

Elevated, sustained, and yet reversible biotoxicity effects of lead on cessation of exposure: Melatonin is a potent therapeutic option

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Abstract

Melatonin (Mel) is known to prevent and mitigate lead (Pb)-induced gonadotoxicity. However, there is no report in literature on the endogenous levels of different biomarkers after the cessation of Pb exposure, with or without treatment with Mel. Fifty adult male Wistar rats were divided into five groups ($N = 10$), which included control ((vehicle (normal saline) - treated) – 0.1 ml/day); lead chloride (PbCl_2) untreated (3 weeks vehicle + 3 weeks Pb); Pb recovery (3 weeks Pb + 3 weeks vehicle); Pb + Mel (3 weeks Pb + 3 weeks Mel); and Mel (3 weeks vehicle + 3 weeks Mel) groups. Pb and Mel were administered at 50 and 10 mg/kg B.W. (*p.o.*), respectively. The results showed that Pb caused significant decreases in total bilirubin (TB), phospholipids (PLP), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC), but significant elevations in alkaline phosphatase (ALP), aspartate aminotransferase (AST), triglyceride (TG), and malondialdehyde (MDA). Although the adverse effects of Pb on TB, ALP, AST, SOD, MDA, and TAC were sustained after the cessation of exposure, a reversal was observed in total cholesterol (TC), TG, PLP, CAT, and c-reactive protein (CRP) results. Nevertheless, the detrimental effects of Pb on alanine aminotransferase (ALT), albumin, and globulin were only expressed post-exposure. Treatment with Mel caused no significant effect on TB and albumin levels. However, unlike TAC and CRP, the hormone significantly reduced ALP, AST, ALT, TC, low-density lipoprotein cholesterol, PLP, SOD, CAT, MDA, and globulin to levels comparable to the control group. In conclusion, following the cessation of Pb exposure, alterations in physiological balance could be elevated, sustained, or reversible. However, Mel enhanced the reestablishment homeostatic status after Pb administration.

Keywords

Lead, melatonin, hepatic, antioxidant, inflammation

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Introduction

Lead (Pb), a nonbiodegradable element, remains a major public health concern and has become a constant threat to the ecosystem. The soft, malleable, inelastic heavy metal is considered as the second most hazardous environmental poison, as indicated in the Agency for Toxic Substances and Disease Registry's Priority Substance List (ATSDR, 2017). Humans are exposed to this heavy metal through various sources, among which include Pb-based paints, leaded

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gasoline (Lovei, 1996), Pb-containing water pipes, Pb smelting, to mention a few. Nevertheless, the general populace is exposed to this ubiquitous metal primarily through contaminated food or water (ATSDR, 1999). Efforts to eradicate various sources of human exposure to Pb seemed to be frustrated because there is no lowest safe concentration for the heavy metal (World Health Organization (WHO), 2009). Reports in the literature showed that Pb adversely affects many systems in the body. Exposure to the metal is accompanied by impaired functioning of cardiovascular system (Vaziri, 2002), nervous system (Baghurst et al., 1992), immune system (Dietert and Piepenbrink, 2006), reproductive system (Bellinger, 2005), bones and teeth tissues (Hu et al., 1998), hepatic tissue (Khaki and Khaki, 2010), among others.

Studies have revealed that Pb causes oxidative stress by increasing the generation of reactive oxygen species and as a result depletes the antioxidant defense system (Flora, 2002; Olayaki et al., 2019). Pb binds to amide and sulfhydryl groups in enzymes structures, changing their conformation and attenuating their activities (Flora et al., 2007). Aside from oxidative stress, it is known that the heavy metal could augment inflammatory events by influencing some endogenous enzymes involved in the inflammatory pathways (Chang et al., 2011). For example, Pb increases the production of inflammatory mediators by activating the transcription of genes encoding cytosolic phospholipase A2 and cyclo-oxygenase-2 (Chang et al., 2011). Moreover, Pb exposure has been reported to be associated with significant dose-dependent elevations in alanine aminotransferase (ALT) (Cave et al., 2010), alkaline phosphatase (ALP), and α -glycerophosphate-dehydrogenase (Jarrar and Taib, 2012), which are indications of compromised hepatic function. Kristal-Boneh et al. (1999) reported on the positive association between blood levels of Pb and levels of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C).

