$AlCl_3$-Induced Toxicity and Oxidative Stress in Liver of Male Rats: Protection by Melatonin

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Abstract: Aluminum is a ubiquitous element with known toxicity for both human and experimental animals. It has been implicated in the pathogenesis of several diseases. The present study investigates the possible hepatoprotective role of melatonin in modulating the toxicity and the oxidative stress induced by chronic exposure to aluminum chloride ($AlCl_3$) in the liver of male rats. 40 male rats were divided into four groups (10 rats each): vehicle control group treated with alcoholic saline, $AlCl_3$ group treated with 20 mg/kg of $AlCl_3$, melatonin group treated with 5 mg/kg of melatonin, and melatonin+$AlCl_3$ group treated with the previous doses of both $AlCl_3$ and melatonin. Rats were treated orally once daily for 30 consecutive days. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total lipids, total cholesterol, triglycerides, glucose and total proteins were measured in the plasma to assess the liver functioning. Liver specimens were also collected for histopathological examination and also for assessment of hepatic level of malondialdehyde (MDA), reduced glutathione (GSH) in addition to the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). The results showed that the oral administration of $AlCl_3$ caused significant ($p<0.05$) increases in the plasma level of the ALT, AST, ALP, total bilirubin, total lipids, total cholesterol, triglycerides and glucose while the level of total proteins was found to be decreased. Moreover, $AlCl_3$ induced oxidative stress as indicated by a significant increase in the level of MDA with a concomitant decrease in the GSH content as well as in the activity of GPx, SOD and CAT in the liver tissue. Histological examination for liver sections revealed marked necrosis and degeneration of hepatocytes, centrilobular necrosis, congestion of the central vein, vacuolization of cytoplasm, infiltration of inflammatory cells, dilatation and congestion of the blood sinusoids. Pretreatment with melatonin in $AlCl_3$-treated rats alleviated the previously mentioned alterations in the biochemical and oxidative stress parameters and restored their values toward the normal value of the control group. Moreover, it improved to a large extent the histological changes induced by $AlCl_3$ in such a way that more or less normal architecture of the liver was observed. Therefore, the data obtained in the present study confirmed the deleterious effects of $AlCl_3$ in the liver. Moreover, it can be concluded that these effects could be overcome or, at least, significantly minimized by the administration of melatonin.

[Wessam M. Abdel-Wahab . **$AlCl_3$-Induced Toxicity and Oxidative Stress in Liver of Male Rats: Protection by Melatonin.** *Life Sci J* 2012; 9(4): 1173-1182] (ISSN: 1097-8135). http://www.lifesciencesite.com. 174

Key words: Aluminium chloride, Toxicity, Oxidative stress, Melatonin, Liver, Rat

1. Introduction

Aluminium (Al) is ubiquitous in the environment, it makes up close to 8% of the Earth's crust by weight. It is released to the environment either naturally through weathering and erosion processes or from various anthropogenic sources. This element has a myriad of uses in daily life throughout the world and in particular in developing countries. It is alloyed with other metals and used in many industries including ship building, electrical building and motor vehicle industries. Exposure to Al is almost inevitable, humans are frequently exposed to Al by the inhalation of ambient air and the ingestion of food and water (Soni *et al*., 2001). Consumption of processed food and water purified using Al-containing additives is the main route for this metal to enter the human body (Newairy *et al*., 2009). Another source of exposure is the use of Al-containing compounds such as antiperspirants, cosmetics, internal analgesics, anti-ulcerative medications, astringents, and antacids (Zhou and Yokel, 2005). Industrial waste water and particulate matters emitted from cement-producing factories also contain high amount of Al which results in environmental pollution (Fatima *et al*., 2001).

Aluminum has the potential to be toxic for both human and animals. It was included in the priority list of hazardous substances identified by The Agency for Toxic Substances and Disease Registry (ATSDR, 2007). It accumulates in various mammalian tissues such as the kidneys, liver, heart, brain and is related to cardiotoxicity, nephrotoxicity, neurotoxicity and hepatic dysfunctions (Reinke *et al*., 2003). Intoxication with Al exacerbate reactive oxygen species (ROS) formation (Bondy *et al*., 1998) and has been found to cause oxidative damage of lipids, proteins and DNA (Gonzalez *et al*., 2007; Newairy *et al*., 2009). Salts of Al may inhibit enzymes like acid and alkaline phosphatases, phosphodiesterase and phosphooxydase (Ochmanski

and Barabasz, 2000). The toxicological effects of Al in humans include encephalopathy (Alfrey *et al*., 1976), bone disease (Ward *et al*., 1978), anemia (Short *et al*.,1980). Furthermore, Al is possibly a contributing factor in the development of Alzheimer's disease (Campbell, 2002).

Melatonin (5-methoxy-N-acetyltryptamine) is a natural compound of almost ubiquitous occurrence. Its presence has been demonstrated in all major taxa of organisms, as far as tested, including bacteria, unicellular eukaryotes, algae, fungi and invertebrate animals *(*Hardeland and Fuhrberg, 1996*)*. Moreover, several studies dealt with melatonin in edible plants (Reiter and Tan, 2002). It is relatively nontoxic and highly lipophilic molecule that can easily cross cell membranes and blood-brain barrier (Hardeland, 2005). It displays a wide spectrum of metabolic and physiological effects including hypothermic (Dolberg *et al*., 1998), analgetic, cardio-and neuroprotective effects (Lagneux *et al*., 2000), anti-apoptotic activity (Sainz *et al*.,1999) and antidepressive (Ergün *et al.*, 2008). Melatonin acts as a regulator for many physiological functions such as endocrine rhythm, antigonadotropic effects and stimulation of the immune system (Csernus and Mess, 2003). Melatonin is a very powerful endogenous antioxidant with a known ability to remove ROS and reactive nitrogen species (Mahieu *et al*., 2009). Moreover, metabolites of melatonin have been found to protect tissues against oxidative damage generated by a variety of toxic agents and metabolic processes (Tan *et al*., 2007). Melatonin upregulates several antioxidant enzymes, most frequently, glutathione peroxidase (Barlow-Walden *et al*., 1995) and sometimes glutathione reductase (Liu and Ng, 2000). It is also essential for avoiding radical formation through downregulation of prooxidant enzymes, in particular nitric oxide synthases (Storr *et al*., 2002).

