

Melatonin prevents and ameliorates lead-induced gonadotoxicity through antioxidative and hormonal mechanisms

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Abstract

We investigated the effects of melatonin on sperm parameters and some biochemical markers in lead-exposed male Wistar rats. Lead (50 mg/kg bw/day) and/or melatonin (4 mg/kg or 10 mg/kg bw/day) was administered for 4 weeks, while 2-week lead exposure was preceded by or followed by 2-week treatment with both doses of melatonin in other groups. Lead reduced glutathione, catalase, adjusted testes weight, semen parameters but did not change malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase, and total antioxidant capacity. Though independent of prolactin, lead-induced gonadotoxicity was both centrally and peripherally mediated, as it reduced gonadotropin-releasing hormone and testosterone levels, while gonadotropin levels did not change significantly probably due to negative feedback by elevated estradiol. However, pre-, simultaneous, or posttreatment of lead-exposed rats with melatonin reduced MDA, SOD, and estradiol but dose-dependently increased other parameters. Conclusively, lead causes male gonadotoxicity through oxidative stress and endocrine mechanisms, and these could be dose-dependently prevented and ameliorated by melatonin.

Keywords

Gonadotoxicity, lead, melatonin, oxidative stress, reproductive hormone, sperm

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Introduction

The nonbiodegradable nature of lead presents it as a constant threat to our environment and a major public health concern. Human exposure to lead occurs through various sources, for example, leaded gasoline (Lovei, 1996), lead-based paints, lead smelting, and lead-containing water pipes (Epinfo, 1995), among others. However, the general populace is exposed to lead basically by contaminated drinking water or food (ATSDR, 1999). Its exposure is accompanied by deficits in the functioning of various body systems and tissues, such as nervous system (Baghurst et al., 1992; Baker et al., 1984), cardiovascular system (Vaziri, 2002), immune system (Dietert and Piepenbrink, 2006), gastrointestinal system (Steenland and Boffetta, 2000), kidneys (Gonick, 2002), bones and

teeth tissues (Hu et al., 1998), as well as reproductive system (Bellinger, 2005).

Lead inactivates endogenous antioxidants (Ahamed and Siddiqui, 2007) and could act as a substitute for the zinc ion, which serves as a cofactor in

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the catalytic site of different antioxidant enzymes. This results in the inhibition of antioxidant enzymes (Flora et al., 2007) and the enhancement of prooxidative events. In addition, lead can replace other monovalent and divalent cations affecting major biological functions of the body, for example, cell adhesion, cellular signaling, release and uptake of neurotransmitters, enzyme regulation (Garza et al., 2006), and as such, bringing about physiological imbalance. Although a desirable level of reactive oxygen species (ROS) is necessary for the regulation of normal sperm functions (Agarwal and Allamaneni, 2004), excess production of these free radicals in the male reproductive system as a result of imbalance in the antioxidant/prooxidant status would result in oxidative stress, which is the primary factor that precipitates male infertility (Agarwal et al., 2008). The latter has been shown to be accompanied by irregularities in the endogenous levels of reproductive hormones, for example, gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, testosterone, and prolactin (Anawalt, 2013). Therefore, it could be suggested that any pharmacological agent that may possibly intercept any of the pathological pathways instigated by lead could be used to ameliorate its toxicity.

Studies have shown that nutrients with antioxidant property can offer protection from lead-induced toxicity, and as such could restore the imbalance between prooxidant and antioxidant events in a biological system (Hsu and Guo, 2002). Melatonin has been reported to have antioxidant and anti-inflammatory effects (Alamili et al., 2014). It directly scavenges ROS, stimulates the synthesis and the activation of antioxidant enzymes, and enhances the efficacy of mitochondrial functions by increasing oxidative phosphorylation in the mitochondrial respiratory chain and improving the mitochondrial permeability transition pore (Chabra et al., 2014). These series of events result in decreased peroxidation of membrane lipids (Acuna-Castroviejo et al., 2007). Because of the antioxidant action of melatonin, it could reverse oxidative stress-induced sperm dysfunction, and hence improve sperm quality (Agarwal and Majzoub, 2016). Nevertheless, there are reports about the prooxidant effects of melatonin in certain conditions (Alagbonsi and Olayaki, 2017; Alagbonsi et al., 2016; Bejarano et al., 2009).

Therefore, the present study investigated the effects of melatonin on the antioxidant profile, some

gonadal parameters, and hormonal status in lead-exposed male Wistar rats.

Materials and methods

Drugs and chemicals

Lead chloride and melatonin were purchased from Sigma Chemical Company (St. Louis, MO), while sodium pentobarbital was obtained from Korea United Pharm. Inc. (Korea). All the other chemicals used were of analytical grade and procured from British Drug House (Poole, Dorset, UK).

Animal care

Fifty adult male Wistar rats (weight range: 160–180 g) were obtained from a trusted commercial breeder. They were housed in wooden cages, maintained under standardized conditions (12-h light/dark cycle, 27–30°C, 50–80% relative humidity), and were acclimatized in the laboratory for 2 weeks before the commencement of the study. The rats were fed with standard rodent pelletized diet (Ace Feeds, Ibadan, Nigeria) and water *ad libitum*. All the animals were well-cared for according to criteria outlined in the “Guide for the Care and Use of Laboratory Animals,” prepared by the National Academy of Science and approved by the Ethical Research Committee of the University of Ilorin, Nigeria.

