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# Pleiotropic protective roles of melatonin against aluminium-induced toxicity in rats

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**Abstract.** This study aimed to investigate the potential effects of melatonin on aluminium-induced toxicity in a rat model using a set of biochemical, inflammatory, oxidant, lipid profile criteria and hepatic integrity (verified by hematoxylin-eosin staining). The results indicated that $AlCl_3$ administration during 60 days (100 mg/kg b.w.) significantly increased the activities of transaminases AST and ALT by 46% ($p < 0.001$) and 21% ($p < 0.01$), lactate dehydrogenase (LDH) by 30% ($p < 0.001$), the levels of bilirubin by 85% ($p < 0.001$), total cholesterol by 115% ($p < 0.001$), triglycerides by130% ($p < 0.001$), LDL-cholesterol by 413% ($p < 0.001$), oxidized LDL (oxLDL) by 51% ($p < 0.01$) and apolipoprotein B100 (apoB100) by 63% ($p < 0.001$), as compared to controls. The inflammatory markers (TNF-α, IL-2, and IL-6) were significantly increased ($p < 0.001$), associated to higher lipid peroxidation (TBARS) level. Also, both plasma HDL-cholesterol level and hepatic LDL receptors ($p < 0.01$) expression and antioxidant protein (SOD, CAT, and GPx) activities are decreased. Those physiological disturbances were, however, noted to alleviate following the co-administration of melatonin (10 mg/kg b.w.). Overall, the present study is the first to provide evidence on the anti-inflammatory, anti-oxidant, anti-lipidic and, hence, therapeutic effects of melatonin with regard to the control and prevention of aluminium-intoxication.

**Key words**: Aluminium chloride — Melatonin — Lipid profile — Inflammation — Oxidative stress

## Introduction

Recent reports have raised concerns over the increasing rates of human exposure to aluminium due to its ubiquitous presence and large scale applications in modern times. It is widely used in medicines (as antiacids), in consumer products (cooking utensils, wrapping paper, deodorants, etc.), water treatment, and food processing, which constitute the most important routes for aluminium-toxicity (Yokel 2000; Chen et al. 2010). This metal has been reported to induce several physiological disorders, particularly neurodegenerative changes (Prakash et al. 2013; Sharma et al. 2013; Allagui et al. 2014b). Lipid and inflammation disorders may also be linked to Al-toxicity, though no definite mechanisms have yet been identified. Nevertheless, aluminium administration was previously reported to induce an increase in total lipid, cholesterol, triglycerides and LDL-cholesterol and a decrease in HDL-cholesterol decreased (Newairy et al. 2009). These effects may lead to severe cardiovascular events, since the augmentation of plasma triglycerides, low density lipoprotein (LDL), oxidized LDL (oxLDL), and apolipoprotein B100 (apoB100) have often been described as important risk factors for cardiovascular diseases. The increase in plasma HDL-cholesterol, on the other hand, is known to reduce the occurrence of major cardiovascular events.

Reactive oxygen species (ROS) and pro inflammatory cytokines have been reported to play important roles in several aluminium-reducing processes (Prakash et al. 2013). Free radicals might also have a crucial incidence in several

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physiological disorders (Arbos et al. 2008). In fact, during inflammatory injury, several cytotoxic agents, including ROS, inflammatory cytokines, tumor necrosis factor (TNF-α), interleukin1-2 (IL-2), and interleukin-6 (IL-6), are over expressed and actively involved in the promotion of the decline of several physiological disorders, such as immune system and brain damages and hepatotoxicity (Miller 1995; Hachani et al. 2012; Dab et al. 2013; Prakash et al. 2013). The over production of ROS is, however, often reported to aggravate these physiological changes and to induce cell damages through the increase of oxidative stress.

Melatonin, N-acetyl-5-methoxytryptophan, is a hormone secreted primarily by the pineal gland (Tan et al. 2007) but synthesized by several other tissues and cells such as those of the retina and gastrointestinal tract (Bubenik and Konturek 2011). It is useful for the treatment of several physiological disorders, namely diabetic and neurodegenerative diseases (Srinivasan et al. 2005; Espino et al. 2011, Allagui et al. 2014a, 2014b). It has also been described to exhibit potent antioxidant and anti-oxidative actions against high levels of ROS (Hardeland et al. 1995; Reiter 2000; El-Sokkary et al. 2002) and inflammatory mediators (Cuzzocrea and Reiter 2002).

The data currently available on the effects of aluminium on the lipid profile and levels of oxidative stress and pro-inflammatory cytokines are very limited. Accordingly, the present study was undertaken to investigate the potential protective role of melatonin against aluminium-induced changes pertaining to lipid profile, inflammation and oxidative stress. The total cholesterol, LDL-cholesterol, HDL-cholesterol, native and oxLDL concentrations in the plasma were, therefore, determined. Oxidative stress markers (TBARs concentration) and antioxidant enzyme activities (catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) were also evaluated. The study also focused on hepatic injury markers (AST, ALT, and LDH activities), bilirubin level, hepatic histology, and hepatic LDL receptor (LDLR).