Therefore, the present study firstly aimed to investigate the possible $AlCl_3$-induced changes in enzyme activities, oxidative stress biomarkers and some biochemical parameters in the plasma and liver of male rats. Since there is always need for a successful therapeutic approach that might inhibit the initiation and progression of diseases, the present study also evaluates the potential hepatoprotective effect of exogenous melatonin in ameliorating these possible alterations.

2. Material and Methods

2.1. Chemicals:

All chemicals used in the present study were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. (St Louis, Mo, USA). $AlCl_3$ was dissolved in physiological saline while melatonin was dissolved just before use in a vehicle composed of 1% ethanol (96%) and 99% saline. All kits used in the present study were the products of Biodiagnostic Co. (Egypt), Biosystems CO. (Spain) and Randox Laboratories (United Kingdom).

2.2. Animals and Experimental Protocol:

Forty male albino Sprague-Dawley rats (weighing 170-190 gm) were purchased from the animal house of the High Institute of Public Health, Alexandria University, Alexandria, Egypt. Rats were housed in stainless steel cages (5 rats/cage). The animals were kept under controlled conditions of 12 h light-dark cycle, room temperature of 22-25ºC, relative humidity of 40-60%. The animals were allowed free access to standard pelleted food and water. After two weeks of acclimatization to the laboratory conditions, rats were randomly divided into 4 experimental groups (10 rats in each) as follows: Group 1 (vehicle control): was administered alcoholic (1%) saline, Group 2 ($AlCl_3$): given $AlCl_3$ at a dose of 20 mg/kg bw (1/20 LD_{50} of $AlCl_3$ as reported by Krasovskii *et al*., 1979), Group 3 (Melatonin): administered melatonin alone at a dose of 5 mg/kg bw, Group 4 (Melatonin+$AlCl_3$): given the previous doses of melatonin and $AlCl_3$ in groups 3 and 2. Doses were administered orally by gavage (since the main route of human exposure is the oral) once daily for 30 consecutive days. Melatonin was administered at 18:00, 30 minutes before the administration of $AlCl_3$ since it is rapidly metabolized (Vakkuri *et al*.,1985). The doses of $AlCl_3$ and melatonin were calculated according to the animal's body weight before treatment. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

2.3. Blood Sampling:

At the end of the experimental duration, rats were fasted overnight, with free access to water. Under light anesthesia with diethyl ether, rats were sacrificed by cervical decapitation and the blood was collected into heparinized tubes. The collected blood was then centrifuged at 4000 rpm for 15 minutes, the obtained plasma was stored at - 20ºC till analysis.

2.4. Preparation of Liver Homogenate:

One gram of the liver tissue was washed in ice-cold isotonic saline containing 1 mM EDTA. The tissues were then homogenized separately in 8 mL of cold buffer (50 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) using a Potter-Elvejham homogenizer at 4°C. The crude tissue homogenate was then centrifuged at 8,000 rpm for 15 main at 4°C and the supernatant was removed and kept at -20°C for estimation of malondialdehyde (MDA), reduced glutathione (GSH) and the activity glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT).

2.5. Biochemical Assays:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was assayed according to the method described by Belfield and Goldberg (1971). Total bilirubin was assayed according to the method of Walter and Gerade (1970).

MDA, as a marker for lipid peroxidation (LPO) was measured colorimetrically in liver homogenate according to the method of Ohkawa *et al.* (1979). The non-enzymatic antioxidant GSH in liver was determined by the method of Ellman (1959).

The activity of GPx was determined as described by Paglia and Valentine (1967). According to the method of Nishikimi *et al*. 1972), the activity of SOD was assayed. The CAT activity in the liver was assayed according to the method of Aebi (1984).

Total Lipids were assayed according to the method of Zollner and Kirsch (1962). Total cholesterol was determined after enzymatic hydrolysis and oxidation according to the method of Richmond (1973). Triglycerides were measured using the method of Fossati and Principle (1982).

Glucose level in the plasma was measured according to the method of Trinder (1969). Total protein was assayed in plasma and liver homogenate according to Lowry *et al.* (1951).

2.6. Histological Examination:

For histological examinations, small pieces of the liver were quickly removed and fixed in 10% neutral buffered formalin solution. Following fixation, specimens were dehydrated in graded ethanol, embedded in wax, sectioned to 5 microns thickness. The sections were stained with Haematoxylin and Eosin (Banchraft *et al*., 1996) and examined under Olympus BX41 light microscope.

2.7. Statistical Analysis:

Data were analyzed using Statistical Package for Social Science (SPSS/Version 17.0) software. Significance was calculated using one-way analysis of variance (ANOVA). Values at $p<0.05$ were considered statistically significant.

3. Results

3.1. The Activity of Liver Function Enzymes in the Plasma

Data presented in Table 1 denoted that the administration of $AlCl_3$ at a dose level of 20 mg/kg for 30 days induced a significant ($p<0.05$) increase in the activity of ALT, AST, ALP and the level of bilirubin as compared to the vehicle control group (by 83.1%, 80.8%, 51.3% and 144.2% respectively) indicating hepatic damage caused by $AlCl_3$. No change in the activity of these liver marker enzymes and in the level of total bilirubin was observed in rats administered melatonin alone as compared to the vehicle control group. Exogenous melatonin, when administered 30 minutes prior to $AlCl_3$, demonstrated a potent effect in protecting rats against $AlCl_3$-induced liver damage as evidenced by the reduction in the activity of these liver function biomarker enzymes compared to the $AlCl_3$ group (Table1).

