

The Role Of Melatonin In Aluminium Induced Genotoxicity In Female Albino Rats

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Abstract

Aim of the work, In spite of the wide range of use of aluminium. With the increasing awareness of possible health hazards associated with their use, more attention have been focused on their biological and biochemical activity. Aluminium salts are used as the active antiperspirant in under cosmetics, but the effects of widespread, long term and increasing use remain unknown. Aluminium is known to have genotoxic profile, capable of causing both DNA alterations and epigenetic effects. Melatonin reduces the free radical generation and metal detoxification. This study was planned to determine the effects of melatonin on aluminium induced toxicity in the chromosomes of bone marrow cell.

Material and Methods ,Forty female albino rats were used in the present work for the chromosomal analysis. Four groups of adult female albino rats were used in the following way: Group -I, control group; Group-II, 50 mg /kg b.wt aluminium orally; Group-III, 50µg / kg b.wt melatonin orally and Group- IV, 50 mg /kg b.wt aluminium orally (am) +50 µg / kg b.wt melatonin orally(pm). Doses were given 30 days. All animals were sacrificed and the bone marrow of femur bones was taken for subsequent cytogenetic studies.

The results, Aluminium treatment induced a significant increase in the structural as well as numerical chromosomal aberrations. The frequency of chromosomal aberrations showed significant decrease when rats treated with melatonin and aluminium. **In conclusion,** the results indicated that melatonin showed a significant improvement of genetic damages induced by aluminium.

Introduction

Numerous environmental and industrial are capable of causing cytogenetic damage in experimental animal and human [Heddle *et al.*, 1983]. Aluminium (Al) chloride (10 – 200 µ M) increased the aluminium content in hepatocytes isolated from fed male rats in a time -and concentration - dependent manner. After 60 min of incubation with 100 µ M aluminium about 45 % of cellular aluminium was found each in the mitochondrial and the post mitochondrial fraction of hepatocytes, whereas about 5% of aluminium sedimented with nuclei and cell debris. [Muller and Wilhelm 1987]. Aluminium linked inhibition of several enzymes, such as ATP ase, hexokinase, phosphofructokinase, and lactate dehydrogenase (LDH) in brain fractions [Riedel and Christensen 1979; Bock and Ash 1980; Lai and Blass, 1984 and Ganrot, 1986].

Aluminium is known to react with

targets within the cell similar to those of known toxic metals, such as cadmium [Nieboer and Richardson 1980 and Muller and Wilhelm 1987].

Melatonin is the principal secretory product of the pineal gland. The molecule endocrine actions on other glands, but it also has been shown to function as a free radical scavenger, antioxidant and in immunomodulation [Conti and Maestroni 1995; Reiter *et al.*, 1995 and Vijayalaxmi *et al.*, 2004]. Melatonin is highly lipid soluble and enters cells and subcellular compartments with ease [Reiter 1991]; melatonin has not been shown to be toxic. Melatonin and its precursors, tryptophan and serotonin, were examined for their metal binding affinities for both essential and toxic metals: aluminium, cadmium, copper, iron, lead and zinc [Limson *et al.*, 1998].

Melatonin, together with other free radical scavenger in the brain, reduces the

free radical damage caused by aluminium. Since melatonin is produced by the pineal gland only in the dark, the excess of electric light in developed countries may help explain why Alzheimer's disease (AD) is more prevalent in these countries than in rural Africa [Van – Rensburg *et al.*, 1997; Limson *et al.*, 1998; Lack *et al.*, 2001 and Maharaj *et al.* 2005]

Melatonin is considered a biological-response modifier of the immune system with broad application in veterinary medicine. Melatonin enhances a defined immune response in vivo opens new perspectives for the improvement of Th2-biased immune response by alum adjuvants [Regodon *et al.*, 2005].

The administration of melatonin caused a decrease in the frequency of chromosome aberration in irradiated animal [Badr *et al.*, 1999]. Also melatonin decrease the frequency of chromosome aberration and DNA concentration in the diabetic animal [Hanafy 2005]. Therefore, the present study was designed to determine the effects of melatonin on aluminium-induced toxicity on the chromosomes in female albino rats.

Material and Methods

Material:

Aluminium hydroxide (ALOH) was supplied by Sigma Chemical Co., USA; while melatonin was obtained from Amoun. Ltd. Co. Egypt.

Animals:

Forty female albino rats approximately 160 - 200 gm of weight were used for this study.

The rats were divided into four groups:

Group -I: control group, administered distilled water by stomach tube.

Group -II: Aluminium hydroxide (ALOH) was dissolved in distilled water and given in dose of 50 mg /kg b.wt. orally by stomach tube.