Clinically, chelation therapy is used in the management of Pb poisoning. Chelating agents such as calcium disodium versenate, dimercaprol, unithiol, succimer, for example, have been used for decades in the treatment of heavy metal poisoning. However, even at therapeutic dose levels, their administrations have been associated with side effects such as renal damage, allergic reactions, transaminase elevation, and increased excretion of copper and zinc (Bradberry and Vale, 2009; Dimaval, 2008; Takeda and Tamano, 2009). Therefore, there is the need to

investigate alternative therapies that could be used to mitigate the toxic effects of exposure to heavy metals.

There are several reports on the pharmacological benefits of melatonin (Mel) in various disease conditions including drug toxicity (Zhang et al., 2017). The main pathway for the metabolism of the hormone in the hepatic tissue is through hydroxylation at the C-6 position by 6-hydroxylase and P450 cytochromes (Facciola et al., 2001), resulting in the synthesis of 6-hydroxymelatonin, which is conjugated with glucuronide and sulfate to 6-hydroxymelatonin glucuronide or sulfate. During this process, Mel and its metabolites exert antioxidant activity. Alternatively, Mel could be oxidized to *N*-acetyl-5-methoxykynuramine and *N*-acetyl-formyl-5-methoxykynuramine. The kynurenine metabolic pathway of Mel results in the formation of free radical scavengers (Harthé et al., 2003). Apart from the antioxidant properties of the hormone, it also decreases the production of pro-inflammatory cytokines (Laliena et al., 2012) and has anti-dyslipidemic (Esquifino et al., 1997) and chelating (Romero et al., 2014) properties. Therefore, the aim of the present study was to investigate the effects of Mel on selected biomarkers in Pb-exposed adult male Wistar rats.

Materials and methods

Drugs and chemicals

Lead chloride (PbCl₂) and Mel were purchased from Sigma Chemical Company (St. Louis, Missouri, USA) and Actiza Pharmaceutical Private Ltd (Surat, Gujarat, India), respectively, while sodium pentobarbital was purchased from Nicholas Piramal Ltd (Thane, Maharashtra, India).

Diagnostic kits for the determination of total bilirubin (TB), ALP, aspartate aminotransferase (AST), ALT, albumin, globulin, total protein, blood urea nitrogen (BUN), uric acid (UA), and c-reactive protein (CRP) were purchased from Fortress Diagnostics Limited, UK. Assay kits for the determination of TC, triglyceride (TG), HDL-C and low-density lipoprotein cholesterol (LDL-C), free fatty acids (FFA), phospholipids (PPL), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), lactate dehydrogenase (LDH), and total antioxidant capacity (TAC) were purchased from Elabscience Biotechnology Company Ltd (Wuhan, Hubei, China). The biochemical assays were performed according to the manufacturers' instruction.

Experimental animals and care

Forty adult male Wistar rats weighing between 180 and 200 g were used for this experiment. They were purchased from reputable commercial breeders and were housed in plastic cages at a room temperature of 27–30°C and photo-periodicity of 12 h light/12 h dark. After 2 weeks of acclimatization, the rats were randomly allotted to separate groups. They were given standard rat chow (manufacturer: Ace Feed PLC, Ibadan, Nigeria) and water *ad libitum* daily, and were weighed weekly. The animals that were used in this study were cared for in accordance with the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” reported by the National Academy of Sciences (2011).