Experimental design

The 50 rats were blindly randomized into four groups: normal saline (control; $n = 5$), lead (50 mg/kg bw; $n = 5$), melatonin ($n = 10$), and a combination of lead and melatonin ($n = 30$). In order to ascertain the possible dose-dependent effect of melatonin, the animals in the melatonin group were subdivided into two subgroups ($n = 5$ each) so that some received 4 mg/kg and others received 10 mg/kg of melatonin (Olayaki et al., 2017). In order to also determine if melatonin could be useful as a preventive and/or curative agent against lead-induced toxicity, the 30 animals in the combination group were subdivided into six treatment subgroups ($n = 5$ each) in such a way that melatonin was administered at a dose of 4 mg/kg or 10 mg/kg bw, respectively, 2 weeks before, simultaneously with, or 2 weeks after lead exposure. All treatments were given once daily between 8 am and 10 am via oral gavage.

Determination of biochemical parameters

Animals were anesthetized a day after the last treatment with sodium pentobarbital (40 mg/kg bw, *im*). Thereafter, they were dissected in order to collect blood by cardiac puncture. Whole blood for the determination of biochemical markers was collected in heparinized sample bottles that were centrifuged at 3500 r/min for about 10 min at -4°C using a cold centrifuge (Model 8881, Centurion Scientific Ltd, UK). The separated plasma samples were collected into separate plain bottles and were stored at -20°C prior to biochemical analyses.

Diagnostic kits for the determination of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), reduced glutathione (GSH), catalase, and total antioxidant capacity (TAC) were obtained from Fortress Diagnostics Limited, UK. In addition, analytic kits for the determination of GnRH, LH, FSH, prolactin, testosterone, and estradiol were procured from Elabscience Biotechnology Company Ltd, Wuhan, Hubei, China. The analyses were done with a spectrophotometer (Spectramax Plus; Molecular Devices, Sunnyvale, CA) according to the manufacturer's instruction.

Determination of gonadal parameters

The paired testes were carefully removed and weighed. The paired testicular weight was adjusted for body weight for testes–body weight ratios (TBWR) as previously described (Alagbonsi et al., 2016). The epididymal sperm parameters such as sperm count, sperm motility, sperm morphology, and sperm life to death ratio were estimated as previously described (Afolabi et al., 2014; Salman et al., 2016).

Data analyses

Statistical evaluations were done using statistical package for social sciences (SPSS) version 16.0 (IBM Corporation, Armonk, NY). Values were expressed as mean $\pm$ SEM of the variables measured. Comparisons among the groups were done by one-way analysis of variance, followed by *post hoc* Tukey's multiple comparison test. Lead-treated and melatonin-treated groups were compared to the control group, while the groups that received a combination of melatonin and lead were compared to the lead-treated group. Statistical significance was considered at $p < 0.05$.

Results

Effects of melatonin on oxidative stress status in lead-treated rats

The plasma MDA level, SOD, and GPx activities were not significantly affected by lead and both doses of melatonin when compared to control. Pretreatment with 4 mg/kg of melatonin reduced MDA but increased GPx in lead-exposed rats, while posttreatment with both doses of melatonin also reduced the MDA level in these rats when compared to rats that received lead only. Moreover, the SOD activity was lower in lead-exposed rats simultaneously treated with, pretreated with, or posttreated with both doses of melatonin when compared to rats that received lead only (Figure 1(a) to (c)).

The plasma activities of GSH and catalase were reduced by lead but unchanged by both doses of melatonin when compared to control. However, both of these parameters were higher in lead-exposed rats pretreated with both doses of melatonin, while simultaneous treatment or posttreatment with 4 mg/kg of melatonin additionally increased the GSH activity when compared to rats that received lead only (Figure 1(d) and (e)).

The plasma TAC was increased by both doses of melatonin but unchanged by lead when compared to control. Moreover, it was also higher in lead-exposed rats either simultaneously treated with, pretreated with, or posttreated with both doses of melatonin when compared to rats that received lead only (Figure 1(f)).

Effects of melatonin on gonadal parameters in lead-treated rats

The TBWR was reduced by lead and 10 mg/kg of melatonin but unchanged by 4 mg/kg of melatonin when compared to control. Lead-exposed rats that were simultaneously treated with 10 mg/kg of melatonin or posttreated with 4 mg/kg of melatonin had significantly higher TBWR than lead-exposed rats that received vehicle (Table 1).

Lead decreased while both doses of melatonin increased the sperm count when compared to control. The sperm motility, morphology, and viability were all reduced by lead but unchanged by both doses of melatonin (except 10 mg/kg of melatonin that decreased sperm viability) when compared to control. However, all these sperm parameters were higher in lead-exposed rats either simultaneously treated with,