Group -III: melatonin was dissolved in distilled water (1:500) and given in dose of 50 µg /kg b.wt. orally by stomach tube.

Group - IV: female rats were given 50 mg /kg b.wt aluminium (am) and 50 µg

/kg b.wt of melatonin (pm).

Doses were given 30 days orally by stomach. All animals were sacrificed and the bone marrow of femur bones was taken for subsequent cytogenetic studies

Assays to detect chromosomal aberrations :

The bone marrow tissue was subjected to colchicine treatment (0.5 solution 0.1ml/culture), hypotonic treatment (KCl, 5.6 g /l), fixed in acetomethanol, spread, and stained by Giemsa stain, in 6.8 phosphate buffer [Evans,1987].

To analyze the frequency and type of chromosomal aberrations, 100 well spread metaphases were analyzed under the microscope for each rat in either the therapeutic or double therapeutic dose-exposed animals. The chromosomal aberrations observed were classified as follows: Structural chromosomal aberrations which include gap, break, deletion and centromeric attenuation and numerical chromosomal aberrations which include endomitosis and polyploidy.

Statistical Analysis:

Analysis was performed using the Statistical Package for Social Sciences (SPSS, Version 10).

Medians were compared using the Kruskal wallis H-test (non-parametric ANOVA) followed by Q-test to show the difference between individual groups. P-Value less than 0.05 was considered significant [Kruskal and Wallis,1952].

Results:

Structural chromosomal aberrations

In bone marrow cells of rats of melatonin group (group III), no significant difference was observed as regards structural chromosomal aberrations when compared with the control group (Table 1).

However, the mean percentage of both the individual and total structural chromosomal aberrations showed significant increases in aluminium group (20%) when compared with the control groups (1%) (Table 1).

On the other hand, rats treated simultaneously with aluminium + melatonin (group IV) showed highly significant

decrease of the total structural chromosomal aberrations (11%) in comparison is with aluminium- treated rats (20%), however, the mean percentage of the total structural chromosomal aberrations was significantly in group IV than the corresponding values of the control groups (Table 1, Fig. 1).

Numerical chromosomal aberrations

In the melatonin group, no significant differences were observed when compared with the control groups as regards the numerical chromosomal aberrations (Table 1).

Meanwhile, the frequency of the total numerical chromosomal aberrations was a significant increased in aluminium - treated rats (7%) when compared with the control groups (1%)(Table 1).

Rats treated with aluminium + melatonin (group IV) showed a significant decrease of total numerical chromosomal aberrations (5% via 7% in aluminium – treated only) . However, the mean percentage of the total numerical chromosomal aberrations was significantly higher in group IV than the corresponding values of the control groups (Table 1, Fig. 2) .

Table (1): Chromosomal aberrations in the different studied experimental groups.

Experimental groups	Chromosomal aberrations							
	Gap	Break	Deletion	Centromere attenuation	total	ploidy	endomytosis	total
Control	0 a (0-1)	0 a (0-0)	0 a (0-1)	1 a (0-1)	1 a (0-2)	1 a (0-2)	1 a (0-1)	1 a (1-3)
Aluminium	5 b (4-5)	7 c (6-7)	4 c (3-5)	4 c (3-5)	20 d (19-21)	4 c (3-5)	3 c (2-4)	7 c (5-8)
Melatonin	1 a (0-2)	2 b (2-3)	2 b (1-3)	0 a (0-0)	5 b (4-6)	1 a (0-1)	1 ab (0-3)	1 a (1-3)
Aluminium + Melatonin	3 b (3-4)	3 b (3-4)	2 b (2-4)	2 b (1-3)	11 c (10-12)	2 b (1-3)	2 b (2-3)	5 b (3-6)
Chi-square	17.14	17.82	15.48	16.88	18.02	13.81	12.18	15.857
p-value	0.0006	0.0004	0.001	0.0007	0.0004	0.003	0.006	0.001

Data are expressed as median and range of five readings (out of 100 metaphase spreads) in each experiment. The different letters indicate statistically significant median values according to Kruskal Waalis H-test (non-parametric ANOVA)

