,[5] is secreted in the pineal gland[6] and other extra-pineal sources like retina, gut, skin, bone marrow, lymphocytes, and ovaries.[7] Its ability to scavenge free radicals like hydroxyl radical ($^{\bullet}OH$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet-}$), hypochlorous acid (HOCl), peroxynitrite anion ($ONOO^-$), nitric oxide ($NO^{\bullet}$), and others in many conditions[8] directly by free radical scavenging actions;[9] indirectly by enhancing anti-oxidative enzymes activities;[10] reducing electron leakage from the mitochondrial electron transport chain;[11] stimulating the synthesis of glutathione;[12] and its synergistic interactions with other anti-oxidants[13] has been extensively documented. However, its pro-oxidant action has also been extensively reported in many conditions.[14,15]

The effects of melatonin on male reproductive functions are controversial and inconclusive as both beneficial[16] and detrimental[17] effects have been well reported. Saalu et al.[18] reported that the cryptorchidism-induced azoospermia and asthenospermia were improved by melatonin, while testosterone level was unchanged. However, there was no data to support their claim that the ameliorative effect of melatonin was mediated by its anti-oxidative action, neither was there any hormonal explanation for the effect which this study sought to provide.

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2. Materials and method

2.1. Animals

Thirty six (36) male albino rats (150–170 g) were obtained from the Animal House of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Kwara State, Nigeria. They were housed at room temperature with free access to food and water *ad libitum* and were maintained on the daily light/dark cycle. "Principles of laboratory animal care (NIH publication No. 85-23, revised 1985)" were followed. All experiments have been examined and approved by our institutional ethics committee.

2.2. Experimental protocol

After 2 weeks acclimatisation to their new environment with standard laboratory diet and water given *ad libitum*, the animals were randomly divided in a blinded fashion into sham-operated (n = 18) or bilaterally cryptorchid (n = 18) group. Each of these groups was then subdivided into 3 oral treatment groups (n = 6 rats each) that received normal saline, low dose (4 mg/kg)[19,20] melatonin (Bulk supplements, Henderson, Nevada, USA) or high dose (10 mg/kg)[21,17] melatonin for 30 days.

Animals were sacrificed a day after the last treatment under light ketamine anaesthesia and serum was collected from each animal and preserved at −20 °C.

2.3. Determination of epididymal sperm parameters

The epididymal sperm parameters (count, motility, morphology and viability) were determined as previously described.[22,23]

2.4. Determination of reproductive hormones

Enzyme-linked immunosorbent assays of Testosterone (Monobind Inc., Lake Forest, CA, USA. Product Code: 3725-300), Oestadiol (Monobind Inc., Lake Forest, CA, USA. Product Code: 4925-300), Luteinising Hormone (Monobind Inc., Lake Forest, CA, USA. Product Code: 625-300), and Follicle Stimulating Hormone (Monobind Inc., Lake Forest, CA, USA. Product Code: 425-300) were done spectrophotometrically (Spectramax plus, Molecular devices, Sunnyvale, CA, USA) following the kits' manufacturer procedures.

2.5. Determination of glutathione peroxidase and lactate dehydrogenase activities

Colourimetric assays of lactate dehydrogenase (product code BXC0243; Fortress Diagnostics, UK) and glutathione peroxidase activities were done spectrophotometrically (Spectramax Plus; Molecular Devices, Sunnyvale, CA, USA) following the kits manufacturer's procedures.

2.6. Data analysis

Data were analysed using SPSS version 16.0 for windows (IBM Corporation, Armonk, NY, USA). All values given were the Mean ± S.E.M of the variables measured. Significance was assessed by the one-way Analysis of Variance (ANOVA), followed by a post-hoc Least Significance Difference (LSD) test for multiple comparisons. *p*-Values of 0.05 or less were taken as statistically significant

