Protective effects of Selenium and/or Melatonin against Cadmium Chloride induced Chromosomal aberrations in Swiss Albino mice Mus musculus BALB/c

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The present study was aimed to investigate the protective role of selenium and melatonin with concentration (0.2 mg/kg b.wt./day) for selenium and (2.5 mg/kg b. wt./day) for melatonin. The doses were administered for five weeks five day per week against the genotoxic effect of orally given Cadmium chloride (1mg/kg b.wt./day) to male albino mice for five weeks (five day per week). Thirty five male albino mice were divided into seven groups; each group contained five mice. Chromosomal studies in bone marrow cells were used as parameters in this evaluation. It was observed that the administration of Cadmium chloride generally produced more chromosomal aberrations such as (gap, break, ring, dicentric, centric fusion, end to end association, aneuploidy and polyplody) compared with control. Chromosomal aberrations were reduced in treatment with the antioxidants (Selenium, melatonin) and their combinations. Finally, it was concluded that selenium and melatonin can serve as possible anti-genotoxic agent against the clastogenic effect of Cadmium chloride.

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1. INTRODUCTION
A heavy metal is a member of an ill-defined subset of elements that exhibit metallic properties, which would mainly include the transition metals, some metallioids, lanthanides, and actinides. Heavy metals have a high atomic weight and a density much greater (at least 5 times) than water. There are more than 20 heavy metals, but lead (Pb), cadmium (Cd), and inorganic arsenic (As) are of special concern (Flora, 2009). Human beings are exposed to various types of environmental contaminants at different stages of their life span; the majority of these are harmful. Cadmium (Cd) is considered to be one of the most toxic (Verma et al., 2009), non essential heavy metal that is dispersed throughout the environment (Thévenod, 2003). Cadmium has biological half life of about 10 years in human bodies. So symptoms of cadmium intoxication may occur several years after the exposure (Kowalczyk et al., 2003). Cadmium is the 48th element and a member of group 12 in the Periodic table of elements. The most common oxidation number of cadmium is +2. About 13,000 tons of cadmium is produced yearly worldwide, mainly through directly contact with cadmium via occupational worker in industrials such as for nickel-cadmium batteries pigments, metal coatings, alloys (Shimada et al., 2008), phosphate fertilizers (Grant, 2011), and indirectly, humans are exposed to cadmium unconsciously such as food, water and cigarette smoke induces uptake of Cadmium from the environment into the body through pulmonary and enteral pathways (Waisberg et al., 2003). Cytogenetic markers such as chromosomal aberrations (CAs) in vivo rodents involving gross alterations of the genetic material have been regarded as a
sensitive endpoint for detecting genotoxic effects induced by heavy metals and toxic chemicals (Topashka-Ancheva et al., 2003; Sarkar et al., 1993). Selenium, an essential trace element of fundamental importance for animals and humans as a cancer chemopreventive agent, is obtained from dietary sources including cereals, grain products, vegetables, seafood, meat and nuts (Tapiero et al., 2003). Selenium (Se) compounds are considered as “Janus compounds” i.e. products with a double face, due to their contrasting behavior, which depends on the concentration used. Selenium in low concentrations may have anticarcinogenic effect, whereas in high concentrations it can be a genotoxic and carcinogenic agent (Biswas et al., 1999).

Melatonin (N-acetyl-5-methoxytryptamine), a hormone of the pineal gland produced from the amino acid tryptophan in minute quantities, has been known to be a chemopreventive and anticancer agent in vitro and in vivo studies (Mirunalini et al., 2010). Melatonin is able to modulate chromosome damage (chromosomal aberrations and sister chromatid exchanges) induced by genotoxic agents, by modulating oxidative processes, thereby reducing DNA damage. Melatonin is able to decrease H2O2-induced chromosomal aberrations (Salvia et al., 1999). The main aims of the present work are to study the protective effects of selenium and melatonin against the genotoxic effect of orally given Cadmium chloride to male albino mice.