Experimental design

Forty adult male Wistar rats were used in this study. They were divided into four groups of 10 rats each, which included group 1—control; group 2—Pb untreated; groups 3—Pb recovery (Pb rec); and group 4—Pb + Mel. PbCl₂ salt, which was dissolved in warm normal saline was administered at 50 mg/kg B.W./day (*p.o.*) (Olayaki et al., 2018a), while Mel was administered at 10 mg/kg B.W./day (*p.o.*) (Olayaki et al., 2018a). The control group was administered normal saline (0.1 ml/day) throughout the 6 weeks duration of the experiment, while the Pb untreated group was administered normal saline during the first 3 weeks and then PbCl₂ during the last 3 weeks of the experiment; however, in groups 3 and 4, Pb was administered during the first 3 weeks, afterward, the rats were administered normal saline (0.1 ml/day, *p.o.*) and Mel (10 mg/kg B.W./day, *p.o.*), respectively, during the subsequent 3 weeks.

Biochemical analyses

Twenty-four hours after administration at the end of the 6 weeks of the experiment, the rats were anesthetized with sodium pentobarbital (40 mg/kg, *i.m.*) (Adeyemi and Olayaki 2017a, 2017b, 2019a). Afterward, they were necropsied, and blood was collected by cardiac puncture into heparinized sample bottles, which were centrifuged at $1372 \times g$ for 15 min, at –4°C using a cold centrifuge (Benchmark Scientific, model: C3100, Sayreville, New Jersey, USA) (Olayaki et al., 2020). The supernatant plasma samples were collected into separate plain bottles prior to the analyses.

Data analyses

Statistical package for social sciences (SPSS) version 18.0 (SPSS Inc., Chicago, Illinois, USA) was used to analyze the data that were obtained from this study. Statistical evaluations of the group mean differences were tested by one-way analysis of variance and then Tukey *post hoc* test. The results were expressed as mean $\pm$ standard error of mean (SEM), and statistical significance was considered at $p < 0.05$.

Results

Effects of Mel on antioxidant and inflammatory markers in Pb-exposed Wistar rats

Compared to the control group, there was a significant elevation in MDA, but a significant decrease in TAC in Pb untreated, Pb rec, and Pb + Mel groups ($p = 0.00, 0.00, 0.00$; $p = 0.00, 0.00, 0.00$, respectively) (Table 1). In addition, relative to the control group, we recorded a significant decrease in SOD activity in Pb untreated and Pb rec groups ($p = 0.00$ and 0.00 , respectively), and in catalase (CAT) activity in Pb untreated group ($p = 0.05$); but a significant increase in CRP level in Pb untreated group ($p = 0.00$) (Table 1). In comparison to the latter, there were significant increases in CAT, SOD, and TAC, but a significant decrease in MDA ($p = 0.01, 0.00, 0.04$, and 0.02 , respectively) in Pb + Mel group. Moreover, relative to the former, we observed a significant increase in CRP level in Pb rec and Pb + Mel groups ($p = 0.05$ and 0.00 , respectively). Relative to Pb rec group, there was a significant increase in SOD activity, but significant decreases in MDA and uric acid ($p = 0.00, 0.01$, and 0.05 , respectively) in Pb + Mel group (Table 1).

Effects of Mel on lipid indices in Pb-exposed Wistar rats

In comparison to the control group, there were significant elevations in TG and PLP in Pb untreated group ($p = 0.01$ and 0.00 , respectively), and in FFA in Pb rec group ($p = 0.04$); however, a significant decrease was observed in TG level in Pb + Mel group ($p = 0.02$) (Table 2). Relative to Pb untreated group, there were significant elevations in TC and PLP in Pb rec group ($p = 0.05$ and 0.01 , respectively), and in PLP in Pb + Mel group ($p = 0.00$), but a significant decrease in TG level in Pb rec and Pb + Mel groups ($p = 0.01$ and 0.00 , respectively) (Table 2). Compared to the latter, there were significant increases in TC, TG, and

Table 1. Effects of Mel on antioxidant and inflammatory markers in lead-exposed Wistar rats.^a