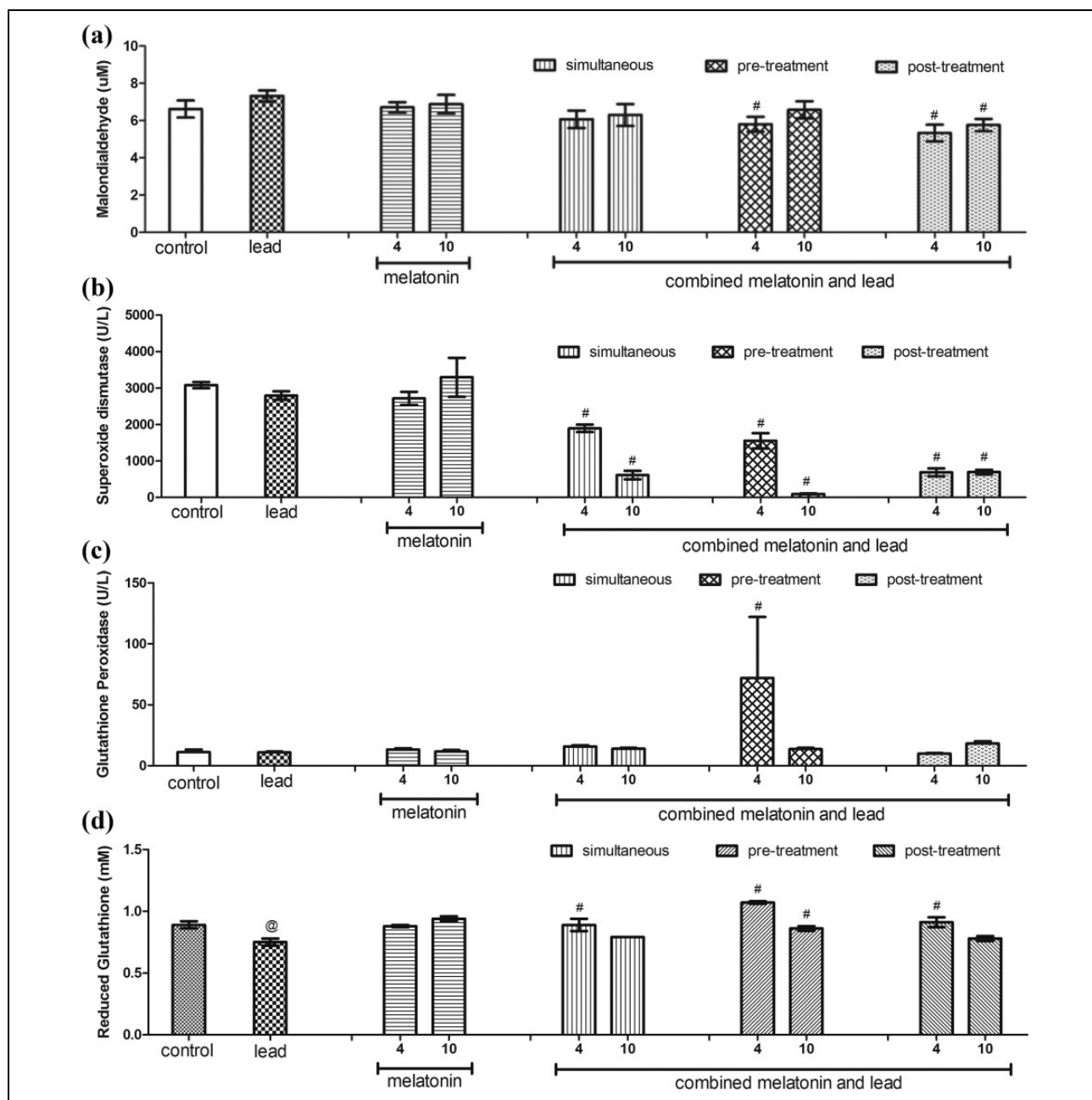


Figure 1. Effect of melatonin on MDA level in lead-treated rats. [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (b) Effect of melatonin on SOD activity in lead-treated rats. [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (c) Effect of melatonin on GPx activity in lead-treated rats. [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (d) Effect of melatonin on GSH activity in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (e) Effect of melatonin on catalase activity in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (f) Effect of melatonin on TAC in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. MDA: malondialdehyde; SOD: superoxide dismutase; GPx: glutathione peroxidase; GSH: reduced glutathione; TAC: total antioxidant capacity.