## Material and Methods

### *Animals housing and treatment*

Two-month-old male Wistar rats ($n$ = 40) were purchased from the Pharmacie Centrale de Tunisie (Tunisia) and maintained for an adaptation period of one-month under the same conditions of temperature (22 ± 2°C), relative humidity (70 ± 4%), and 12-h light/dark cycle. The animals were fed a commercial pellet diet and had access to tap water *ad libitum*. They were treated in accordance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes.

### *Experimental groups and treatment*

After the adaptation period, the animals were randomly divided into four groups ($n$ = 10/group). The first served as the control group. The second group, designated as the Al group, received a daily supplementation (*per* gavage) of aluminium chloride (at 100 mg $AlCl_3$/kg b.w.). The third group, Mel group, consisted of rats that received only melatonin supplementation (at 10 mg/kg b.w./day, intraperitoneally injected (i.p.)). The fourth group, Al+Mel group, was co-treated with aluminium chloride (at 100 mg $AlCl_3$/kg b.w.) and an i.p. injection of melatonin (at 10 mg/kg b.w., dissolved in 5% ethanol v/v) (Jangra et al. 2013; Allagui et al. 2014a, 2014b).

After 60 days of treatment, the animals were sacrificed by decapitation, and the blood was collected in two tubes. The first tube was dry and served for serum collection; the second was heparinized and served for the determination of hepatic parameters. The liver tissues were also removed. All samples were then stored at –80°C until further use.

### *Evaluation of liver dysfunction parameters*

Serum samples were obtained by the centrifugation of the blood collected in the dry tubes at 1000 × $g$ for 10 min at 4°C. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities and bilirubin levels were measured using commercial kits from Bio Maghreb (Tunis, Tunisia) and Biomerieux (Lyon, French).

### *Cytokines measurements*

The levels of IL-2, IL-6 and TNF-α were determined in triplicate using commercially available Multi-Analyte ELISArray Kits for Rats (QUIAGEN Company, USA) according to the manufacturer's instructions. The plate included a standard curve and known positive and negative controls. Absorbance was read at 450 nm and at 570 nm using a microtiter ELISA reader (Vadhana et al. 2011).

### *Cholesterol and lipid profile determination*

Plasmatic LDL fraction was extracted by sequential ultracentrifugation as previously described elsewhere (Itabe et al. 1996). The apoB100 and oxLDL concentrations in the extract were then determined using appropriate sandwich ELISA kits for rats (Uscn Life Science Inc., Wuhan) according to the manufacturer's instructions. The plasmatic LDL-cholesterol concentration was determined enzymatically after LDL extraction using a Hitachi 717 automated analyzer (Boehringer). The total cholesterol, triglycerides and HDL-cholesterol concentrations in the plasma were determined enzymatically using the same relevant automated kits.

*Preparation of solubilized membrane proteins*

Solubilized membrane proteins were prepared from three separate experiments in each group (control, Al, Mel and Al+Mel), where protein samples were prepared from 1 g of liver as previously described elsewhere (Vaziri and Liang 1996). In brief, rat liver was homogenized in 20 mM Tris-HC1 (pH 7.5) containing 2 mM $MgCl_2$, 0.2 M sucrose, 5 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, 10 µg/ml aprotinin, and 3 µg/ml pepstatin A. The crude extract was centrifuged at 3000 × *g* for 10 minutes at 4°C. The supernatant was then centrifuged at 35,000 × *g* for 30 minutes. The membrane preparation was then washed with the above buffer and centrifuged at 35,000 × *g* and 4°C for 45 minutes. The protein concentrations in the protein samples were determined by the Lowry method using BSA (Bovine Serum Albumin) as a standard protein. Soluble membrane proteins were used for the quantification of hepatic LDL receptors by Western blot.

*Western immunoblot analysis of LDL receptor*

An amount of 20 µg of proteins was taken from each group and simultaneously loaded onto 10% SDS-PAGE. After electrophoresis, proteins were transferred to a nitrocellulose membrane (Sigma, St. Louis, MO, USA), which was blocked with 5% non-fat dry milk in TBST (Tris-buffered saline with 0.1% Tween 20) for 1 h at room temperature. Membranes were then incubated for 1 h at room temperature in the same blocking buffer containing the primary rabbit polyclonal anti-LDL Receptor antibody (1:500; from Abcam). After three washes with TBST for 10 min each, membranes were incubated in TBST with horseradish peroxidase-conjugated goat anti-rabbit antibody IgG (1:1000; from Abcam) for 1 h at room temperature. Membranes were washed 3 times for 10 min and then incubated with chemiluminescent peroxidase substrate-3 (Sigma, St. Louis, MO, USA) for 5 min at room temperature. Proteins were detected by enhanced chemiluminescence reagents on Kodak BioMax Films. The developed films were scanned, and proteins were quantified by densitometry analysis using ImageJ software (NIH). Finally, the amount of each protein was normalized to the amount of glyceraldehyde phosphate dehydrogenase (GAPDH) that was revealed by rabbit monoclonal anti-GAPDH IgG (1:2000; from Abcam) using the same immunoblotting method described above.