Groups/parameters	SOD (U/ml)	CAT (μmol/min/ml)	MDA (μM)	LDH (U/l)	TAC (mM Trolox equivalent)	CRP (mg/dl)	UA (mg/dl)
Control	1.71 ± 0.89	47.50 ± 4.23	5.02 ± 0.42	40.02 ± 0.45	9.44 ± 3.57	4.62 ± 2.45	10.53 ± 3.01
Pb untreated	0.87 ± 0.72 ^b	18.02 ± 0.99 ^b	10.11 ± 0.93 ^b	80.15 ± 0.79 ^b	2.03 ± 0.04 ^b	5.02 ± 2.21 ^b	9.89 ± 4.14
Pb rec	0.92 ± 0.77 ^b	39.21 ± 2.45	9.44 ± 0.65 ^b	78.89 ± 0.41 ^b	2.25 ± 0.07 ^b	3.85 ± 2.79 ^c	14.75 ± 2.97
Pb + Mel	1.54 ± 0.21 ^d	59.77 ± 3.45 ^c	7.55 ± 1.33 ^{b,c,d}	59.82 ± 0.79 ^{b,c,d}	4.80 ± 0.76 ^{b,c}	1.68 ± 4.57 ^c	7.40 ± 1.09 ^d
Mel	1.68 ± 0.67	45.25 ± 4.43	4.93 ± 0.07	39.94 ± 0.22	8.92 ± 3.46	4.29 ± 2.17	10.09 ± 2.57

SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde; LDH: lactate dehydrogenase; TAC: total antioxidant capacity; CRP: c-reactive protein; UA: uric acid; Pb: lead; Mel: melatonin.

^aValues are expressed as mean ± SEM.

^bp < 0.05 is significant compared to control group.

^cp < 0.05 is significant—Pb untreated vs. Pb rec and Pb + Mel group.

^dp < 0.05 is significant—Pb rec vs. Pb + Mel.

Table 2. Effects of Mel on lipid indices in lead-exposed Wistar rats.^a

Groups/parameters	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	FFA (μmol/l)	PLP (mg/dl)
Control	115.52 ± 4.85	78.45 ± 4.48	7.31 ± 1.14	126.18 ± 4.09	781.11 ± 16.78	149.47 ± 11.44
Pb untreated	112.92 ± 3.50	102.90 ± 5.99 ^b	6.91 ± 0.58	128.33 ± 5.25	949.29 ± 21.17	77.45 ± 2.54 ^b
Pb rec	126.52 ± 3.06 ^c	78.90 ± 5.06 ^c	6.94 ± 0.53	136.42 ± 2.92	985.90 ± 86.59 ^b	123.59 ± 9.95 ^c
Pb + Mel	108.71 ± 2.36 ^d	55.78 ± 2.26 ^{b,c,d}	5.51 ± 0.63	114.69 ± 2.79 ^d	955.61 ± 30.41	161.45 ± 9.37 ^{c,d}
Mel	114.47 ± 4.45	77.55 ± 4.40	7.30 ± 1.04	125.19 ± 4.11	780.10 ± 15.77	148.45 ± 10.45

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FFA: free fatty acids; PLP: phospholipids; Pb: lead; Mel: melatonin.

^aValues are expressed as mean ± SEM.

^bp < 0.05 is significant compared to control group.

^cp < 0.05 is significant—Pb untreated vs. Pb rec and Pb + Mel group.

^dp < 0.05 is significant—Pb rec vs. Pb + Mel.

LDL-C ($p = 0.01$, 0.01 , and 0.01 , respectively), but a significant decrease ($p = 0.04$) in PLP in the Pb rec group. However, there was no significant difference in HDL-C level when comparisons were made across the groups (Table 2).