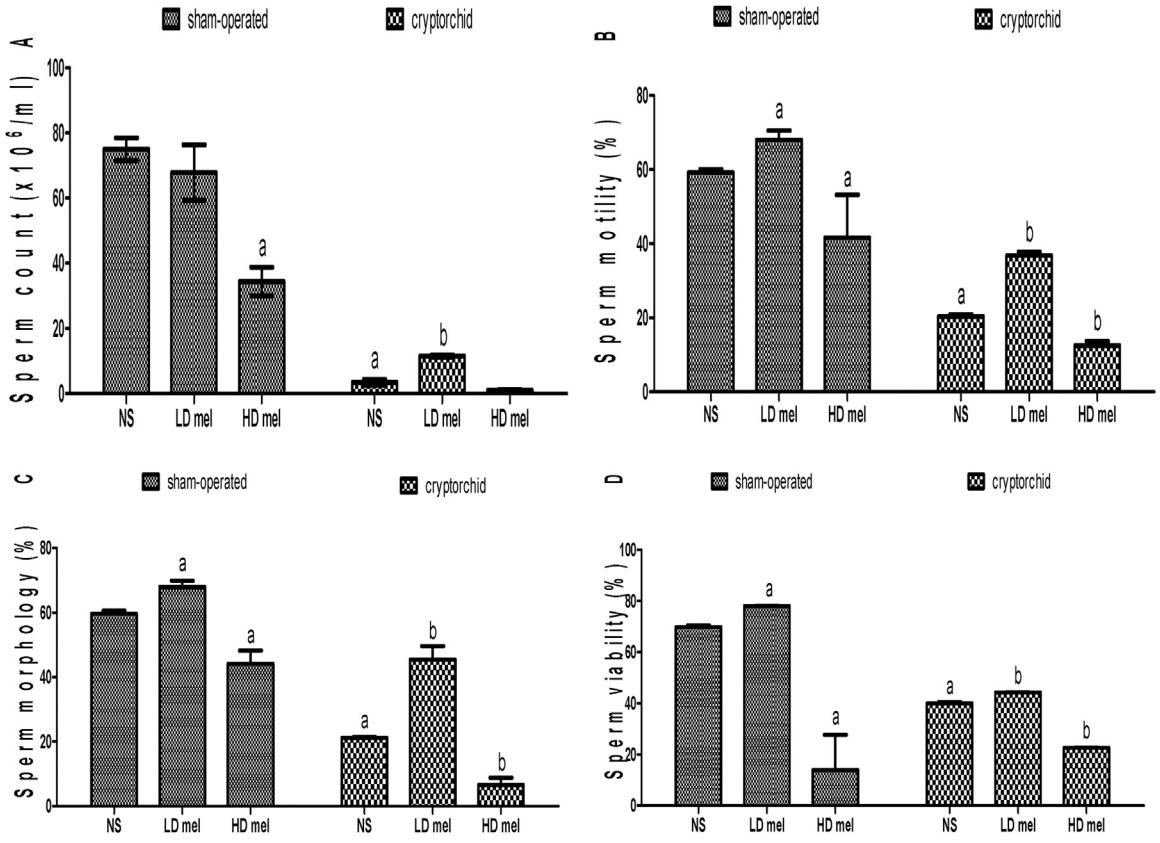

Fig. 1. Effects of melatonin on epididymal sperm parameters in cryptorchid rats.
Values are expressed as Mean ± S.E.M. (n = 6). [a]$p < 0.05$ vs. sham-operated + normal saline; [b]$p < 0.05$ vs. cryptorchid + normal saline. NS = normal saline, LD mel = low dose melatonin, HD mel = high dose melatonin.

3. Results

3.1. Melatonin had dose-dependent dual effects on cryptorchidism-induced spermatotoxicity

The sperm parameters were lower in cryptorchid than in sham-operated rats given normal saline. The sperm parameters of sham-operated and cryptorchid rats were increased by low dose (4 mg/kg) of melatonin but decreased by its high dose (10 mg/kg) when compared to normal saline (Fig. 1A–D).

3.2. Effects of melatonin on plasma reproductive hormones in cryptorchid rats

The plasma testosterone concentration (T) was higher in cryptorchid rats than in sham-operated rats given normal saline. The T of sham-operated rats was unchanged by low dose (4 mg/kg) of melatonin but reduced by its high dose (10 mg/kg) when compared to normal saline, while both doses caused reduction in T of cryptorchid rats (Fig. 2A).

The plasma oestradiol concentration (E_2) was lower in cryptorchid rats than in sham-operated rats given normal saline. The E_2 of sham-operated rats was unaffected by low dose (4 mg/kg) of melatonin but reduced by its high dose (10 mg/kg) when compared to normal saline, but the effects of both doses were vice-versa in the cryptorchid rats (Fig. 2B).

The plasma concentrations of LH and FSH were higher in cryptorchid rats than in sham-operated rats given normal saline. Low dose (4 mg/kg) and high dose (10 mg/kg) of melatonin did not affect the LH but increased the FSH of sham-operated rats, while decreasing both LH and FSH in cryptorchid rats (Figs. 2C and D).