a : non significant b : significant c : significant d : highly significant

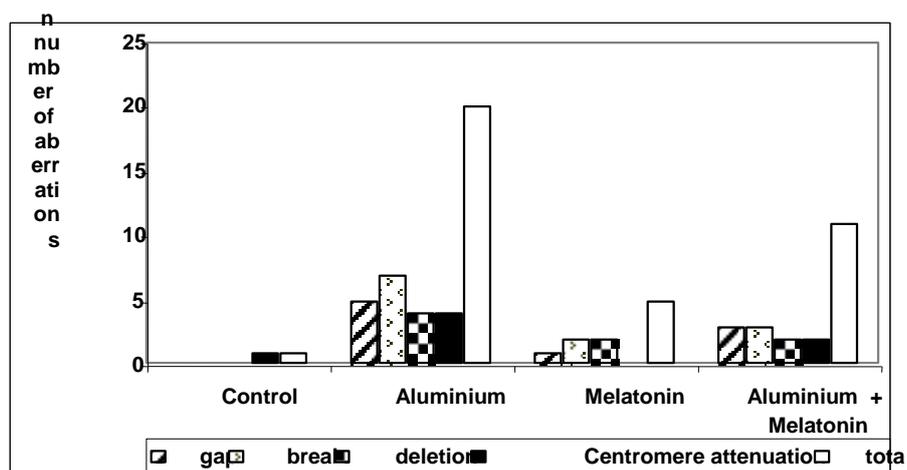


Fig. (1): Median number of chromosomal aberrations (gap, break, deletion, centromere attenuation and total) in the metaphase spreads taken from female albino rats of the different experimental groups

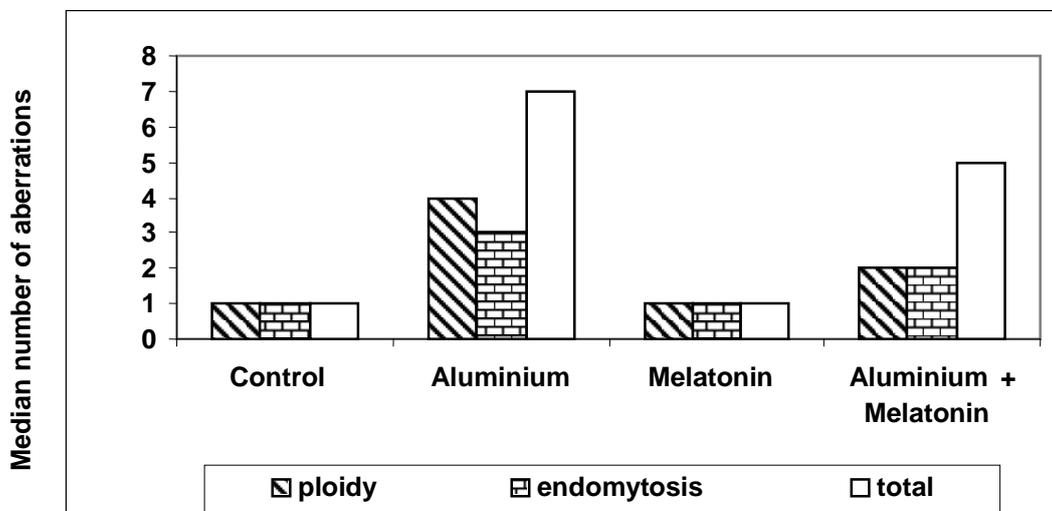


Fig. (2): Median number of chromosomal aberrations (ploidy, endomytosis and total) in the metaphase spreads taken from female albino rats of the different experimental groups

Discussion

The results of the present study clearly showed that aluminium is a genotoxic agent. This was evidenced by the increased incidence of chromosomal aberration particularly gaps and break in aluminium treated rats.

The importance of recording gaps in assessing the genotoxic effects of compound is controversial. Some authors considered gaps to be a sensitive indicator of chemically induced chromosomal

damage, while others considered the scoring of gaps to be highly subjective and therefore an unsuitable indicator of genotoxicity [Hassan,1997]. However, since some gaps could be deletions, it is appropriate to analyze the data including and excluding gaps [Preston *et al.*, 1987]. In the present study ,aluminium gave a highly significant increase in the number of aberrant cells, even after excluding gaps.

Aluminium induced genotoxicity is

usually considered to involve free radicals, while melatonin reduced this effect. [Menendez – Pelaez and Reiter 1993 and Van –Rensburg *et al.*, 1997].

Aluminium caused lipid peroxidation in the presence of iron(II) ion(Fe^{2+}). Hydrogen peroxide (H_2O_2) greatly exacerbated the toxicity of aluminium. H_2O_2 induced chromosomal aberration. Melatonin prevented lipid peroxidation by aluminium in the absence of H_2O_2 , but only reduced the process when H_2O_2 was present. [Van – Rensburg *et al.* , 1997 and De-Selvia *et al.*, 1999]

Millan-Plano *et al.*, (2003) suggest that the indoleamine melatonin may potentially act as neuroprotectant agents in the therapy of those diseases with elevated aluminium concentrations in the tissues.

