



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.

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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 gland⁶ and other extra-pineal sources like retina, gut, skin, bone marrow, lymphocytes, and ovaries.⁷ Its ability to scavenge free radicals like hydroxyl radical

(•OH), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), superoxide anion (O₂•⁻), hypochlorous acid (HOCl), peroxyxynitrite anion (ONOO⁻), nitric oxide (NO•), and others in many conditions⁸ 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-oxidants¹³ 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 beneficial¹⁶ and detrimental¹⁷ 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 \pm 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

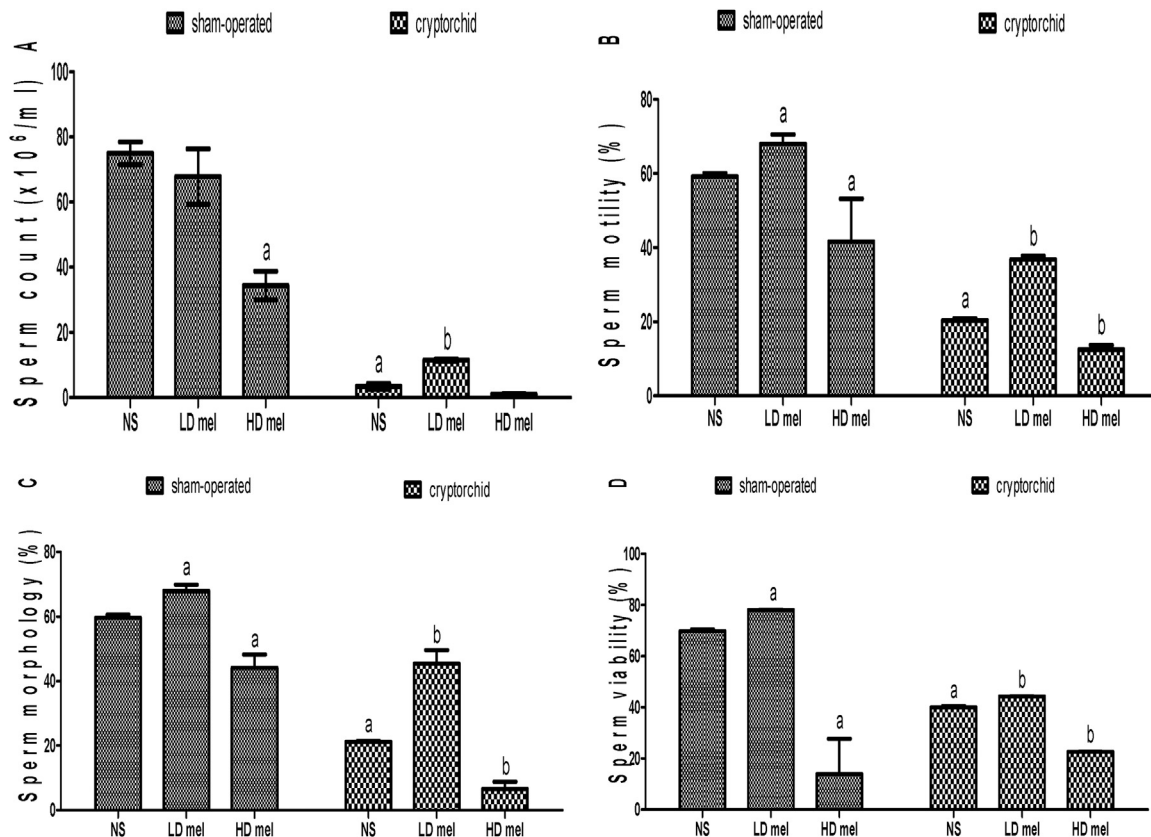


Fig. 1. Effects of melatonin on epididymal sperm parameters in cryptorchid rats. Values are expressed as Mean \pm S.E.M. ($n = 6$). ^a $p < 0.05$ vs. sham-operated + normal saline; ^b $p < 0.05$ vs. cryptorchid + normal saline. NS = normal saline, LD mel = low dose melatonin, HD mel = high dose melatonin.

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