3.3. Effects of melatonin on glutathione peroxidase and lactate dehydrogenase activities in cryptorchid rats

The glutathione peroxidase activity (GPx) was lower in cryptorchid rats than in sham-operated rats given normal saline. The GPx activity was increased in sham-operated rats and decreased in cryptorchid rats by low dose of melatonin (4 mg/kg), but was reduced by its high dose (10 mg/kg) in both sham-operated and cryptorchid rats when compared to normal saline (Fig. 3A).

The lactate dehydrogenase activity (LDH) was higher in cryptorchid rats than in sham-operated rats given normal saline. Low dose of melatonin (4 mg/kg) reduced the LDH of sham-operated and cryptorchid rats while high dose of melatonin (10 mg/kg) increased their LDH when compared to normal saline (Fig. 3B).

4. Discussion

In the present study, sperm parameters (count, motility, morphology and viability) were lower while plasma oxidative stress was higher in experimentally cryptorchid rats. This observation is consistent with previously reported oligoteratoasthenozoospermia and oxidative stress in cryptorchid rats[24,22] and

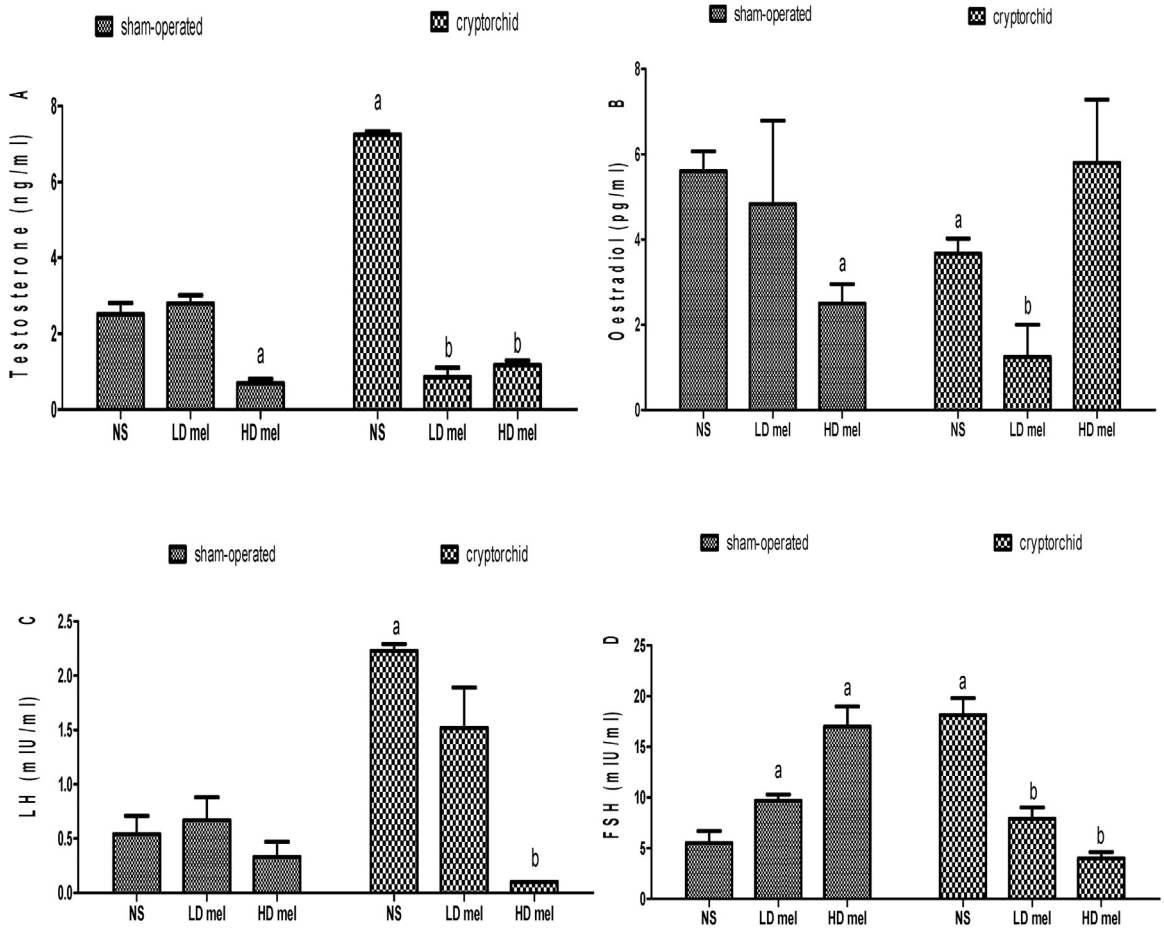

Fig. 2. Effects of melatonin on serum reproductive hormones in cryptorchid rats.
Values are expressed as Mean ± S.E.M. (n = 6). [a]$p < 0.05$ vs. sham + normal saline; [b]$p < 0.05$ vs. cryptorchid + normal saline. NS = normal saline, LD mel = low dose melatonin, HD mel = high dose melatonin; LH = luteinising hormone, FSH = follicle stimulating hormone.