This study revealed that in bone marrow cell, no significant chromosomal aberration was observed in animals treated with melatonin only. While melatonin produced a significant decrease of the total structural chromosomal aberration at a percentage of 11% via 20% in animals treated with aluminium only and the numerical chromosomal aberration showed a decrease at a percentage of 5% via 7% in animals treated with aluminium only. The present results were in agreement with Sewerynek *et al.*, 1996 and Vijayalaxmi *et al.* (1998) showed that melatonin ingestion exhibited a significantly decreased extent of primary DNA damage and reduced frequencies of chromosomal aberration and micronuclei.

Also Badr *et al.* (1999) found that no mutagenic effect of melatonin on chromosomal aberration frequency in spermatogonial chromosomes. Administration of one single dose of melatonin to animals before irradiation lowered total chromosomal aberration from 46% to 32% .

Pretreatment of buffalo heifers with melatonin reduced the incidence of chromosomal abnormalities and sister chromatid exchange (SCE) during superovulation (Kandil *et al.* , 2001) .

In bone marrow cell, melatonin caused a decrease in the frequency of chromosomal aberration at a percentage of 7% via 17% in diabetic animals. whereas, giving melatonin before diabetic treatment was more effective than after diabetic

occurrence [Hanafy 2005].

Melatonin has been shown to protect DNA from damage inflicted by a physical agent, i.e., ionizing radiation [Cagnoli *et al.*, 1995] and the bacterial toxin, lipopolysaccharide [Vijayalaxmi *et al.*, 1995], both of which are believed to damage the genetic material by free radical mechanisms. Melatonin has been shown to scavenge not only The-oh [Hayashi *et al.* , 1992], but also possibly the peroxy radical [Tan *et al.* , 1993 and Pieri *et al.*, 1994] and single oxygen [Scaiano 1995]. Additionally, melatonin seems to increase the concentration of the endogenous antioxidant glutathione and stimulate the antioxidative enzymes superoxide dismutase and glutathione peroxidase [Melchiorri *et al.*, 1995; Sewerynek *et al.*, 1996 and Antolin *et al.*, 1996].

Lower dose chronic treatment of melatonin suggests that the regulation of antioxidant enzymes gene expression is likely to be receptor mediated [Bhatia and Manada, 2003 and Manada and Bhatia 2004]. In conclusion, melatonin produced a significant decrease of the total structural and numerical chromosomal aberration induced by aluminium.

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دراسة تأثير الميلاتونين على التشوهات الكروموسومية الناتجة من الآثار الضارة للألمونيوم في إناث الجرذ الأبيض

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يعتبر الألمونيوم احد المركبات الشائعة الاستخدام في كثير من الآنية المستعملة في حفظ الأطعمة . ويدخل في تركيب بعض الأدوية و العديد من الأطعمة والمشروبات وخاصة البان الأطفال المجففة. وتستخدم أملاح الألمونيوم في العديد من مستحضرات التجميل و خاصة مضاد العرق . وأثبتت عدة أبحاث مدى الآثار الضارة للألمونيوم .

وقد تم تصميم هذا البحث لمعرفة التأثير الواقي للميلاتونين لتقادي هذه الآثار الضارة للألمونيوم. أجرى هذا البحث على أربعين من الجرذان البيضاء البالغة وقسمت إلى أربع مجموعات هي:-

1- المجموعة الأولى : مجموعة ضابطة

2- المجموعة الثانية : مجموعة تم معالجتها بالألمونيوم (بجرعة 50 مجم/كجم).

3- المجموعة الثالثة : مجموعة تم معالجتها بالميلاتونين (بجرعة 50 ميكروجم/كجم).

4- المجموعة الرابعة: مجموعة تم معالجتها بالميلاتونين والألمونيوم معا.

وبعد شهر من المعالجة تم تشريح الجرذان و تحضير الكروموسومات من خلايا نخاع العظمي.

وتتلخص نتائج هذا البحث فيما يلي:-

1- أظهرت هذه الدراسة تغيرات تركيبية و عددية في كروموسومات خلايا نخاع العظمي نتيجة تأثير الألمونيوم وتصل نسبة الشذوذ الكروموسومي التركيبي إلى 20% و الشذوذ الكروموسومي العددي تصل النسبة إلى 11%.

2-المعالجة بالميلاتونين أدى إلى انخفاض نسبة الشنوذ الكروموسومي التركيبي الناتجة من الألمونيوم وتصل إلى 7%. ويقل الشنوذ الكروموسومي العددي وتصل النسبة إلى 5%. وهذه الدراسة توضح مدى فائدة الميلاتونين في الوقاية من الآثار الضارة للألمونيوم على الكروموسومات .