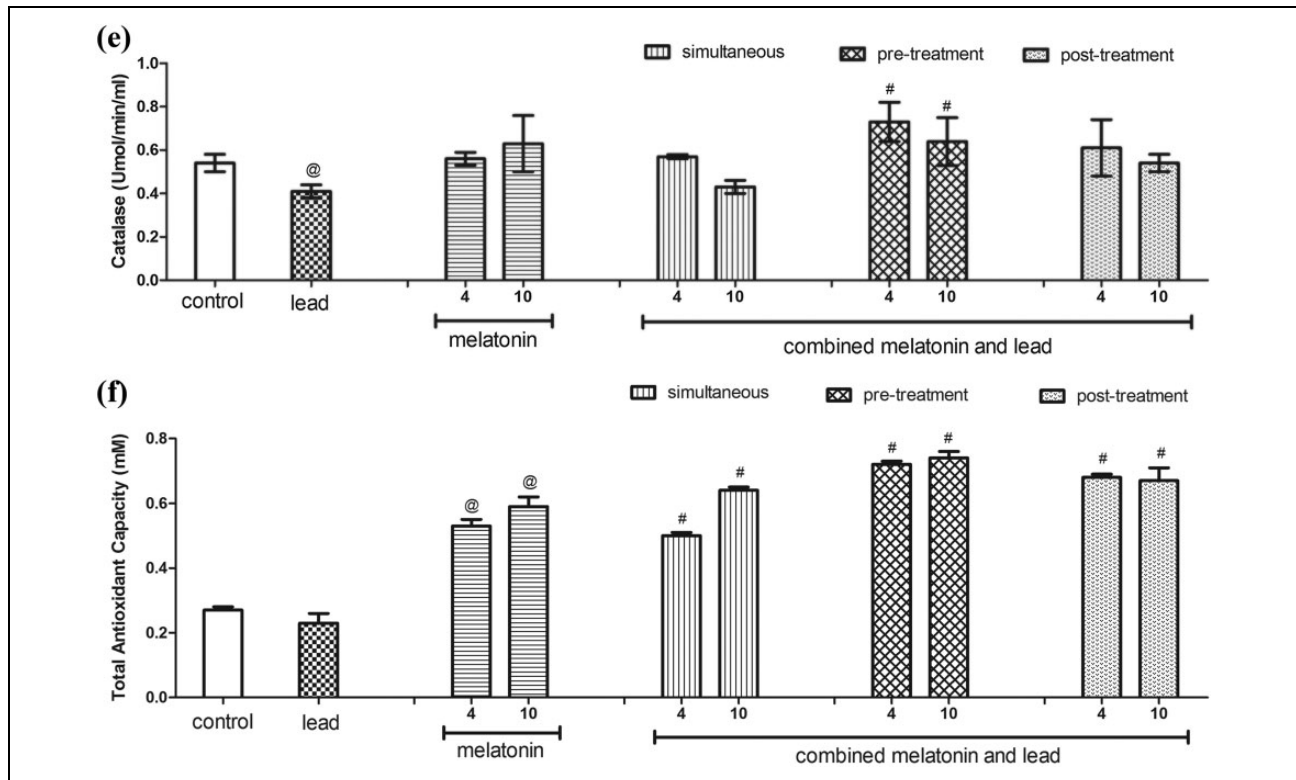


Figure 1. (continued).

pretreated with, or posttreated with both doses of melatonin when compared to rats that received lead only (Table 1).

Effects of melatonin on reproductive hormones in lead-treated rats

The GnRH was reduced by lead but unchanged by both doses of melatonin when compared to control. It was higher in lead-exposed rats either simultaneously treated with or posttreated with 10 mg/kg of melatonin, and in those pretreated with both doses of melatonin when compared to rats that received lead only (Figure 2(a)).

The LH and FSH were unchanged by lead and both doses of melatonin when compared to control (Figure 2(b) and (c)). The LH was higher in lead-exposed rats either pretreated with or posttreated with 10 mg/kg of melatonin, and in those simultaneously treated with both doses of melatonin when compared to rats that received lead only (Figure 2(b)). The FSH was higher in lead-exposed rats either simultaneously treated with or pretreated with 4 mg/kg of melatonin, and in those posttreated with both doses of melatonin when compared to rats that received lead only (Figure 2(c)).

Neither lead nor melatonin affected the plasma prolactin concentration. None of the modes of supplementation of lead-exposed rats with melatonin had significantly different prolactin concentration when compared to rats that received lead only (Figure 2(d)).

The testosterone was reduced by lead and 4 mg/kg of melatonin but unchanged by 10 mg/kg of melatonin when compared to control. The testosterone in lead-exposed rats was not affected by either simultaneous treatment, pretreatment (except with 10 mg/kg of melatonin that increased it), or posttreatment with both doses of melatonin (Figure 2(e)).

The estradiol was increased by lead and both doses of melatonin when compared to control. It was, however, reduced in lead-exposed rats simultaneously treated with or posttreated with both doses of melatonin, but unchanged in those pretreated with both doses of melatonin when compared to rats that received lead only (Figure 2(f)).

Discussion

Lead inactivates endogenous antioxidants (Ahamed and Siddiqui, 2007; Kasprczyk et al., 2004) and, as such, causes an imbalance in the antioxidant/prooxidant status and hence oxidative stress. It has been

Table 1. Effects of melatonin on sperm parameters in lead-treated rats.

		Testes–body weight ratio	Sperm count ($\times 10^6/\text{mL}$)	Sperm motility (%)	Sperm morphology (%)	Sperm viability (%)
Control		0.02 ± 0.0005	74.85 ± 2.67	82.74 ± 1.91	85.47 ± 1.52	89.95 ± 1.04
Lead		0.01 ± 0.0009^a	50.33 ± 4.72^a	72.03 ± 1.79^a	64.54 ± 1.52^a	69.99 ± 1.49^a
Melatonin	4 mg/kg	0.02 ± 0.0017	88.40 ± 1.14^a	82.45 ± 0.40	84.73 ± 0.49	88.74 ± 0.64
	10 mg/kg	0.01 ± 0.0007^a	89.80 ± 2.61^a	81.22 ± 0.31	84.12 ± 0.31	86.60 ± 0.16^a
Combination of melatonin and lead	Simultaneous administration of lead and 4 mg/kg of melatonin	0.01 ± 0.0000	82.70 ± 2.52^b	81.40 ± 0.49^b	84.33 ± 0.70^b	87.62 ± 0.56^b
	Simultaneous administration of lead and 10 mg/kg of melatonin	0.02 ± 0.0012^b	96.10 ± 2.20^b	81.43 ± 0.61^b	85.15 ± 0.24^b	86.39 ± 0.17^b
	Pretreatment with 4 mg/kg of melatonin for 2 weeks prior to lead exposure	0.01 ± 0.0015	86.73 ± 1.62^b	78.41 ± 0.18^b	83.29 ± 0.19^b	87.33 ± 0.51^b
	Pretreatment with 10 mg/kg of melatonin for 2 weeks prior to lead exposure	0.01 ± 0.0017	104.20 ± 0.96^b	77.79 ± 0.72^b	80.23 ± 0.60^b	81.48 ± 0.17^b
	Posttreatment with 4 mg/kg of melatonin after 2 weeks of lead exposure	0.02 ± 0.0015^b	92.40 ± 1.28^b	78.74 ± 0.60^b	81.74 ± 0.83^b	82.52 ± 0.47^b
	Posttreatment with 10 mg/kg of melatonin after 2 weeks of lead exposure	0.01 ± 0.0015	90.90 ± 4.13^b	79.28 ± 0.62^b	84.12 ± 1.02^b	84.86 ± 0.59^b