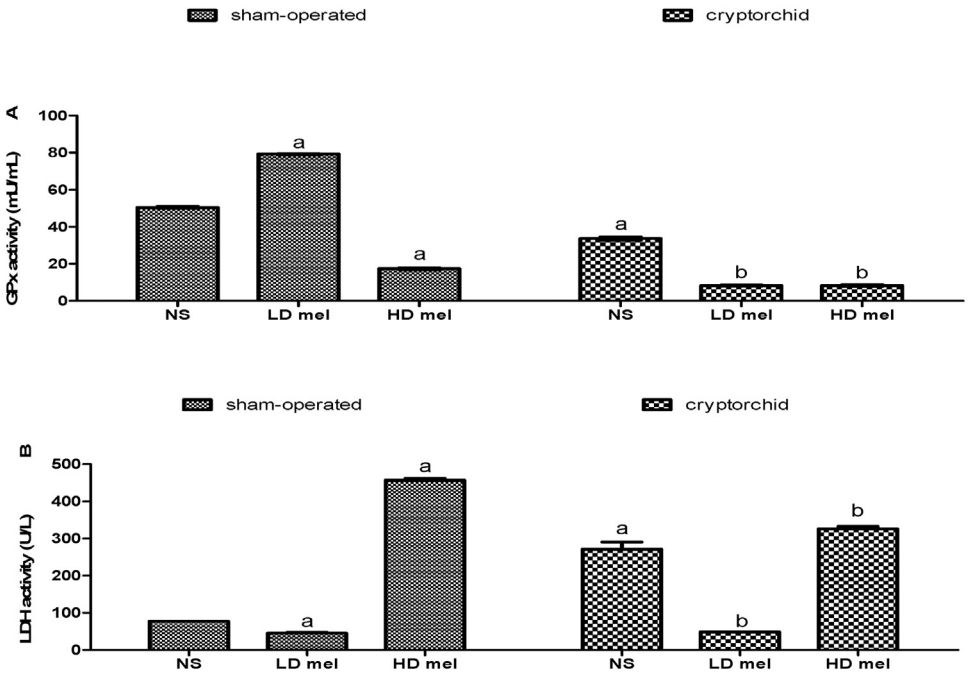

Fig. 3. Effects of melatonin on serum glutathione peroxidase (A) and lactate dehydrogenase (B) activities in cryptorchid rats.
Values are expressed as Mean ± S.E.M. (n = 6). [a]$p < 0.05$ vs. sham + normal saline; [b]$p < 0.05$ vs. cryptorchid + normal saline. NS = normal saline, LD mel = low dose melatonin, HD mel = high dose melatonin; GPx = glutathione peroxidase, LDH = lactate dehydrogenase.

humans.[25] The present study provides additional information that 4 mg/kg of melatonin ameliorated cryptorchidism-induced spermatotoxicity in rats.

Oestradiol, a potent oestrogen produced from the testes and peripheral conversion of androgens or their precursors, has been shown to potently inhibit LH much more than testosterone.[19] The reduction in oestradiol in cryptorchid rats might have suppressed its strong feedback inhibition on LH and FSH, leading to increased gonadotropins that will stimulate testosterone production to enhance spermatogenesis as a compensation for reduced sperm parameters associated with cryptorchidism. Thus, the increase in testosterone and gonadotropin levels in cryptorchid rats observed in this study is in agreement with previous reports[26–28] and could be mere physiological responses which could not normalise the sperm parameters due to high testicular temperature and oxidative stress associated with cryptorchidism.[22]