Table 1. The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the level of total bilirubin in the plasma of rats treated with $AlCl_3$ or melatonin or both.

Experimental group	Parameter			
	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)
Control	45.6±2.65	78.2±4.10	162.3±8.21	0.86±0.06
$AlCl_3$	83.5±5.10[a]	141.4±7.64[a]	245.5±10.63[a]	2.10±0.10[a]
Melatonin	43.0±3.85	74.5±3.89	157.6±5.82	0.80±0.04
Melatonin +$AlCl_3$	52.2±2.10[b]	92.1±5.71[b]	180.3±6.40[b]	1.05±0.06[b]

- Values are expressed as means ± SE, n=10 for each experimental group.
- [a] Significant difference from control group ($p<0.05$). - [b] Significant difference from $AlCl_3$ group ($p<0.05$).

3.2. Hepatic MDA Level and GSH Content

Results presented in Table 2 indicated that the level of MDA, an end product of LPO, was significantly ($p<0.05$) increased in $AlCl_3$-treated rats as compared to the vehicle control group. Conversely, hepatic GSH content showed significant ($p<0.05$) reduction in rats treated with $AlCl_3$ at a dose of 20 mg/kg bw as compared to the vehicle control group. Administration of melatonin alone resulted in an insignificant increase in the level of GSH when compared to the vehicle control group. Pretreatment with melatonin in $AlCl_3$-intoxicated rats restored the values of MDA and GSH toward the normal value of the control (Table 2).

3.3. The Activity of GPx, SOD and CAT in the Liver

The change in the activity of the enzymatic antioxidants namely GPx, SOD and CAT in liver of control and experimental animals are shown in Table 2. The oral administration of $AlCl_3$ was associated with a significant ($p<0.05$) decrease in

the activities of these radical scavenging enzymes by 57.5%, 50.2% and 56.6% respectively compared to the vehicle control. Rats treated with melatonin alone showed an insignificant increase in the measured antioxidant enzymes compared to the vehicle control group. Administrating melatonin prior to $AlCl_3$ enhanced the enzymatic antioxidative status as demonstrated by the significant (*p<0.05*) increase in the activity these enzymes in the liver in comparison with that of $AlCl_3$ group.

3.4. Level of Some Biochemical Parameters in the Plasma

The administration of $AlCl_3$ for 30 days was associated with some alterations in the biochemical parameters in the plasma of rats in different experimental groups as shown in Table 3. Total lipids, total cholesterol, triglycerides and glucose showed a significant (*p<0.05*) increase (by 42.8%, 121.6%, 81.2% and 84.7% respectively) as compared to the vehicle control group. On the other hand, total proteins showed significant (*p<0.05*) decrease by 26.2% as compared to the vehicle control group. Administrating melatonin alone did not induced significant changes in these biochemical parameters. The toxic effect of $AlCl_3$ was alleviated to a large extent by the pretreatment with melatonin as shown by restoring the values of these altered parameters toward the value of the control group (Table 3).

Table 2. Changes in the level of malondialdehyde (MDA), reduced glutathione (GSH) content, protein content and the activity of the antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in liver homogenate of rats treated with $AlCl_3$ or melatonin or both.

Experimental group	Parameter					
	MDA (nmol/mg protein)	GSH (nmol/ mg protein)	GPx (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	Protein (mg/g tissue)
Control	0.34±0.03	47.26±2.42	74.10±3.01	68.71±3.81	58.50±3.10	156.9±4.2
$AlCl_3$	3.11±0.24[a]	18.71±1.17[a]	31.45±1.84[a]	34.22±2.56[a]	25.40±2.85[a]	113.5±3.3[a]
Melatonin	0.40±0.04	51.15±3.21	79.51±4.30	74.10±3.30	63.75±3.40	160.0±6.2
Melatonin +$AlCl_3$	0.68±0.08[b]	38.32±2.83[b]	63.18±3.52[b]	58.81±3.92[b]	47.80±3.51[b]	143.7±5.0[b]

- Values are expressed as means ± SE, *n*=10 for each experimental group.
- [a] Significant difference from control group (*p<0.05*). - [b] Significant difference from $AlCl_3$ group (*p<0.05*).

Table 3. Changes in the concentration of total lipids, total cholesterol, triglycerides, glucose and total proteins in the plasma of rats treated with $AlCl_3$ or melatonin or both.

Experimental group	Parameter				
	Total Lipids (mg/dl)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	Total Proteins (mg/dl)
Control	365.2 ± 9.4	81.32±3.11	74.6±2.60	97.43±3.83	6.91± 0.05
$AlCl_3$	521.5±12.1[a]	180.25±7.3[a]	135.2±6.65[a]	179.97±7.27[a]	5.10±0.07[a]
Melatonin	352.1±10.2	74.60±4.49	77.5±3.85	102.43±4.30	7.00±0.09
Melatonin +$AlCl_3$	400.4±12.4[b]	102.31±5.39[a]	91.64±4.10[b]	110.77±1.97[b]	6.51±0.10[b]

- Values are expressed as means ± SE, *n*=10 for each experimental group.
- [a] Significant difference from control group (*p<0.05*). - [b] Significant difference from $AlCl_3$ group (*p<0.05*).

3.5. Histopathological examinations:

Microscopical examination of liver sections from the vehicle control group showed no evidence of histological abnormalities. The examination revealed regular hepatocytes architecture with distinct central vein, polygonal hepatocytes arranged in strands running radially from the central vein with blood sinusoids in between these hepatic strands (Figure 1). On the other hand, liver sections from rats administered $AlCl_3$ showed distorted liver architecture. Marked necrosis and degeneration of hepatocytes, centrilobular necrosis and congestion of the central vein (Figure 2), vacuolization of hepatocytes, dilatation and congestion of the blood sinusoids (Figure 3) in addition to infiltration of inflammatory cells (Figure 4) were observed. No histological changes were observed in the liver of rats treated with melatonin alone indicating non-toxic effect of melatonin (Figure 5). Administration of melatonin at a dose of 5 mg/kg prior to $AlCl_3$ improved to a large extent the hepatic damage induced by $AlCl_3$ as indicated by less degeneration and necrosis of hepatocytes, minimal vacuolization of hepatocytes, disappearance of congestion in the central vein and the blood sinusoids. The examined sections revealed more or less normal architecture of the liver (Figure 6).