Materials and Methods:
Laboratory Mice:
Adult male laboratory mice Mus musculus BALB/C, (8-10 weeks) in age, weighing (25-30) gm, were kept in the animal house / Dept. of Biology, Faculty of Science and Education Science, Sulaimani University / Iraqi Kurdistan Region, and maintained under room temperature (22 ± 2). And were kept under constant environmental condition with a 12:12 light- dark cycle. A standard diet was prepared to feed the mice.

Cadmium Chloride:
Cadmium chloride(CdCl2) obtained from Faculty of Medicine University of Sulaimani / Iraqi Kurdistan Region and were prepared by dissolving 1mg CdCl2/ in 2ml double distilled water (Abdel-moneim and Ghafeer.,2007) and administered 1mg CdCl2/kg b.w. (Singh et al.,2007) by gavage syringe to experimental animals.

Melatonin and Selenium:
Melatonin and Selenium, they were purchased from Daik Pharmacy (USA Company) it was administrated orally to the experimental animals 2.5mg/kg b.w. melatonin (Hafez, 2009), and 0.2mg/kg b.w selenium (Mohammadi et al., 2008) by gavage syringe to experimental animals.

Animal treatments:
In present study, (35) male mice were divided into (7) groups (each group contained) 5 animals), all groups treated orally through gavages syringe five days per week at the beginning of each week for five weeks.

Group I: Mice were treated with distilled water.

Group II: Mice were treated with cadmium chloride (1 mg/kg B.W)

Group III: Mice were treated with 2.5 mg/kg B.W of melatonin

Group IV: Mice were treated with 0.2 mg/kg B.W of Selenium

Group V: Mice were pre-treated with 2.5 mg/kg B.W of melatonin and 1mg/kg B.W of cadmium chloride one hour gap between treatment.

Group VI: Mice were pre-treated with 0.2 mg/kg B.W of Selenium and 1mg/kg B.W of cadmium chloride one hour gap between treatment.

Group VII: Mice were pre-treated with 2.5 mg/kg B.W of melatonin + 0.2 mg/kg B.W of Selenium and 1mg/kg B.W of cadmium chloride one hour gap between each treatment.
Chromosome preparation:
Chromosomal preparations from bone marrow cells were done by standard method of (Evans, 1966).

Microscopic Examinations and counting
Microscopic examinations for prepared chromosome (100 metaphase) for each mice slides done by using light microscope (Motic) with ocular lens 10X and objective lens 100X and the photos were taken using digital camera (Samsung 10.2 Mega Pixels).

Statistical Analysis
Using factorial experiment statistical analysis done by using the statistical packages for the social science (SPSS version 16) which is a computer program used for statistical analysis in scientific fields.

Results and Discussion:
Table (1) summarizes the treatment effects of melatonin and selenium on clastogenic effect of cadmium chloride.
A significant increase (p<0.05) is found in cadmium chloride treated group with respect to the total abnormal metaphase and most aberrations were studied like (centromeric gap, centromeric break, ring, acentric fragment, dicentric chromosome and aneuploidy) compared with the control, but there were non-significant effect (p<0.05) regarding other aberrations such as (chromatid break, centric fusion, polyploidy, end to end association and chromatid break with fragment) in five weeks exposure to cadmium chloride. These results were in agreement with the results obtained by (Singh et al.,2007) reported in their study that mice treated with cadmium chloride resulted in extensive chromosomal aberrations as manifested by aneuploidy, breaks, gaps, centromeric fusion resulting in formation of submetacentric and metacentric chromosomes and El-Refaiy & Eissa,2012 have indicated that the treatment of rats with cadmium chloride significantly increased the frequency of chromosomal aberrations such as: break, ring). It is reported that DNA double-strand breaks are responsible for the induction of various types of chromosome aberrations (Natarajan and Obe, 1978). Moreover, it has also been reported that cadmium could have direct interactions with DNA, inhibits of DNA repair, generation of reactive oxygen and the interference with the cellular response to DNA damage. Cadmium also induces cell proliferation, inactivates negative growth stimuli, such as the tumor suppressor protein p53. (Filipic et al., 2006; Hartwig, 2010). It has been suggested that cadmium can cause aneuploidy, and it poisons the mitotic spindle. The mitotic spindle has a great importance in the formation of DNA damage through the period of exposure. Bone marrow could be more sensitive to cadmium chloride due to high level of proliferation as this metal interferes with a number of enzymes involved in DNA repair and replication (Hartwig, 1994, 1995).
micronuclei (Bérces et al., 1993). It has been documented that the loss of p53 activity leads to aneuploidy along with gene amplification since genotoxic metal is known to alter p53 activity (Livingstone et al., 1992; Reznikoff et al., 1994; Rossman, 2003). In addition, cadmium alters p53 protein and the expression of the p53 protein is significantly depressed by cadmium. Cadmium is involved in the disruption of many genomic processes, the mechanisms of which are being gradually understood. Changes in DNA methylation may be induced by cadmium leading to epigenetic alterations (Méplan et al., 1999; Anetor, 2012).