*Evaluation of TBARS and antioxidant enzymes*

About 1 g of excised liver was homogenized into 2-ml ice-cold lyses buffer (pH 7.4), sonicated twice, and centrifuged for 20 min at 3000 × *g* and 4°C. The collected supernatants were used for the determination of TBARS concentration and antioxidant enzyme activities. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS), according to the method of Buege and Aust (1984), and expressed as nmol of MDA/mg protein content. SOD activity was assayed by the method of Marklund and Marklund (1974), and CAT activity was determined by measuring hydrogen peroxide decomposition at 240 nm according to the method of Aebi (1984). GPx activity was determined using the method of Flohe and Gunzler (1984) by the subsequent oxidation of NADPH at 240 nm. Protein contents were determined as previously described in the literature (Lowry et al. 1951) using bovine serum albumin as a standard.

*Liver histopathological studies*

Liver tissues were cut into 5-cm-thick slices and fixed with 10% phosphate-buffered formalin (pH 7.4). They were then dehydrated through ascending grades of alcohol and embedded in paraffin. Tissue sections were made using microtome and stained with hematoxylin-eosin solutions (H&E). Tissue preparations were observed and micro-photographed under a light Olympus CX31 microscope.

*Statistical analysis*

The results were expressed as mean ± standard deviation (S.D.). Comparisons between values were made using one-way analysis of variance (ANOVA) followed by Fisher test when necessary. Statistical significance was accepted at $p < 0.05$.

**Table 1.** Effects of melatonin treatments on the activities of hepatic markers enzymes

| Parameters | Control | Al | Mel | Al+Mel |
|---|---|---|---|---|
| AST (U/l) | 89.11 ± 9.20 | 129.35 ± 12.3*** | 86.33 ± 7.21 | 97.22 ± 7.9### |
| ALT (U/l) | 175. 62 ± 13.32 | 212.34 ± 12.88** | 178.12 ± 16.72 | 186. 37 ± 11.78## |
| LDH (U/l) | 967. 25 ± 61 | 1249.75 ± 49.51*** | 838.67 ± 89.73* | 890. 63 ± 88.31### |

Values are expressed as means ± SD, for 10 rats in each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* control; ## $p < 0.01$, ### $p < 0.001$ *vs.* Al+Mel group. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; Al, aluminium-treated group; Mel, melatonin-treated group; Al+Mel, aluminium and melatonine-treated group.