^a $p < 0.05$ compared to control.^b $p < 0.05$ compared to lead.

reported that oxidative stress coupled with the abnormal profile in reproductive hormones induces sperm dysfunction and impairs sperm quality (Agarwal and Majzoub, 2016). Therefore, any pharmacological agent with antioxidant property and central and peripheral effect on the hypothalamic–pituitary–gonadal axis could improve fertility in males exposed to lead.

In the present study, there was a slight but insignificant elevation in the lipid peroxidative marker—MDA in the rats that received lead only relative to those that received normal saline. This could be related to the administered dose or the duration of the experiment. Melatonin is produced in numerous body cells and its production may be enhanced when the physiological system is exposed to oxidative stress (Arnao and Hernandez-Ruiz, 2013). Therefore, it

could be that the expression of pro-lipid peroxidative action of lead was ameliorated by the intervention of endogenous adaptive mechanisms. However, the in-built homeostatic system could be annulled by persistent exposure and increased synthesis of prooxidant species. Although melatonin has been said to prevent the oxidative deterioration of polyunsaturated fatty acids of the cell membrane (Tan et al., 2007), it was found in this study to have no effect on the endogenous status of MDA either when it was administered alone or simultaneously with lead. However, the lipid peroxidative effect of lead was negated by pretreatment with 4 mg/kg of melatonin and posttreatment with graded doses of melatonin. A molecule of melatonin has been predicted to scavenge about 10 ROS or reactive nitrogen species. This effect of melatonin