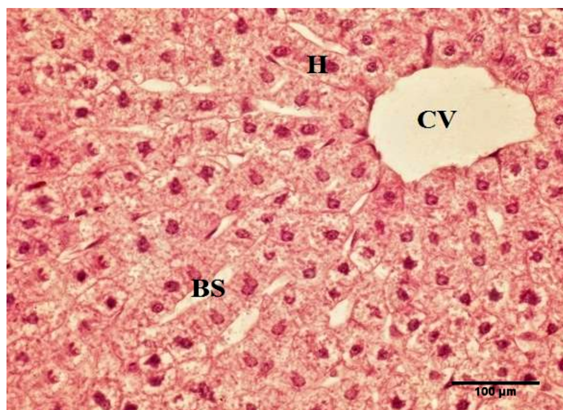

Figure 1: Liver section from vehicle control group showing normal histological structure: central vein (VC), hepatocyte (H), blood sinusoid (BS) (H&E x 400).

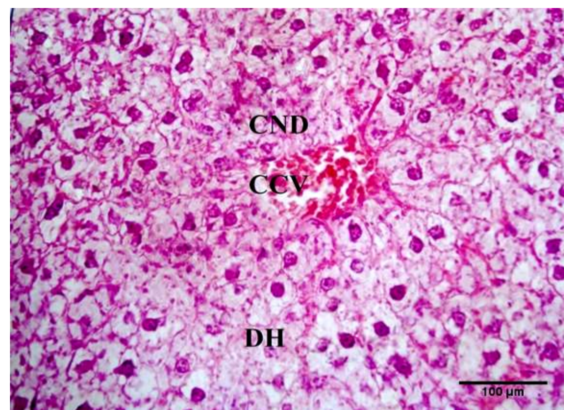

Figure 2: Liver section from $AlCl_3$-treated group showing congestion of central vein (CCV), centrilobular necrosis and degeneration (CND) and degeneration of hepatocytes (DH) (H&E x 400).

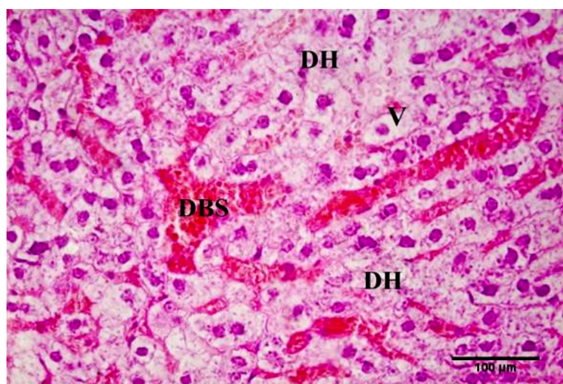

Figure 3: Liver section from $AlCl_3$-treated group showing dilatation and congestion of blood sinusoids (DBS), vacuolization of cytoplasm (V) and degeneration of hepatocytes (DH) (H&E x 400).

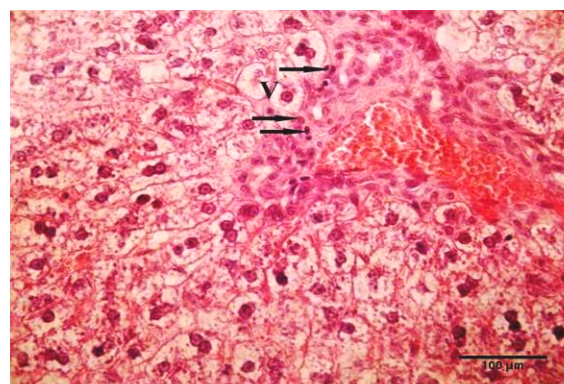

Figure 4: Liver section from $AlCl_3$-treated group showing infiltration of inflammatory cells (arrows) and vacuolization of cytoplasm (V) (H&E x 400).

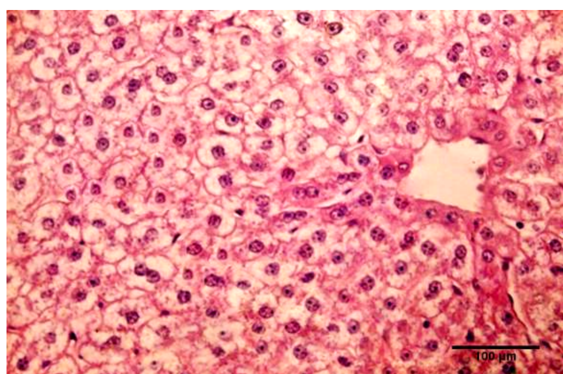

Figure 5: Liver section from melatonin group showing nearly normal liver structure (H&E x 400).

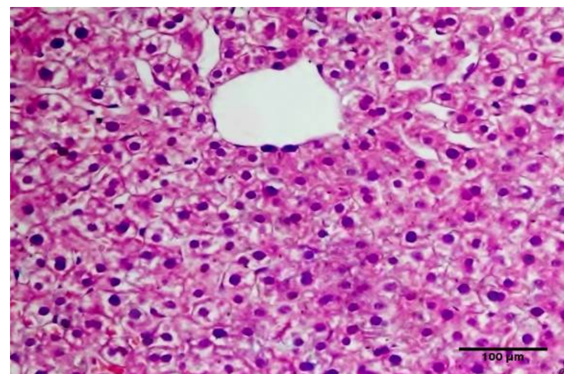

Figure 6: Liver section from $AlCl_3$+melatonin group showing less pathological changes and improved liver architecture (H&E x 400).