The generation of ROS in sperm comes from the plasmatic membrane (NADPH route) and mitochondrion (NADH region) and is elevated in immature sperm cells due to spermiogenesis defect.[29] The teratozoospermic (immature) sperm cells are characterised by the presence of cytoplasmic residues at the middle piece due to incomplete cytoplasmic reduction which is necessary for sperm cells to regain their elongated shape. The cytoplasmic residues have elevated levels of cytosolic enzymes (lactate dehydrogenase [LDH], creatinine phosphokinase, glucose-6-phosphate dehydrogenase), the activities of which are positively correlated with sperm dysfunctions.[30] The decrease in glutathione peroxidase (GPx) and increase in LDH of cryptorchid rats with abnormal sperm function in this study suggests that cryptorchidism-induced sperm abnormality might have led to an increase in the activity of LDH, which could have played a significant role in the generation of NADPH, a fuel for ROS generation.[20,23] This supports the contention that oxidative stress is one of the consequences of and a cause of spermatotoxicity in cryptorchidism[24,22] and that the oxidative stress could arise from the elevated cryptorchidism-induced tetatozoospermia.

Previous studies have shown a dual dose-dependent effect of melatonin on reproductive function and oxidative stress. Its pro-oxidant[31,20] and anti-oxidant[32,33] effects have been well-reported. It has been shown to protect testicular tissue against injury at low dose but adversely affect spermatogenesis at a dose of 9 mg/kg or more.[34,17] Similarly in this study, cryptorchidism-induced spermatotoxicity was ameliorated by low dose (4 mg/kg) of melatonin but exacerbated by its high dose (10 mg/kg). This contrasts the previous study of Fahimeh et al.[21] where 10 mg/kg of melatonin protected against busulfan-induced testicular damage but in agreement with those of others[34,17] where ≥ 9 mg/kg of melatonin negatively affected male reproductive indices. Fahimeh et al.[21] assessed Johnsen's score, Leydig cell volumes and germ cell counts while we assessed sperm parameters as reproductive indices. In addition, they induced reproductive toxicity chemically by busulfan (an antineoplastic chemotherapy) while we surgically induced it by bilateral cryptorchidism. Moreover, their study was performed in mice while ours was performed in rat. These differences in experimental designs might be responsible for the discrepancy observed with the effects of 10 mg/kg of melatonin in both studies.

Saalu et al.[18] suggested that melatonin reduced the detrimental effect of cryptorchidism on testes by alleviating the free radicals generated from the testes exposed to core body temperature, though they did not provide any data to support this claim. Since the testes of sham-operated rats are not exposed to core body temperature as in cryptorchid rats, the interesting observation of ameliorative effect of low dose of melatonin but detrimental effect of its high dose in sham-operated and cryptorchid rats in this study suggest that the hypothermic property of melatonin may not be involved in its ameliorative effect (at low dose) on sperm parameters in cryptorchidism. Rather, the status of sperm parameters is a reflection of the oxidative stress status of the organism. This is similar to the previous observation that infertile men have lower reserve of anti-oxidant than their fertile counterpart[35] in addition to the reported observation that reactive

oxygen species level can predict male factor infertility with an accuracy of ≥80%.[36]

Sperm production and functions including count, motility, morphology and viability are generally known to be favoured by high intratesticular testosterone level, the secretion of which is regulated by pituitary gonadotropins.[37] Likewise, oxidative stress has been extensively shown to play a pivotal role in male factor infertility.[38] However, it is noteworthy that the dual dose-dependent effect of melatonin on sperm and oxidative stress parameters are not manifested in the reproductive hormones. For instance, both doses of melatonin caused reductions in the plasma testosterone and follicle stimulating hormone despite varying effect on sperm oxidative stress parameters. This suggest that the dual dose-dependent effect of melatonin on sperm parameters is related more to oxidative stress and less to endocrine influence.

5. Conclusion

In conclusion, low and high dose of melatonin could ameliorate and exacerbate the cryptorchidism-induced spermatotoxicity respectively. Moreover, the pro- and anti-oxidant effects of melatonin in cryptorchidism are dose-dependent. In addition, melatonin's effect on sperm parameters in cryptorchidism is not determined by core body temperature that the testes is exposed to, but determined by the oxidative stress status of the organism.

Conflict of interests

None declared.

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Authors' contributions

L.A.O. and I.A.A. conceived and designed the study, and are joint first/lead authors. All authors participated in the conduct of the experiment, data analysis and interpretation. I.A.A. drafted the manuscript. L.A.O. revised the manuscript. All authors have approved the final article.

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