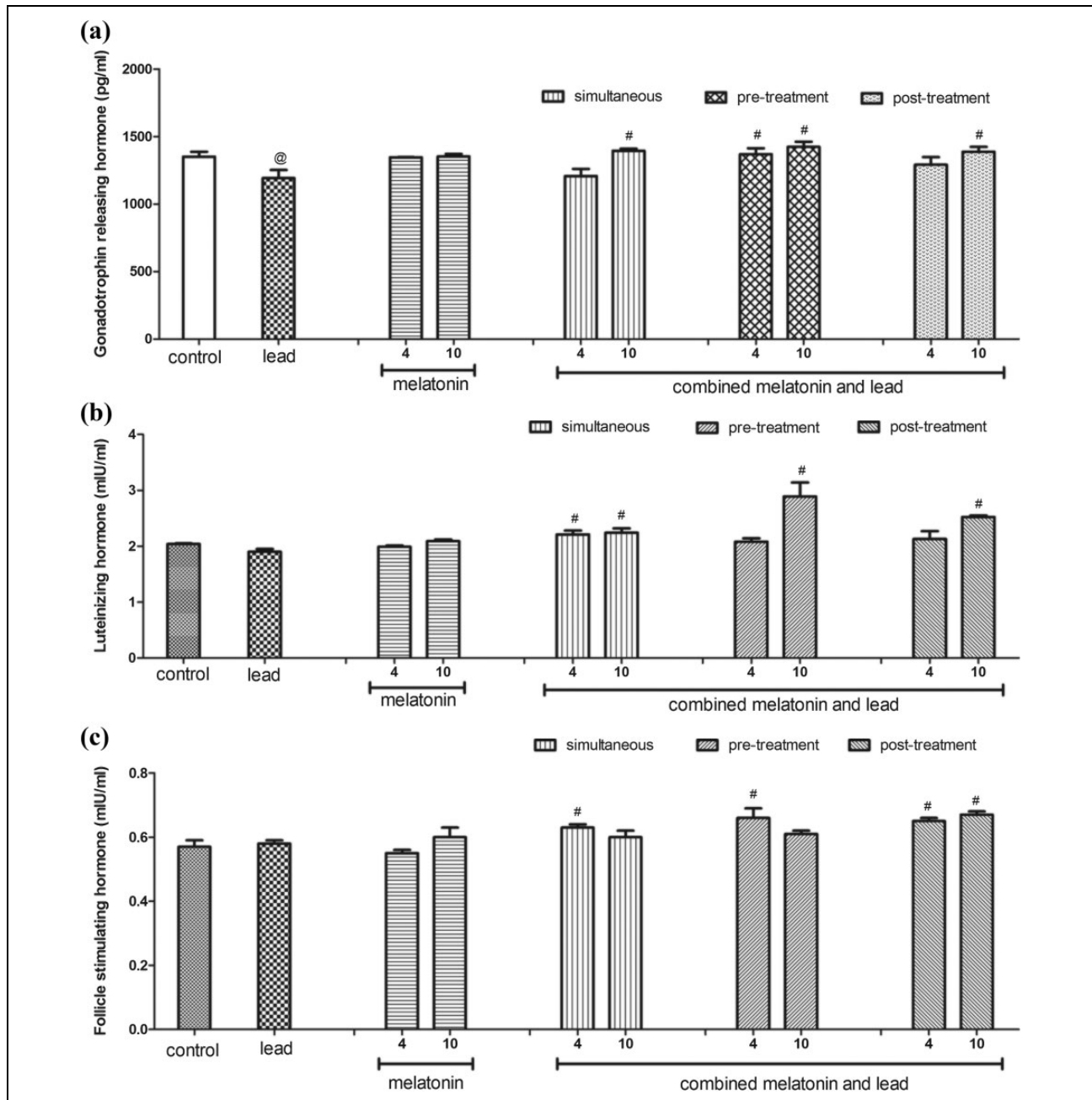


Figure 2. Effect of melatonin on GnRH in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (b) Effect of melatonin on LH in lead-treated rats. [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (c) Effect of melatonin on FSH in lead-treated rats. [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (d) Effect of melatonin on prolactin in lead-treated rats. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (e) Effect of melatonin on testosterone in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. (f) Effect of melatonin on estradiol in lead-treated rats. [@] $p < 0.05$ compared to control; [#] $p < 0.05$ compared to lead. The numbers 4 and 10 on the x-axis denote 4 mg/kg and 10 mg/kg of melatonin, respectively. GnRH: Gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

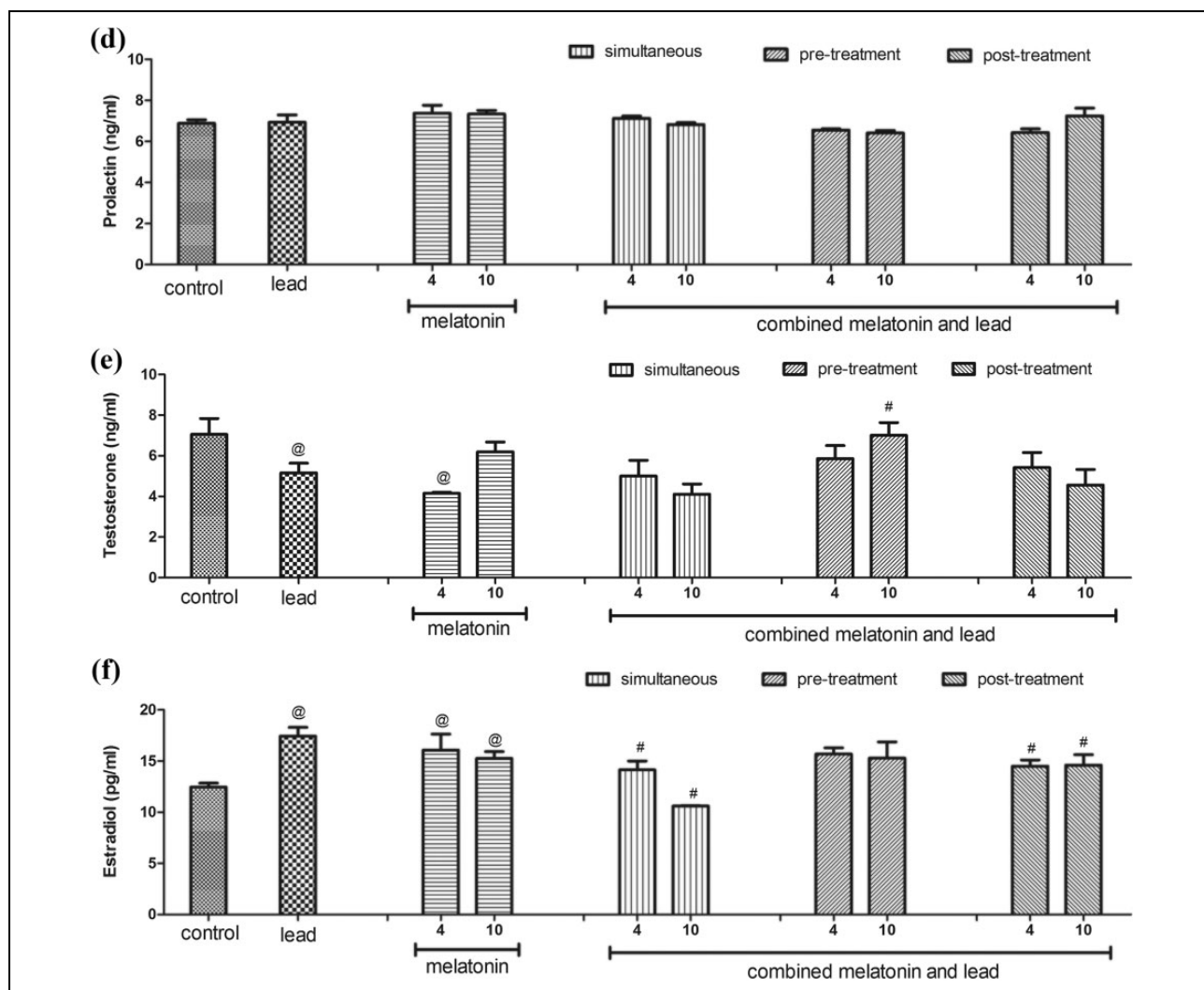


Figure 2. (continued).

is no doubt contributed by its metabolites, for example, cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, and N1-acetyl-5-methoxykynuramine, which are well-recognized for their free radical scavenging actions (Reiter et al., 2014). The failure of the present study to determine endogenous melatonin and ROS levels is considered as part of its limitations.