Effects of Mel on liver function markers in Pb-exposed Wistar rats

Relative to the control group, there was a significant decrease in TB in Pb untreated, Pb rec, and Pb + Mel groups ($p = 0.04$, 0.01 , and 0.02 , respectively), but significant increases in ALP and AST activities in Pb untreated and Pb rec groups ($p = 0.02$, 0.01 and $p = 0.00$, 0.04 , respectively) (Table 3). Moreover, in comparison to the control group, there were significant elevations in ALT and albumin levels ($p = 0.01$ and 0.04 , respectively), but a significant decrease in globulin ($p = 0.00$) in Pb rec group (Table 3). Compared to the former, a significant ($p = 0.00$) increase in albumin level was recorded in the Pb + Mel group. Relative to the Pb untreated group, there were significant decreases in ALP and AST activities ($p = 0.00$ and 0.04 , respectively), but a significant ($p = 0.00$) increase in globulin level in Pb + Mel group. In addition, compared to the Pb rec group, significant decreases in ALP and ALT activities ($p = 0.00$ and 0.04 , respectively), but a significant ($p = 0.00$) elevation in globulin level were observed in the Pb + Mel group (Table 3).

Discussion

El-Sokkary et al. (2005) reported on the protective action of Mel on hepatic and renal tissues in Pb-exposed rats. The limitations in scope of their study were considered in this manuscript. In the present study, we went further to estimate the biochemical status of markers of lipid metabolism, hepatic-based enzymes and protein molecules, inflammatory indices, and TAC. Moreover, we reported possible biochemical changes that could accompany cessation of Pb exposure.

In the present study, lead exposure was characterized by significant inhibition of antioxidant enzymes and reduction of TAC. The heavy metal causes an imbalance in the delicate antioxidant/pro-oxidant balance, compromising the antioxidant defense system and instigating the production of reactive species (Hsu and Guo, 2002). Nevertheless, there are incongruent reports in the literature on the effects of Pb on

Table 3. Effects of Mel on liver function markers in lead-exposed Wistar rats.^a

Groups/parameters	TB (mg/dl)	ALP (U/l)	AST (U/l)	ALT (U/l)	Albumin (mg/dl)	Globulin (mg/dl)	Total protein (g/dl)	BUN (U/l)
Control	23.25 ± 1.94	57.64 ± 5.10	49.20 ± 5.69	73.58 ± 3.68	2.33 ± 0.09	3.13 ± 0.16	5.80 ± 0.20	10.20 ± 0.94
Pb untreated	18.21 ± 0.66 ^b	78.32 ± 5.01 ^b	93.63 ± 5.69 ^b	93.66 ± 6.93	2.42 ± 0.12	2.81 ± 0.06	5.53 ± 0.08	10.18 ± 0.79
Pb rec	16.68 ± 0.69 ^b	81.64 ± 3.06 ^b	79.70 ± 9.66 ^b	108.88 ± 9.27 ^b	2.70 ± 0.08 ^b	2.53 ± 0.03 ^b	4.82 ± 0.49	9.96 ± 0.88
Pb + Mel	17.50 ± 1.10 ^b	53.08 ± 3.53 ^{c,d}	63.40 ± 7.36 ^c	81.55 ± 3.84 ^d	3.01 ± 0.04 ^b	3.36 ± 0.07 ^{c,d}	5.60 ± 0.06	9.51 ± 0.57
Mel	23.14 ± 1.97	55.66 ± 4.95	47.55 ± 5.60	70.45 ± 2.88	2.31 ± 0.05	3.05 ± 0.10	5.65 ± 0.11	9.75 ± 0.88

TB: total bilirubin; ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: Blood urea nitrogen; Pb: lead; Mel: melatonin.

^aValues are expressed as mean ± SEM.

^b $p < 0.05$ is significant compared to control group.

^c $p < 0.05$ is significant—Pb untreated vs. Pb rec and Pb + Mel group.