4. Discussion

Aluminum is broadly used in daily life throughout the world. The present study investigated the potential protective effects of melatonin against the possible toxic effects of $AlCl_3$ on the liver male rats through the evaluation of some biochemical parameters and enzymes in the plasma in addition to LPO and oxidative stress in this organ.

Exposure to high concentrations of Al can result in its accumulation in the liver and in turn to alterations in the liver function (Nikolov *et al*., 2010). Transaminases are intracellular enzymes, released into the circulation after damage and necrosis of hepatocytes (Sallie *et al*., 1991). The current study revealed a significant increase in the activity of ALT and AST in the plasma of $AlCl_3$-intoxicated rats which may be a sign of impaired liver function. The obtained data are in agreement with the earlier work of Chinoy and Memon (2001) and El-Demerdash (2004). They found that exposure to $AlCl_3$ caused necrosis to the liver with the subsequent release of AST from the injured hepatic cells to the plasma. Plasma ALT level increases when cellular degeneration occurs which in turn indicates the existence of liver diseases. ALP is a membrane-bound enzyme related to the transport of various metabolites so it is a sensitive biomarker for liver disease (Lakshmi *et al*.,1991). In the present investigation, $AlCl_3$ caused a significant elevation in the activity of ALP (Table 1). This observation is in agreement with the earlier findings of Ochmanski & Barabasz (2000) and El-Demerdash (2004). Furthermore, Esmaeili *et al*. (2009) reported a similar increase in the activity of ALP and they attributed it to severe damage to cell membranes or increased permeability of plasma membrane. Cell necrosis and subsequent release of membrane bound enzymes into the blood circulation following Al intoxication could also explains its elevated level (Bansal *et al.*, 2005).The impaired liver function observed in the present study is thought to be mediated by LPO which causes damage to cell membranes. LPO of cell membranes leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability (Nehru and Anand, 2005), all of which lead to leakage of the enzymes from the liver cells. Moreover, Al shows high affinity for phosphate groups and binds to the phospholipid head through electrostatic forces, which may induce conformational changes in the lipid bilayer of the plasma membrane (Martin, 1986). Another possible mechanism for the observed elevation in the enzymes may be liver dysfunction and disturbance in the biosynthesis of these enzymes which all are indicative of liver damage and thus impaired liver function. The observed increase in the concentration of total bilirubin in $AlCl_3$ group may be due to the fact that Al exposure can result in its accumulation in the liver which can be toxic to the hepatic tissue at high concentrations and may lead to the increase in bilirubin level (Gonzalez *et al*., 2007). Pretreatment with melatonin in $AlCl_3$ intoxicated rats resulted in improvement in ALT, AST, ALP and total bilirubin indicating improved liver function and protection against $AlCl_3$ toxicity. The hepatoprotective effect of melatonin can be attributed to its antioxidative effect. Melatonin may hamper $AlCl_3$-induced LPO and in turn protects cellular membrane integrity from oxidative damage and prevents the leakage of hepatic enzymes (Carla *et al.*, 2009).

Reactive oxygen species (ROS) have been implicated in the etiology of several diseases including atherosclerosis, inflammatory conditions, neurodegenerative diseases, cancer, diabetes mellitus, renal, pulmonary, cardiac diseases and the process of aging (Young and Woodside, 2001). It has been reported that the toxic effects associated with Al are related to the generation of ROS which results in oxidative damage to cellular lipids, proteins and DNA (El-Demerdash *et al*., 2004). LPO, as one of the main manifestations of oxidative damage, has been found to be a major contributor in the pathogenesis of many diseases and in the toxicity of many xenobiotics (Anane and Creppy, 2001). The data obtained in the present study revealed elevation in the level of hepatic MDA as a marker for LPO. Several investigations reported that Al has the ability to potentiate iron-mediated LPO (Oteiza, 1994; Ohyashiki *et al*.,1998). Disruption in mineral balance through replacing iron ions with Al and the subsequent increase in the amount of the free iron can explain the increased LPO. The free iron ions have a strong catalytic power to generate highly reactive hydroxyl radicals from hydrogen peroxide through Fenton's reaction (Ward *et al*., 2001). These radicals are able to initiate LPO and cellular damage.

The body has antioxidative mechanisms to stabilize oxidative molecules, control lipid oxidation and keep these radicals in balance. When free radicals are generated, the body defends itself from these radicals by endogenous antioxidants (Halliwell, 1994). However, when endogenous antioxidants become insufficient in defense against oxidants, exogenous antioxidants are needed to restore the balance. Endogenous antioxidants, either non-enzymatic (as GSH) or enzymatic (as GPx, SOD and CAT) represents the first line of defense against free radical damage and are crucial for preventing or at least slowing the incidence and progression of diseases (Jacob, 1995). The findings of the present study showed that the administration of $AlCl_3$ at a dose of 20 mg $AlCl_3$/kg induced a status of oxidant/antioxidant imbalance as indicated by the increased MDA level with a concomitant depletion in GSH content and in the activity of GPx, SOD and CAT in the liver tissue. These findings are consistent with previous studies that reported Al intake to be related to alteration in the activity of tissue antioxidant enzymes and promotion of oxidative stress (Yousef, 2004; Nehru & Anand, 2005;

Newairy *et al*., 2009). $AlCl_3$ induced free radicals and it may inhibit the antioxidant defense system. Orihuela *et al*. (2005) reported that high doses of Al are able to induce free radicals and resulted in reduced GSH synthesis by decreasing glutathione-synthase activity. The reduction in the activity of GPx, SOD and CAT observed in the present study may be attributed to the reduced synthesis of these enzymes due to higher intracellular concentrations of Al and/or accumulation of free radicals. The inhibition of the activities of these enzymes may also be referred to the effect of Al in declining the expression of mRNA of endogenous antioxidants (Gonzalez *et al*., 2007). The data obtained in the present study illustrated that melatonin restored the oxidant/antioxidant balance as reflected by the decrease in MDA level and the stimulation of the antioxidants GSH, GPx, SOD and CAT in the liver. Melatonin and its metabolites are powerful antioxidants that can directly detoxify free radicals species *via* electron donation (Allegra *et al*., 2003; Reiter *et al*., 2007). It can also defend indirectly against oxidative damage by repair of the antioxidant system through enhancing the activities of a variety of antioxidative enzymes like GPx, SOD, and less commonly CAT (Rodriguez *et al*., 2004).