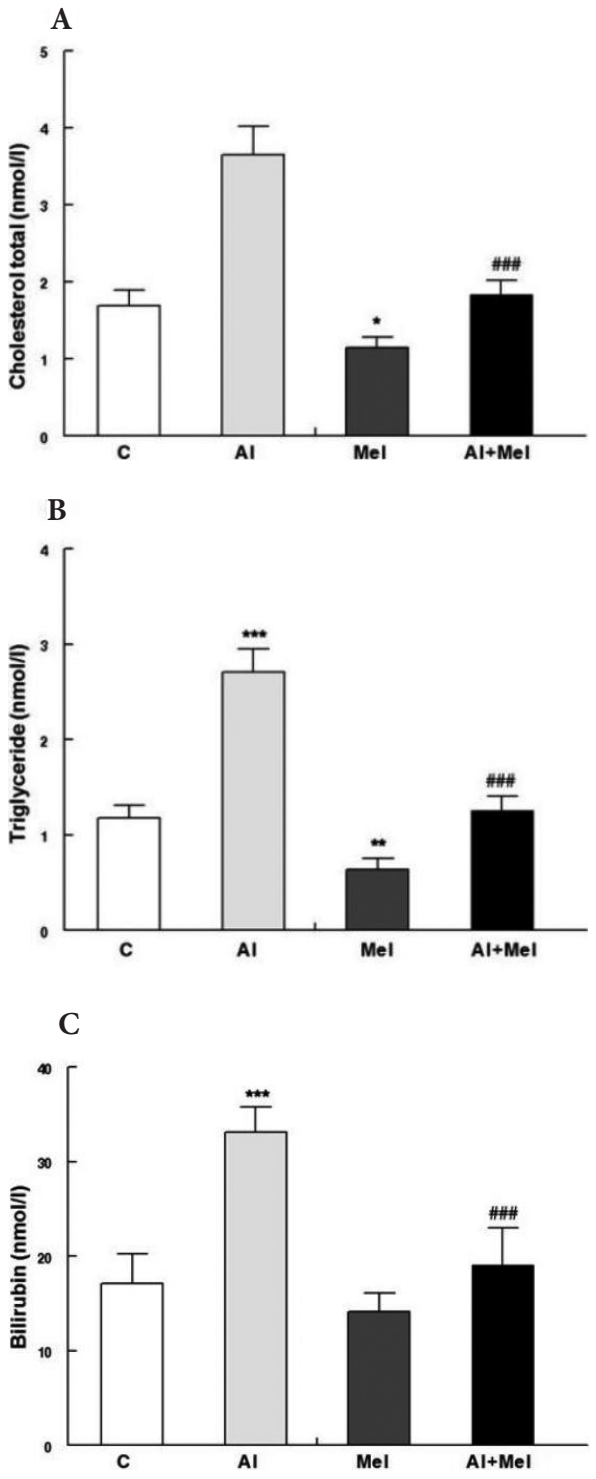



**Figure 1.** Levels of total cholesterol (nmol/l) (**A**), triglyceride (mmol/l) (**B**) and biliburin (mmol/l) (**C**) concentrations in rats treated by aluminium only and/or combined with melatonin supplementation. Data are mean ± SD ($n = 10$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* control; ### $p < 0.001$ *vs.* Al+Mel group. C, control; Al, aluminium-treated group; Mel, melatonin-treated group; Al+Mel, aluminium and melatonin-treated group.

## Results

### *Hepatic markers*

Table 1 shows the effects of melatonin administration on the activity of some hepatic biomarker enzymes in the control and aluminium/melatonin-treated groups of rats. The results revealed that aluminium exposure induced marked increases in the activities of all hepatic damage indicators, AST, ALT, and LDH by 46% ($p < 0.001$), 21% ($p < 0.01$) and 30% ($p < 0.001$), respectively, as compared to the control group. However, the application of melatonin in combination with aluminium was also observed to significantly attenuate the activity of the three hepatic damage marker enzymes as compared to the aluminium-treated group.

### *Bilirubin level*

The findings indicated that the aluminium-treated rats underwent a marked increase of up to 85% ($p < 0.001$) in bilirubin concentration as compared to the control. While the administration of melatonin alone did not exert significant effects on bilirubin concentration, the effect of aluminium was neutralized by co-treatment with melatonin (Fig. 1C).

### *Lipid profile*

The total cholesterol, triglycerides, LDL-cholesterol, oxLDL and apoB100 concentrations in the plasma of the aluminium-treated rats were noted to increase significantly by 115% ($p < 0.001$), 130% ($p < 0.001$), 413% ($p < 0.001$), 51% ($p < 0.01$) and 63% ($p < 0.001$) as compared to the control, respectively (Figs. 1 and 2). Melatonin treatment was, on the other hand, observed to induce a decrease of 32% ($p < 0.05$), 35% ($p < 0.05$), 36% ($p < 0.05$), 72% ($p < 0.001$) and 43% ($p < 0.01$) in all of those parameters as compared to the control, respectively. The treatment involving the co-administration of aluminium and melatonin was noted to neutralize the effects of aluminium toxicity on almost all the parameters under investigation, which underwent a significant decrease by 50% ($p < 0.01$), 53% ($p < 0.01$), 40% ($p < 0.05$), 36% ($p < 0.05$) and 22% ($p < 0.05$) compared to the aluminium-treated rats, respectively.

The results also revealed that aluminium treatment decreased both plasma HDL-cholesterol and hepatic LDL receptors by 55% ($p < 0.01$) and 66% ($p < 0.01$), respectively. Melatonin administration was, on the other hand, noted to induce a significant increase in the LDL receptor ($p < 0.01$) but did not affect the HDL-cholesterol receptor. The two latter parameters were not affected by the treatment involving the combination of melatonin and aluminium (Figs. 2 and 3).