^d $p < 0.05$ is significant—Pb rec vs. Pb + Mel.

the antioxidant system. Pb could either elevate or suppress the activities of antioxidant enzymes (Chiba et al., 1996; Han et al., 2005; Sugawara et al., 1991). After the cessation of Pb exposure in this study, catalase activity was restored to a level comparable to the control group; however, further inhibition in SOD activity and the sustained reduction in the TAC, complemented by a significant elevation of the lipid peroxidative index—MDA, confirmed that the pro-oxidative action of Pb is largely sustained after exposure. Pb is poorly absorbed in the body, but once absorbed, the heavy metal accumulates in several body tissues and usually takes a long time before it could be excreted (Alissa and Ferns, 2011). Mel and its metabolites have free radical scavenging actions and so could foster the functioning of the antioxidant system. In fact, a molecule of the hormone is known to scavenge about 10 reactive species (Reiter et al., 2014). Despite the fact that Mel restored the activity levels of CAT and SOD back to normal, the significant reduction in the TAC in the Pb + Mel group relative to the control group revealed that the antioxidant system was still operating at a suboptimal level. As a result, the prevailing free radicals could no doubt instigate some degree of lipid peroxidation, which was indicated by the significant elevation in MDA level above the basal level even after treatment with Mel. The level of activity of LDH across the groups corresponded with that of MDA (Adeyemi and Olayaki, 2018a; Adeyemi et al., 2019, 2020a, 2020b, 2020c). Reduction in the peroxidation of cell membrane lipids after Mel administration resulted in cells preservation, which was indicated by the reduction in the activity of LDH, an enzyme that is known to be elevated following cellular or tissue damage and death (Shi et al., 2003).

The insignificant difference in UA level in Pb untreated and Pb rec groups, relative to the control group, and the significant reduction in CRP level in groups 3 and 4 versus group 2 indicated that Pb seemed to compromise the antioxidant system to a greater extent than the inflammatory pathways. Oxidative stress is known to activate the pathways that produce inflammatory mediators (Haddad, 2002). Hence, pro-oxidative and pro-inflammatory events often accompany each other (Adeyemi and Olayaki, 2019b). In agreement with this study, Pb exposure was reported to be characterized by a significant elevation in CRP (Khan et al., 2008)—an inflammatory mediator that is produced mainly in the hepatic and fat cells in response to pro-inflammatory factors such as

IL-1 and IL-6 (Boncler et al., 2006). Conversely, UA stimulates the release of IL-6, among other initiators of inflammation (Johnson et al., 2005). The anti-inflammatory action of Mel was strongly expressed by the significant reductions in CRP in the Pb + Mel group relative to the Pb untreated group and UA in the former compared to the Pb rec group. Even though Mel has been reported to have a key regulatory role in the production of cytokines (Guerrero and Reiter, 2002), it was recently documented that the hormone exerts anti-inflammatory effects primarily by inhibiting the activation of the inflammasome (Tarocco et al., 2019).

Aside from the generation of free radicals and inflammatory mediators, in this study, Pb exposure precipitated impaired lipid metabolism, although the evidence was weak. Tarugi et al. (1982) reported an increased serum level of cholesterol following Pb administration; contrarily, Skoczynska et al. (2002) noted a decrease, while Cocco et al. (1995) reported no change. Specifically, a positive correlation between the blood level of Pb and the levels of TC and HDL-C has been documented (Kristal-Boneh et al., 1999); however, it was suggested that the heavy metal decreased PPL levels (Ademuyiwa et al., 2005). In the present study, there was no significant difference in TC, FFA, HDL-C, and LDL-C in the Pb untreated group relative to the control group. Therefore, it seemed that the effect of Pb on markers of lipid metabolism is selective. Compared to what is applicable in a homeostatic state, significant deviations were only recorded for TG and PLP in the Pb untreated group. Moreover, TG and PLP levels in the Pb rec group indicated that the adverse effects of Pb on lipid metabolism could be dampened over time by the intervention of endogenous adaptive mechanisms. The observed association between Pb exposure and imbalance in lipid metabolism could be due to either increased synthesis or reduced removal of lipoproteins. The latter could be secondary to the alteration of cell-surface receptors for lipoproteins (Gerhardsen et al., 1986), or the after-effect of the attenuation of hepatic lipoprotein lipase activity (Chajet-Shaul et al., 1989). Moreover, Pb depresses the activity of cytochrome P-450 (Meredith et al., 1977), resulting in decreased biosynthesis of bile acids, which play a primary role in the elimination of cholesterol from the body. On the other hand, increased synthesis of lipoproteins may be as a result of Pb-induced enhancement of the activity of hepatic enzymes that are involved in *de novo* cholesterol synthesis (Dessi