Findings of the present study showed that oral administration of $AlCl_3$ induced a significant increase in plasma level of total lipids, total cholesterol and triglycerides (Table 3). Accumulation of $AlCl_3$ in the liver may lead to a disturbance of lipid metabolism and in turn to the reported elevation in lipid profile. As mentioned above, administration of $AlCl_3$ resulted in increased LPO and loss of membrane integrity which might be important determinants of altered lipid metabolism and are closely associated with hyperlipidemia and/or hypercholesterolemia in many animals and human studies (Sarin *et al*., 1997; Yousef, 2004). Al has been found to significantly affect various membrane-bound enzymes which confirm the assumption of loss of membrane integrity (Newairy *et al*., 2009). Pretreatment with melatonin improved the altered level of total lipids, total cholesterol and triglycerides in the plasma of rats intoxicated with $AlCl_3$. This anti-hyperlipidimic effect of melatonin may be primarily attributed to its antioxidant activity and the protection of cellular membrane integrity from Al-induced oxidative damage (Carla *et al.*, 2009). Another possible mechanisms for the effect of melatonin on lipid profile may be its action on the gastrointestinal tract and the inhibition of cholesterol and triglycerides uptake, the augmentation of endogenous cholesterol clearance mechanisms through increasing the activity of cholesterol-degrading enzymes and/or its effect on thyroid hormones which in turn affect lipid metabolism (Chan and Tang, 1995; Wakatsuki *et al*., 2001).

In view of the current data, the administration of $AlCl_3$ at a dose of 20 mg/kg resulted in an increase in the blood glucose level which may indicate disruption in carbohydrate metabolism. The observed increase in the blood sugar level in the present study is in agreement with the results of El-Demerdash (2004), Shati and Alamri (2010). This $AlCl_3$-induced hyperglycemia may be attributed to enhanced breakdown of liver glycogen and its subsequent increased glucose production (Yousef, 2004 and Newairy *et al*., 2009). Moreover, it has been proposed that oxidative stress is a major pathogenic link to both insulin resistance and the dysfunction of the pancreatic β-cells through the formation of amyloid proteins, which not only prevents the release of insulin into the circulation, but also destroys the insulin secreting β-cells (Hayden, 2002). Pretreatment with melatonin in the intoxicated rats improved the hyperglycemic effect of $AlCl_3$. Melatonin has been found to influence glucose homeostasis (Reis *et al*.,1996). The effect of melatonin on glucose may be explained by modification of insulin secretion and/or change in cell sensitivity to insulin. Melatonin acts on pancreatic islets and induces regeneration and/or proliferation of β-cells in pancreas which leads to a decrement in blood glucose in diabetic rats (Kanter *et al*., 2006).

The data of the present work showed that Al intoxication caused a significant decrease in the plasma protein content. This result concord with the previous results of Chinoy and Memon (2001) and Newairy *et al*. (2009). The observed decrease in protein content might be attributed to higher intracellular concentration of Al in the liver which could result in reduced protein synthesis as well as reduced enzymes of protein synthesis (Tripathi *et al*., 2009). Free radicals may also be implicated in the observed decline in protein content since exposure to the free radicals leads to protein fragmentation, protein peroxides generation, enzymatic oxidation and degradation of proteins (Albendea *et al*., 2007). Treatment with melatonin before $AlCl_3$ significantly ameliorated the decline in the plasma protein content probably by scavenging the free radicals and improving the antioxidative status and in turn the process of protein synthesis.

5. Conclusion

In conclusion, the present study indicated that oral administration of $AlCl_3$ at a dose of 20 mg/kg daily for 30 days caused hepatic dysfunction, increase in lipid peroxidation and decline in the activity of antioxidant enzymes in the liver. It also

induced histological changes in the liver which all are attributed to free radicals production and oxidative stress. Administration of melatonin at a dose of 5 mg/kg 30 minute prior to $AlCl_3$ intoxication minimized its harmful effects and protected the liver against its toxicity which may be due to the role of melatonin as an antioxidant. Therefore, supplementation with melatonin may be useful as a hepatoprotective therapy in cases of intoxication with aluminium.