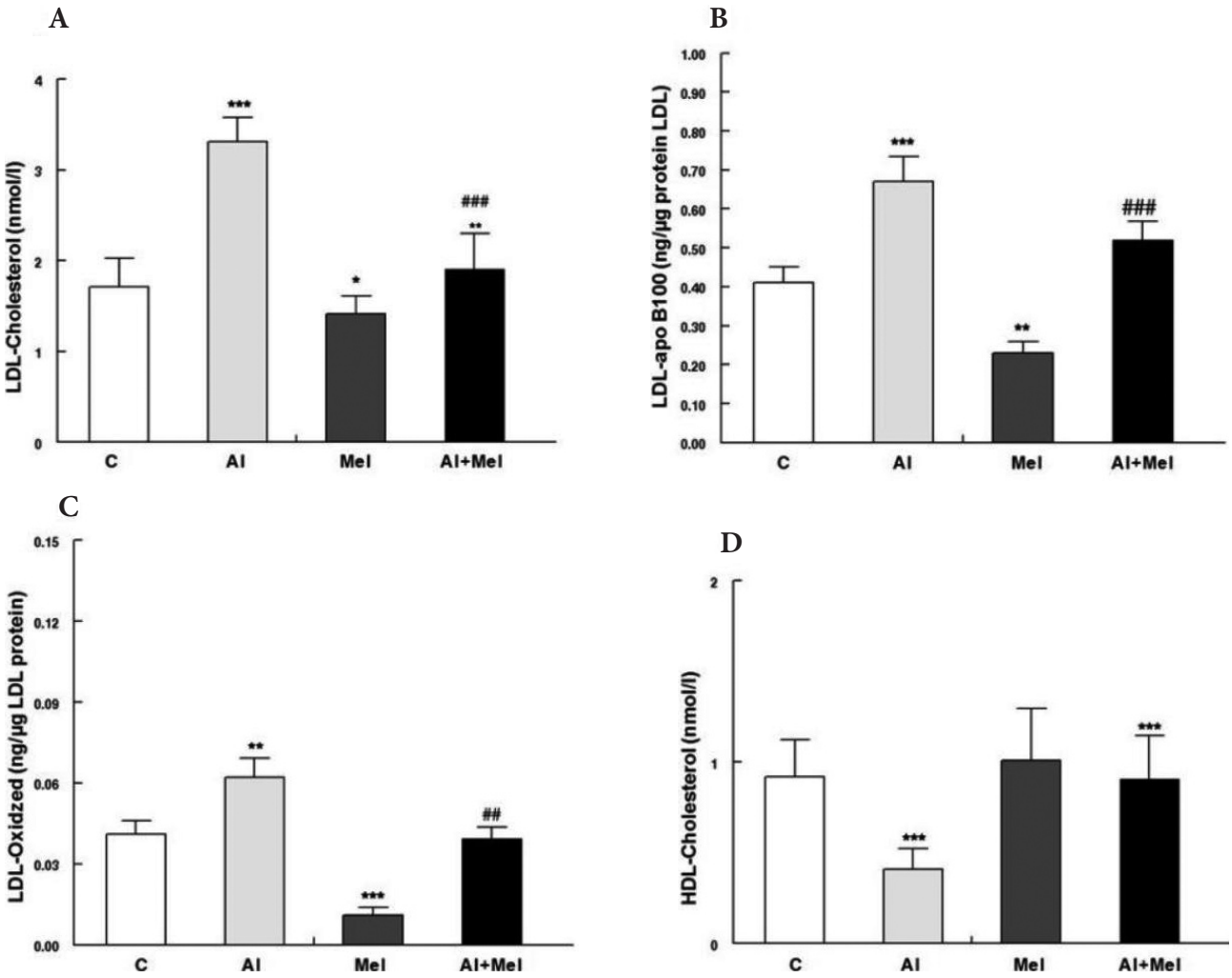
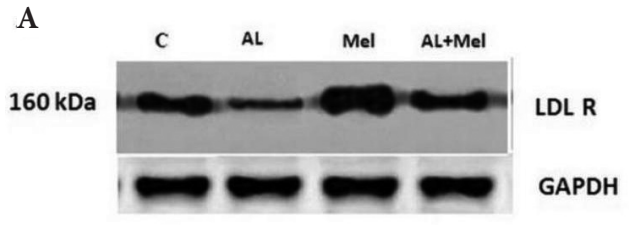



**Figure 2.** Levels of LDL-cholesterol (mmol/l) (**A**), LDL-Apo B100 (ng/µg proteins LDL) (**B**), oxLDL (ng/µg proteins LDL) (**C**) and HDL-cholesterol (mmol/l) (**D**) contents in the plasma of rats treated by aluminium only and /or combined with melatonin injection. Data are mean ± SD ($n$ = 10). * $p$ < 0. 05, ** $p$ < 0.01, *** $p$ < 0.001 *vs.* control: ## $p$ < 0.01, ### $p$ < 0.001 *vs.* Al+Mel group. (For abbreviations, see Figure 1).



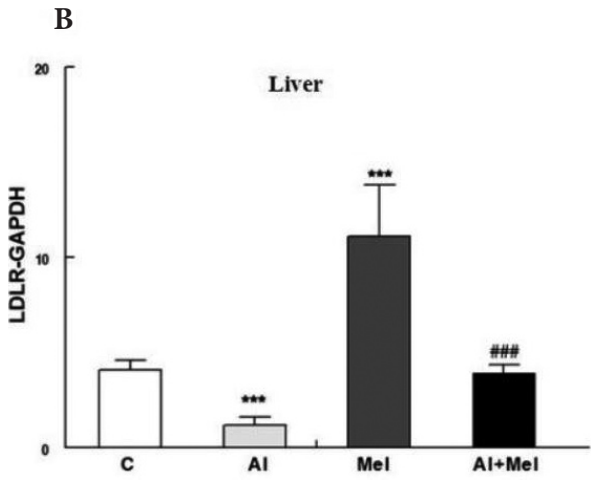




**Figure 3.** Western blot analysis (**A**) and densitometric quantification of the hepatic native LDL receptors (LDLR) (**B**); in control rats, Al group, Mel group and Al+Mel group. Data are shown as mean values (8 rats) ± SD normalized to GAPDH. *** $p$ < 0.001 *vs.* control rats, ### $p$ < 0.001 *vs.* Al group. (For abbreviations, see Figure 1).