An inverse association exists between prooxidative events and antioxidant activities in a physiological system. SOD scavenges superoxide anion radical into hydrogen peroxide, which, in turn, is transformed to water and oxygen by catalase and GPx. These enzymes are altogether referred to as the first line of antioxidant defense enzymes against free radical-induced toxicity (Maksimenko et al., 2010; Salman et al., 2017). The slight reduction, albeit insignificant,

in the plasma SOD activity of lead-exposed rats suggests that superoxide generation has little contribution to lead-induced generation of free radicals. However, the lead-induced reduction of the glutathione level and insignificant change in the GPx activity in this study might be because lead deactivates glutathione reductase, GPx, delta-aminolevulinic acid dehydratase, and glutathione S-transferase enzymes (Ahamed and Siddiqui, 2007), or because lead also inactivates GSH by binding with its sulfhydryl group and results in synthesis of GSH from cysteine through the γ -glutamyl cycle, an event that is usually not effective to make up for the needed GSH (Hultberg et al., 2001). Probably, lead might have elicited oxidative stress by elevation of hydrogen peroxide formation, which could have overused endogenous catalase but not GPx, and consequently caused the reduction of the

catalase. These support the contention that lead causes toxicity in many body systems by eliciting oxidative stress.

Melatonin, a natural hormone secreted by pineal gland and other extra pineal tissues including bone marrow, gut, astrocytes, retina, kidney, platelets, and glia cells (Andrabi et al., 2015), is a therapy for various diseases due to its antioxidative and antiapoptotic properties (Galano et al., 2011; García et al., 2014; Zhang and Zhang, 2014). It stimulates the activities of various antioxidant enzymes, for example, copper-zinc and manganese SOD, glutamylcysteine ligase, GPx, and glutathione reductase (Rodriguez et al., 2004). We, however, observed that it enhances TAC but not antioxidant enzymes when administered alone in rats. This might be because TAC is a better predictor of the antioxidant status of a system than individual enzyme's activity (Alagbonsi and Olayaki, 2017; Kolettis et al., 1999; Sharma et al., 1999). Interestingly, simultaneous treatment, pretreatment, or posttreatment of lead-exposed rats with melatonin ameliorated the lead-induced oxidative stress by increasing antioxidant enzymes (except SOD) and TAC, and consequently reducing the membrane lipid peroxidation assessed by MDA. This reduction in SOD and the corresponding increase in other antioxidants indicate that the system switched to other antioxidants (GSH, GPx, and catalase) when SOD was exhausted. However, the antioxidative effect of melatonin in lead-exposed rats was more evident at the dose of 4 mg/kg than 10 mg/kg. This observation is similar to our previous report that 4 mg/kg but not 10 mg/kg of melatonin ameliorates cryptorchidism-induced spermatotoxicity in rats (Olayaki et al., 2017).

The reduced efficiency of the antioxidant system and hence the imbalance in the prooxidant/antioxidant status have not only been linked to female reproductive diseases, for example, polycystic ovary syndrome, endometriosis, and unexplained infertility (Agarwal et al., 2012), but also male infertility. A minute level of ROS is required for the maintenance of normal sperm functions such as sperm capacitation, sperm-oocyte fusion, and acrosome reaction (Agarwal and Allamaneni, 2004). However, increased production of ROS and hence oxidative stress has been reported to decrease sperm count, sperm motility, sperm-oocyte fusion (Ruder et al., 2008), and ultimately cause male infertility (Agarwal et al., 2008; Agarwal et al., 2014; Ayaz et al., 2015; Du Plessis et al., 2015; Ko et al., 2014). In the present

study, there were significant reductions in TBWR, sperm count, sperm motility, sperm morphology, and sperm viability in lead-exposed rats. These show that lead induces hypogonadism and it agrees with previous reports that higher blood and semen lead levels are associated with a significant decline in semen parameters (Wu et al., 2012). This study additionally shows that lead-induced gonadotoxicity could be prevented and ameliorated by melatonin, irrespective of the dose. It thus supports the recent trends in andrology research that oxidative stress largely contributes to male factor infertility and that antioxidants are of great therapeutic importance in improving male fertility.

Apart from the imbalance in the antioxidant enzyme system, an abnormal level of reproductive hormones is well-known to cause male infertility. The administration of lead caused significant reductions in GnRH and testosterone levels, but insignificant difference in FSH and LH levels. This is in agreement with a previous report that lead initially causes some subclinical testicular damage, followed by hypothalamic or pituitary disturbance when longer periods of exposure take place (Doumouchtsis et al., 2009). Previous reports have also shown that lead inhibits Leydig cell steroidogenesis and ultimately intratesticular testosterone synthesis by lowering the expression of the biosynthetic enzymes involved in the pathway (Liu et al., 2001, 2009; Thoreux-Manlay et al., 1995). The present study also suggests that lead directly inhibits testosterone synthesis in the testes independent of gonadotrophic influence.

It is worthy of note that the plasma concentrations of GnRH and gonadotropins of lead-exposed rats simultaneously treated with, pretreated with, or posttreated with melatonin were significantly higher than in the rats that received lead only. This shows that melatonin prevents and ameliorates lead-induced downregulation of hormones in the hypothalamic-pituitary-gonadal axis. However, the lack of corresponding increase in the testosterone level (except in lead-exposed rats pretreated with 10 mg/kg of melatonin) in response to elevated GnRH and gonadotropin levels further supports the submission that lead has cytotoxic effect on Leydig cells and directly inhibits testosterone synthesis through the mechanisms that are independent of pituitary gonadotropins.