et al., 1984), or it may be due to defective feedback inhibition. Mel facilitated the reversibility of TG and PLP to physiological levels. Moreover, the hormone reversed the escalated level of TC in the Pb rec group to a level comparable to the control group. The anti-dyslipidemic effect of Mel has been attributed to augmentation of cholesterol clearance mechanisms (Chan and Tang, 1995) and enhanced lecithin-cholesterol acyltransferase-mediated cholesterol esterification (Esquifino et al., 1997).

The observed significant increases in plasma activity levels of ALP, AST, and ALT in this study have been interpreted as oxidative stress-mediated hepatic tissue damage (Olayaki et al., 2018b). Damaged hepatic cells release transaminases into the systemic circulation (Aziz and Al Agha, 2006) and cause hyperbilirubinemia (Olayaki et al., 2018b). Contrary to the ALP result obtained in this study, Singh et al. (1994) documented a significant decrease in the activity of ALP following oral administration of Pb to experimental rats, while Fowler et al. (1980) observed no change in the activity of the enzyme after 9 months of Pb administration. Nevertheless, in consonance with the present study, Nehru and Kaushal (1993) reported an increased ALP activity after Pb exposure. Elevated ALP activity as a result of Pb intoxication could be a response to the need to transfer Pb ions across the membranes of hepatic cells in an effort to eliminate the absorbed metal from the hepatic tissue (Sandhir and Gill, 1995). In contrast to expectation, a significant reduction in TB was recorded in group 2, relative to the control group. Elevated levels of bilirubin have been associated with hepatic damage; however, a reduction in its level is usually not a concern. Although bilirubin is one of the indices of hepatic function, the molecule is also considered to have antioxidant properties (Adeyemi and Olayaki, 2017c, 2018b; Sedlak and Snyder, 2004) and anti-inflammatory actions (Stojanov et al., 2013). Therefore, the observed significant reduction in this nonenzymatic biomolecule indicated that Pb impaired both enzymatic and nonenzymatic components of the antioxidant enzyme system. The significant elevations in ALT, albumin, and globulin in Pb rec group, which were not observed in Pb untreated group, suggests that there could be a delayed manifestation of some of the deleterious effects of this heavy metal. The reduced plasma levels of globulin and albumin, which have hepatic origin, alongside with the over-activity of hepatic enzymes, confirmed that the functionality of the hepatic tissue was compromised

following Pb administration. Although there were reductions in total protein level after Pb exposure, there was no significant difference in the level of this marker when comparisons were made among the groups. However, this could have precipitated the observed significant reductions in albumin in groups 3 and 4, relative to group 1, as it is known that amino acids and protein are used in the synthesis of the carrier protein. Albumin is the major protein that provides the colloid osmotic pressure that regulates the passage of diffusible solutes and water across capillaries. Therefore, its deficiency could result in a shift of fluid from the intravascular compartment to the interstitial space, and hence, intravascular volume reduction and edema formation. The insignificant difference in BUN after Pb exposure is in agreement with the findings of Kianoush et al. (2013). The antioxidant action of Mel did not influence the TB level. Moreover, the hormone showed no effect on the plasma level of albumin. However, we submitted that Mel enhanced hepatic function in Pb-treated rats as a result of its anti-oxidative and anti-inflammatory properties. This was evidenced by the reduced expressions of ALP, AST, ALT, and globulin, although not to levels comparable to the control group.

Conclusion

Following the cessation of Pb exposure, alterations in physiological balance could be elevated, sustained, or reversible. However, Mel enhanced the reestablishment homeostatic status after Pb administration.

Authors' note

The rats were purchased from the Animal House of the Biochemistry Department in the University of Ilorin, Ilorin, Nigeria.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was approved by the Ethical Board of the University of Ilorin, Ilorin, Nigeria (no ethical number was given).

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