*Inflammatory parameters*

Table 2 provides the values recorded for the key pro-inflammatory cytokine parameters. TNF-α, IL-2 and IL-6 were evaluated in the control and three treated groups. The results revealed that TNF-α, IL-2, and IL-6 expressions were significantly increased ($p < 0.001$) in the aluminium-treated rats as compared to the control group. Melatonin administration induced a decrease in the plasma concentration of both TNF-α and IL-6 ($p < 0.01$ and $p < 0.05$) but did not affect IL-2. The treatment involving aluminium combined with melatonin was, on the other hand, noted to bring a significant decrease in the three cytokines TNF-α ($p < 0.001$), IL-2 ($p < 0.01$) and IL-6 ($p < 0.01$) as compared to the aluminium-treated group, thus evidencing the protective role of melatonin against aluminium-induced inflammation.

*Oxidant/antioxidant biomarkers*

Table 3 presents the TBARS contents and antioxidant enzyme (SOD, Cat, and GPx) activities estimated in the liver tissues of all groups of rats. The results showed that the aluminium-exposed animals underwent a significant increase in the level of lipid peroxidation (reflected by TBARS) by about 150% ($p < 0.001$) and significant decrease in the hepatic activity of SOD ($p < 0.001$), CAT ($p < 0.01$) and GPx ($p < 0.001$) as compared to the control. The findings showed, however, that the co-administration of melatonin with aluminium considerably attenuated the levels of lipid peroxidation as compared to the control and the aluminium-treated group. Aluminium/melatonin treatment was also observed to induce a significant increase in the activity of the three antioxidant enzymes, indicating a protective role for melatonin against aluminium-induced oxidative stress.

*Histological changes*

Figure 4 summarizes the histological changes of the liver sections of all the treated groups. The histological sections of the liver tissues (Fig. 4A) of the control group showed normal architecture, with hepatocytes arranged in rows that radiate out from the central vein. In the aluminium-treated group (Fig. 4C), the hepatic injury was marked by significant lipid accumulations (steatosis), obliterated intervening hepatic sinusoids and central vein disruption. The administration of melatonin in combination with aluminium was noted to restore the liver tissues and hepatic architecture back to normal states (Fig. 4D).

## Discussion

The present study aimed to investigate the effects of aluminium-induced toxicity in a rat model using a set of biochemical, inflammatory, oxidant, and lipid profile criteria. The potential efficiency of melatonin administration in the alleviation of aluminium toxicity was evaluated. The results demonstrated that aluminium treatment induced a marked increase in the activities of serum AST, ALT and LDH. This may reflect a liver damage since similar increases in plasma transaminases and lactate dehydrogenase activities have

**Table 2.** Effects of melatonin supplementation on the TNFα, IL-2 and IL-6 in the plasma of all experimental groups

| Parameters | Control | Al | Mel | Al+Mel |
|---|---|---|---|---|
| TNF-α (μg/ml) | 27.4 ± 6. 12 | 51.90 ± 5.43*** | 15.91 ± 3.01** | 18.12 ± 4.10### |
| IL-2 (μg/ml) | 5.22 ± 3.28 | 24. 21 ± 4.75*** | 5.01 ± 3.24 | 13.80 ± 3.34## |
| IL-6 (μg/ml) | 5.08 ± 1.76 | 11.90 ± 3. 2** | 2.10 ± 0.94 * | 5.01 ± 1.61## |

Values are expressed as means ± SD, for 10 rats in each group. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ *vs.* control; ## $p \leq 0.01$, ### $p \leq 0.001$ *vs*. Al+Mel group.

**Table 3.** Effects of melatonin supplementation on the lipid peroxidation (TBARs levels) and activities of antioxidant enzymes (SOD, CAT, GPx) in liver of all experimental groups

| Parameters | Control | Al | Mel | Al+Mel |
|---|---|---|---|---|
| TBARs (nmol/mg prot.) | 1.55 ± 0.15 | 3.65 ± 0.13*** | 1.08 ± 0.14 ## | 1.18 ± 0.19### |
| SOD (U/mg prot.) | 8.45 ± 0.52 | 4.55 ± 0.30*** | 7.76 ± 0.63# | 9.34 ± 1.13## |
| CAT (μmol $H_2O_2$/min/mg prot.) | 26.5 ± 2.12 | 18.35 ± 0.22** | 33.34 ± 1.66## # | 29.64 ± 0.81### |
| GPx (μmol GSH ox/min/mg prot.) | 1.98 ± 0.31 | 0.82 ± 0.19*** | 1.72 ± 0.35## | 1.62 ± 0.42## |

Values are expressed as mean ± S.D. for 10 rats in each group. * $p \leq 0, 05$, ** $p \leq 0, 01$, *** $p \leq 001$ significantly different as compared to the control group; # $p \leq 0, 05$, ## $p \leq 0, 01$, ### $p \leq 001$ significantly different as compared to the aluminium-treated group.