Endocrine disruptors are chemicals in our environment that have potentially hazardous effects on male reproductive functions due to their estrogen-like and/or antiandrogenic attributes, and ultimately

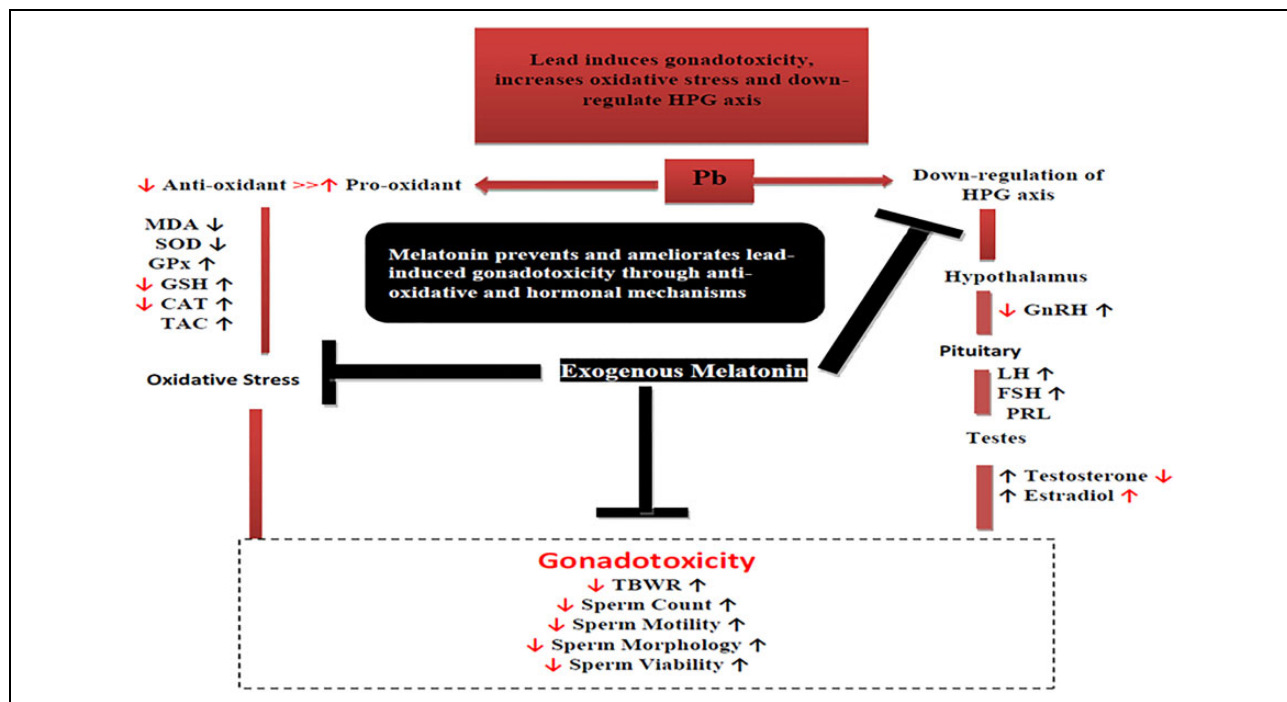


Figure 3. Graphical abstract illustrating the preventive and ameliorative effects of melatonin on lead-induced gonadotoxicity through antioxidative and hormonal mechanisms. ↓ and ↑ indicate decrease and increase, respectively, by lead, while ↓ and ↑ indicate decrease and increase, respectively, by melatonin in lead-treated rats. → indicates the events promoted by lead, while T indicates prevention/inhibition of lead-induced events by melatonin.

resulting in infertility (Sikka and Wang, 2008). We observed that the plasma estradiol concentration was increased by lead or melatonin when each of these was administered alone, but reduced when lead-exposed rats were either simultaneously treated with or posttreated with both doses of melatonin. The observed estrogenic and antiandrogenic activities of lead further support the claim that it is an endocrine disruptor. However, it is interesting to also note that lead-induced endocrine disruption could be prevented or ameliorated by simultaneous treatment or posttreatment with both doses of melatonin.

In contrast to the implication of reduced serum levels of GnRH, FSH, LH, and testosterone on the reproductive status, increased prolactin level is considered as one of the reversible causes of infertility (Buvat, 2003; Masud et al., 2007). It has been reported that azoospermic and oligospermic subjects with normal levels of gonadotropins showed a significantly increased level of prolactin. This report established the gonadotropin-independent role of prolactin in gametogenesis (Masud et al., 2007). Also, the documented normal level of testosterone in almost half of hyperprolactinemic patients with erectile dysfunction confirmed the testosterone-independent

effect of prolactin on male fertility (Bancroft et al., 1984). Sexual improvement upon treatment with a prolactin-lowering drug has been noted to correlate better with reduced serum prolactin than with increased testosterone (Buvat et al., 1985). In this study, lead and/or melatonin caused no significant change in prolactin concentration. This is contrary to the previously reported increase in prolactin level during lead intoxication. This disparity might be as a result of the dose of lead and duration used in this study.

Conclusion

In conclusion, this study supports the claim that lead causes male gonadotoxicity through oxidative stress and endocrine mechanisms. It also suggests that lead-induced gonadotoxicity and its associated mechanisms could be prevented and ameliorated by melatonin in a dose-dependent manner (see graphical abstract in Figure 3).

Authors' note

Luqman Aribidesi Olayaki and Isiaka Abdullateef Alagbonsi contributed to this article equally.

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Declaration of Conflicting Interests

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