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References

1. Aebi H. Catalase in *vitro*. Methods Enzymol. 1984; 105:121-126.
2. Albendea CD, Trullen EM, Broto LE, Miana-Mena FG, Plano SM, Gonzales MC, Balların EM, Garcia JJ. Melatonin reduces lipid and protein oxidative damage in synaptosomes due to aluminum. J. Trace Elem. Med. Biol. 2007; 21:261–268.
3. Alfrey AC, LeGendre GR, Kaehny WD. The dialysis encephalopathy syndrome: Possible aluminum intoxication. New Engl. J. Med. 1976; 294: 184-188.
4. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin interaction with reactive species. J. Pineal Res.2003; 34:1-10.
5. Anane R, Creppy EE. Lipid peroxidation as pathway of aluminum cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase+catalase and vitamins E and C. Hum. Exp. Toxicol. 2001; 20:477-481.
6. ATSDR (Agency for Toxic Substances and Disease Registry). Notice of the revised priority list of hazardous substances that will be the subject of toxicological profiles. Fed. Regist. 2007; 73:12178-12179.
7. Bansal AK, Bansal M, Soni G. Protective role of Vitamin E pretreatment on N-nitrosodiethylamine-induced oxidative stress in rat liver. Chemico-Biological Interactions 2005; 156:101-111.
8. Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD, Poeggeler B. Melatonin stimulates brain glutathione peroxidase activity. Neurochem. Int. 1995; 26:497-502.
9. Banchraft JD, Stevans A and Turnes DR. Theory and Practice of Histological Techniques. 4th ed. 1996, livingstone, Edinburgh, London, Melbourne, New York, Tokyo.
10. Belfield A, Goldberg DM. Revised assay for serum phenyl phosphatase activity using 4-aminoantipyrine. Enzyme 1971; 12:561-73.
11. Bondy SC, Guo-Ross SX, Truong AT. Promotion of transition metal-induced reactive oxygen species formation by beta-amyloid. Brain Res. 1998; 799(1):91-96.
12. Campbell A. The potential role of aluminum in Alzheimer's disease. Nephrol. Dial. Transplant. 2002; 17:17-20.
13. Carla M, Alice S, Ramos A, Azevedo MF, Lima CF, Fernandes-Ferreira M, Cristina Pereira-Wilson. Sage tea drinking improves lipid profile and antioxidant defenses in humans. Int. J. Mol. Sci. 2009; 10: 3937-3950.
14. Chan TY, Tang PL. Effect of melatonin on the maintenance of cholesterol homeostasis in the rat. Endocr. Res. 1995; 21(3):681-96.
15. Chinoy NJ, Memon MR. Beneficial effects of some vitamins and Calcium on fluoride and aluminum toxicity on gastrocnemius muscle and liver of male mice. Fluoride 2001; 34:21-33.
16. Csernus V, Mess B. Biorhythms and pineal gland. Neuro. Endocrinol. Lett. 2003; 24:404-411.
17. Dolberg OT, Hirschmann S, Grunhaus L. Melatonin for the treatment of sleep disturbances in major depressive disorder. Am. J. Psychiat. 1998; 155:1119-1121.
18. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminum. J. Trace Elements in Med. Biol. 2004; 18:113-122.
19. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hematobiochemical parameters and semen quality of male rats. J. Environ. Sci. Health Bull. 2004; 39:443-459.
20. Ellman GL. Tissue sulfhydryl groups. Arch Biochem. Biophys. 1959; 82:70-77.
21. Ergün Y, Orhan FO, Karaaslan MF. Combination therapy of imipramine and melatonin: additive antidepressant effect in mouse forced swimming test. Eur. J. Pharmacol. 2008; 591(1-3):159-6.
22. Esmaeili MA, Sonboli A, Kanani MR, Sadeghi H. *Salvia sahendica* prevents tissue damages induced by alcohol in oxidative stress conditions: Effect on liver and kidney oxidative parameters. J. Med. Plants Res. 2009; 3:276-283.
23. Fatima SK, Prabhavathi PA, Padmavathi P, Reddy PP. Analysis of chromosomal aberrations in men occupationally exposed to cement dust. Mutat. Res. 2001; 490:179-186.

24. Fossati P, Principle L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide, Clin. Chem. 1982; 28:2077-2080.
25. Gonzalez MA, Alvarez ML, Pisani GB. Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminum to rats. Biol. Trace Elem. Res. 2007; 116:329-48.
26. Halliwell B. Free Radicals, Antioxidants and Human Disease: Curiosity, Cause or Consequence. Lancet 1994; 344:721-724.
27. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 2005; 27(2):119-130.
28. Hardeland R, Fuhrberg B. Ubiquitous melatonin-presence and effects in unicells, plants and animals. Trends Comp. Biochem. 1996; 2:25-45.
29. Hayden MR. Islet myeloid, metabolic syndrome and the natural progressive history of type 2 diabetes mellitus. J. Pancreas 2002; 3:126-138.
30. Jacob RA. The integrated antioxidant system. Nutr. Res. 1995; 15(5):755-766.
31. Krasovskii GN, Vasukovich LY, Chariev OG. Experimental study of biological effects of leads and aluminum following oral administration. Environ. Health Perspect. 1979; 30:47–51.
32. Lagneux C, Joyeux M, Demenge P. Protective effects of melatonin against ischemia reperfusion injury in the isolated rat heart. Life Sci. 2000; 66:503-509.
33. Lakshmi R, Kundu R, Thomas E, Mansuri AP. Mercuric chloride induced inhibation of acid and alkaline phosphatase activity in the kidney of Mudskipper; *Boleophthalmus dentatus*. Acta Hydrochim. Hydrobiol. 1991; 3:341-344.
34. Liu F, Ng TB. Effect of pineal indoles on activities of the antioxidant defense enzymes superoxide dismutase, catalase, and glutathione reductase, and levels of reduced and oxidized glutathione in rat tissues. Biochem. Cell Biol. 2000; 78:447-453.
35. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193:265-275.
36. Mahieu S, Maria-Contini M, Gonzalez M, Millen N. Melatonin reduces oxidative damage induced by aluminum in rat kidney. Toxicology Letters 2009; 190:9-15.
37. Martin, RB. The chemistry of aluminum as related to biology and medicine. Clin. Chem. 1986; 32:1797–806.
38. Nehru B, Anand P. Oxidative damage following chronic Aluminum exposure in adult and pup rat brains. J. Trace Elem. Med. Biol. 2005; 19:203-208.
39. Newairy AS, Salama AF, Hussien HM, Yousef MI. Propolis alleviates aluminum-induced lipid peroxidation and biochemical parameters in male rats. Food Chem. Toxicol. 2009; 47:1093-1098.
40. Nikolov IG, Joki N, Vicca S, Patey N, Auchere D, Benchitrit J. Tissue accumulation of lanthanum as compared to aluminum in rats with chronic renal failure: Possible harmful effects after long-term exposure. Nephron Exp. Nephrol. 2010; 115:e112-e121.
41. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 1972; 46:849-854.
42. Ochmanski W, Barabasz W. Aluminum-occurrence and toxicity for organisms. Przegl. Lek. 2000; 57: 665–8.
43. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979; 95:351-358.
44. Ohyashiki T, Suzuki S, Satoh E, Uemori Y. A marked stimulation of Fe^{2+}-initiated lipid peroxidation in phospholipid liposomes by a lipophilic aluminum complex, aluminum acetylacetonate. Biochim. Biophys. Acta. 1998; 1389(2):141-149.
45. Orihuela D, Meichtry V, Pregi N, Pizarro M. Short-term oral exposure to Aluminum decreases glutathione intestinal levels and changes enzyme activities involved in its metabolism. J. Inorg. Biochem. 2005; 99:1871-1878.
46. Oteiza PI. A mechanism for the stimulatory effect of aluminum on iron-induced lipid peroxidation. Arch. Biochem. Biophys. 1994; 308 (2):374-379.
47. Paglia DE, Valentine WN. Determination of Glutathione peroxidase. J. Lab. Clin. Med. 1967; 70:158-169.
48. Kanter M, Uysal H, Karaca T, Sagmanligil HO: Depression of glucose levels and partial restoration of pancreatic beta-cell damage by melatonin in streptozotocin-induced diabetic rats. Arch. Toxicol. 2006;80:362-369.
49. Reinke CM, Breitkreutz J, Leuenberger H. Aluminum in over the-counter drugs: risks outweigh benefits. Drug Saf. 2003; 26:1011-25.
50. Reis LC, Sousa JC, Borges LS, Spadaro F, Santos BM, Collus LO. Metabolic changes and plasmatic biochemistry after pinealectomy in rats. Rev. Méd. Minas. Gerais. 1996; 6(3):101-103.
51. Reiter RJ, Tan DX. Melatonin: an antioxidant in edible plants. Ann. N.Y. Acad. Sci. 2002; 957:341-344.

52. Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnocki Z. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. Acta Biochimica Polinica 2007; 54(1):1-9.
53. Richmond W. Cholesterol enzymatic colorimetric test chop-PAP method of estimation of total cholesterol in serum. Clin. Chem. 1973; 191:1350-1356.
54. Retiman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 1957; 28:56-63.
55. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal Res. 2004; 36:1-9.
56. Sainz RM, Mayo JC, Reiter RJ. Melatonin regulates glucocorticoid receptor: an answer to its antiapoptotic action in thymus. FASEB J 1999; 13:1547-1556.
57. Sallie R, Tredger JM, Williams R. Drugs and the liver. Part I: Testing liver function. Biopharm. Drug Dispos. 1991; 12:251-259.
58. Sarin S, Gupta V, Gill KD. Alterations in lipid composition and neuronal injury in primates following chronic aluminum exposure. Biol. Trace Elem. Res. 1997; 59:133–43.
59. Shati AA, Alamri, SA. Role of saffron (*Crocus sativus L.*) and honey syrup on aluminum-induced hepatotoxicity. Saudi. Med. J. 2010; 31(10):1106-1113.
60. Short AI, Winney RJ, Robson JS. Reversible microcytic hypochromic anemia in dialysis patients due to aluminum intoxication. Proc. Eur. Dial. Transplant. Assoc. 1980; 17:226–233.
61. Soni MG, White SM, Flamm WG, Burdock GA. Safety evaluation of dietary Aluminum. Regulatory Toxicology and Pharmacology 2001; 33(1):66–79.
62. Storr M, Koppitz P, Sibaev A, Saur D, Kurjak M, Franck H, Schusdziarra V, llescher HD. Melatonin reduces non-adrenergic, non-cholinergic relaxant neurotransmission by inhibition of nitric oxide synthase activity in the gastrointestinal tract of rodents in *vitro*. J. Pineal Res. 2002; 33:101-108.
63. Tan DX, Manchester LC, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species. Journal of Pineal Research; 2007; 42(1):28-42.
64. Trinder P. Determination of blood glucose using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 1969; 6:24-27.
65. Tripathi S, Mahdia AA, Nawaba A, Chandera R, Hasanb M, Siddiquib MS, Mahdic F, Mitrad K, Bajpaid VK. Influence of age on aluminum-induced lipid peroxidation and neurolipofuscin in frontal cortex of rat brain: A behavioral, biochemical and ultrastructural study. Brain Res. 2009; 1253:107–116.
66. Vakkuri O, Leppaluto J, Kaupplia A. Oral administration and distribution of melatonin in human saliva and urine. Life Sciences 1985; 37(5): 489- 495.
67. Wakatsuki A, Okatani IY, Kaneda C, Fukaya T. Effect of short-term melatonin administration on lipoprotein metabolism in normolipidemic postmenopausal women. Maturitas 2001; 38:171-177.
68. Walter M, Gerade H. Bilirubin assay. Microchem. J. 1970; 15:231-236.
69. Ward MK, Feest TG, Ellis HA, Parkinson IS, Kerr DN. Osteomalacic dialysis osteodystrophy: Evidence for a water-borne aetiological agent, probably aluminum. Lancet 1978; 22:841-845.
70. Ward RJ, Zhang Y, Crichton RR. Aluminum toxicity and iron homeostasis. J. Inorg. Biochem. 2001; 87:9-14.
71. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol. 2001; 54: 176–186.
72. Yousef MI. Aluminum-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. Toxicology 2004; 199:47-57.
73. Zhou Y, Yokel RA. The chemical species of aluminum influence its paracellular flux across and uptake into Caco-2 cells, a model of gastrointestinal absorption. Toxicol. Sci. 2005; 87(1):15-26.
74. Zollner N, Kirsch K. Serum total lipids determination colorimetrically. Z. Ges. Exp. Meal.1962;1335:54.

9/15/2012