previously been reported to occur in liver injuries (Hassoun and Stohs 1995; Gaskill et al. 2005). In addition, the findings presented in this work also showed that aluminium exposure increased total cholesterol and triglycerides but decreased HDL-cholesterol in both the liver and plasma. The increase in plasma lipid compounds due to aluminium chloride administration is a sign of membrane integrity disruption or loss (Wilhelm et al. 1996). This was further confirmed by the significant effects observed for aluminium treatment on the various membrane-bound enzymes, particularly in terms of altered AST, and ALT activities (Aldana-Madrid et al. 2008; Bhasin et al. 2012). In fact, Wilhelm et al. (1996) have previously reported that aluminium exposure can result in aluminium accumulation in the liver, which may lead to the disruption of lipid metabolism and elevation of serum cholesterol level. These findings were further confirmed by the hepatic histological changes and significant lipid accumulations (steatosis) associated with a major central vein disruption were observed after aluminium treatment (Fig. 4).

Apolipoprotein B100 (ApoB100) is found in native lipoproteins originating from the liver (VLDL, IDL and LDL) and expressed in a single copy *per* particle (Kontush et al. 2003). The plasma apoB100 concentrations recorded in the present study reflected the concentrations of native LDL particles, since they were determined in the LDL fraction. The findings revealed that aluminium intoxication increased LDL-cholesterol by 413%, while native LDL particles (reflected by apoB100) increased by only 63%, suggesting that aluminum exposure enriched LDL particles with cholesterol and, therefore, represent a high risk factor for atherosclerosis.

The increase in both LDL-cholesterol and native LDL particles in the plasma might indicate a reduction in LDL clearance by receptor-mediated pathways. This hypothesis was strengthened by the findings related to hepatic LDL receptors (Fig. 3), previously reported to account for 60–80% of LDL clearance (Stucchi et al. 1995), which were noted to undergo a dramatic decrease following aluminum treatment (Fig. 3), a phenomenon that could facilitate LDL particle uptake and removal from circulation. Furthermore, the cholesterol concentration in the plasma LDL extract normally reflects the concentration of cholesterol linked to the native and oxLDL particles, but the findings of the present study could presumably infer that most of the cholesterol determined in the LDL fractions of aluminium-treated groups was incorporated into the native form. Using sandwich ELISA, this study showed that the plasma of both the control and aluminium-treated groups contained the oxidized form of LDL, though in low amounts when compared to apoB100. These findings are in accordance with previous results in the literature stipulating that oxLDL is also present even in healthy individuals, though at very low rates (Korporaal et al. 2007; Hachani et al. 2012). The results also indicated that aluminium exposure induced a dramatic increase in plasma oxLDL compared to the control. The increased concentration of bilirubin after aluminium exposure is in accordance with the results previously reported by Yousef et al. (2010) and consistent with the oxidative process since a higher bilirubin concentration was previously associated with strong free radical production (Yousef et al. 2004). These findings were further supported by the drastic decreases of SOD, CAT and GPx activities associated with an increase of lipid peroxidation (reflected by TBARS) in liver extract as well

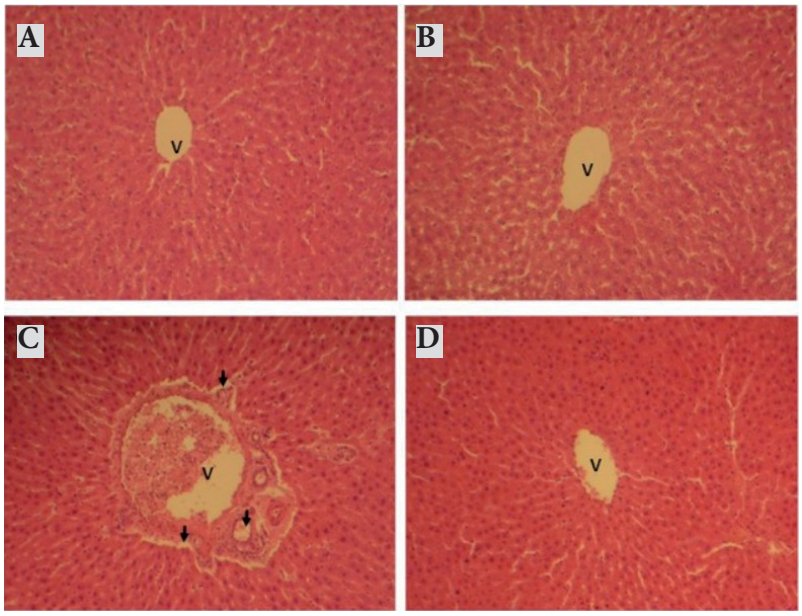



**Figure 4.** Photographs showing histological changes in the liver sections of all-treated groups: in control rats showing normal central vein (V) and radiating cell arrangement (**A**), melatonin only showing the normal histoarchitecture **(B),** in aluminium-treated rats showing a marked lipid accumulation (steatosis, arrows) and central vein disruption (**C**), in Al+Mel group, an improvement in histoarchitecture was clearly observed (**D**). (Hematoxylin-Eosin staining, 200×).

as in plasma and red blood cell extracts (data not shown), in the aluminium-treated group of rats. *Via* oxidative stress mechanisms, this findings supported the participation of free-radical-induced oxidative cell injury in mediating the toxicity of aluminum (Anane and Creppy, 2001; Dua and Gill, 2001). In fact, aluminium may react with superoxide anions forming aluminium superoxide anions, which are more potent oxidants (Exley 2004). In the same context, several studies have also reported that aluminium has the ability to potentiate iron-mediated lipid peroxidation by replacing iron ions with aluminium and that the free iron have a strong catalytic power to generate free radicals despite Fenton's reaction (Oteiza 1994; Ohyashiki et al. 1998; Ward et al. 2001). Conversely, melatonin administration significantly increased antioxidant enzymes activity and decreased TBARS level in the liver tissue and at the circulatory level. The present results related to antioxidative effects of melatonin are consistent with earlier findings on ageing and diabetic circumstances (Allagui et al. 2014a, 2014b).

The findings of the present study also demonstrated that aluminium exposure increased the levels of pro-inflammatory cytokines (IL-2, IL-6 and TNF-α) in plasma. This called for further data on the potential origin or source of this increase. In fact, the answer came from laboratory experiments revealing that aluminium treatment significantly increased the hepatic levels of the three studied proinflammatory cytokines (TNF-α, IL-2 and IL-6) (data not shown). This strongly suggested that the liver, or at least a part of it, was one of the key sources for the increased cytokines after aluminium treatment. This work focused on TNF-α, IL-2 and IL-6, since these pro-inflammatory cytokines were identified as the most important cytokines involved in the activation of other pro-inflammatory cytokines and amplification of other inflammatory mediators (Zhao et al. 2012). It is worth noting that the oxidative stress events observed after aluminum treatment could also result from the increased levels of pro-inflammatory cytokines (IL-2, IL-6 and TNF-α) because, as previously reported in rat models, pro-inflammatory cytokines can induce xanthine oxidoreductase, NADPH oxidase, and iNOS, which would in turn bring an increase in the level of free radicals (Ferdinandy et al. 2000).

Interestingly, the results also demonstrated that melatonin alone or combined with aluminium has an anti-inflammatory action, as evidenced by a marked decrease in plasmatic levels of the three proinflammatory cytokines (TNF-α, IL-2 and IL-6.). The mechanism of action of melatonin on these cytokines is still unclear and warrants further investigation. However, a hypothetic mechanism of action of melatonin *via* its antioxidant propriety, reported herein, could not be excluded. In fact, the production of ROS has previously been reported to cause cellular injury and necrosis through several mechanisms including peroxidation of membrane lipids during inflammation (Szabo et al. 1996). Accordingly, the possibility that melatonin effectively scavenges those ROS, or interfering with their harmful consequences, might be one of the possible mechanisms for the reproducible anti-inflammatory activity reported in the present study or at least an additive pathway to the already involved ones (Mahmood et al. 2010). Furthermore, the anti-inflammatory effects of melatonin have been linked to the modulation of a number of transcription factors, such as nuclear factor kappa B, hypoxia-inducible factor and nuclear factor erythroid 2-related factor 2 (Mauriz et al. 2013). Additionally, it could be hypothesized that melatonin may act on these proinflammatory cytokines indirectly by influencing immune modulators, since proinflammatory cytokines have previously been reported to dominate the early immune response (Babu and Nutman 2003). The presence of both nuclear and membrane receptors for melatonin in the immune system (Guerrero and Reiter 2002) strongly corroborate this hypothesis.

The results presented here also showed that melatonin induced marked decreases in total cholesterol, LDL-cholesterol, oxLDL, apoB100, and triglycerides levels. These findings, confirming an anti-lipidic effect of melatonin, are in agreement with the results previously reported by Hussain (2007) where melatonin was noted to significantly reduce cholesterol absorption and to significantly decrease total cholesterol, triglycerides, LDL-cholesterol and of cholesterol and triglyceride contents in the plasma and the liver of rats fed on high cholesterol diet, respectively. Several studies have previously indicated that the increase of free radicals and pro oxidants molecules could directly or indirectly initiate the oxidation of LDL particles (Tribble 1995; Vaziri and Liang 1996; Heinecke 1997). The results of this work revealed that melatonin significantly reduced oxLDL and increased HDL-cholesterol levels, *via* antioxidative effects, confirming the anti-hyperlipidimic and antioxidative action of melatonin against aluminium-induced oxidative damage.

Last but not least, the present study demonstrated that aluminium has adverse effects on several biochemical, inflammatory, oxidant and lipid profile parameters. The results revealed that melatonin, administered in combination with aluminum, minimized the hazards associated with aluminium exposure. Interestingly, our findings provided interesting evidence on the anti-inflammatory, antioxidant, and anti-lipidic effects of melatonin against aluminium intoxication.

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