Treatment of each of melatonin and selenium alone caused significant decrease (p<0.05) of total aberrant metaphase and most aberrations studied were (centromeric gap, centromeric break, ring, acentric fragment, dicentric chromosome, end to end association and aneuploidy) compared with cadmium chloride group, but there were non-significant effects (p<0.05) with respect to some aberrations such as (chromatid break, centric fusion, polyploidy and chromatid break with fragment) when it is compared with cadmium group.

Co-treatment with melatonin showed significant decrease (p<0.05) of the total chromosomal aberrations and with respect to (dicentric chromosome and aneuploidy) when it is compared with cadmium chloride group but there were non-significant effects (p<0.05) regarding (centromeric gap, centromeric break, ring, acentric fragment, chromatid break, centric fusion, polyploidy, end to end association and chromatid break with fragment).

Co-treatment with selenium showed significant decrease (p<0.05) of the total chromosomal aberrations with respect to (aneuploidy) when it is compared with cadmium chloride group but there were non-significant effects (p<0.05) regarding (centromeric gap, centromeric break, ring, acentric fragment, chromatid break, dicentric, centric fusion, polyploidy, end to end association and chromatid break with fragment).

Co-treatment with melatonin and selenium showed significant decrease (p<0.05) of total chromosomal aberrations, regarding (centromeric break and aneuploidy) when it is compared with cadmium chloride group but there were non-significant effects (p<0.05) with respect to certain aberrations such as (centromeric gap, ring, acentric fragment, chromatid break, dicentric, centric fusion, polyploidy, end to end association and chromatid break with fragment).

The current work showed that co-treatment melatonin and/or selenium in periods of five treatment melatonin with cadmium chloride significantly decreases total abnormal metaphase and other aberrations when compared with cadmium chloride group. The present results are in agreement with the results obtained by (Musatov et al., 1997 reported that melatonin inhibited the formation of chromosomal aberrations in mice; Hanafy, 2007 indicated that melatonin could improve genetic damages, reduced frequency of structural and numerical chromosomal aberration induced by genotoxic agents. Kee et al., 1997 reported that selenium acts as anti-clastogenic agents by preventing oxidative stress, thereby reduce breakage and frequency of chromosomal damage induced by genotoxic metals. Santos and Takahashi, (2008) documented that the mechanism of chemoprotection of selenium may be related to its antioxidant properties as well as its ability to interfere with DNA repair pathways. Therefore, selenium is effective in reducing the frequency of chromosomal aberrations, the number of aberrant metaphases and the frequency of apoptotic cells induced by the antitumoral agent. Cadmium is known to produce DNA damage and lipid peroxide in vitro and in vivo as well as selenium provides a protective effect against cadmium induced
genotoxicity (Hurná et al., 1997). Selenium has ability to increase glutathione peroxidase activity in the elimination of peroxide radicals, thereby providing significant protection against genotoxicity of chemical that induced oxidative DNA damage and apoptosis in aquatic organism (Selvaraj, et al 2012). Cadmium chloride induced their genotoxicity through the induction of oxidative stress, while melatonin reduces this effect by detoxification of oxygen and nitrogen-based free radicals and related non-radical products (Jahangir et al 2006; Reiter et al., 2007, 2010). The administration of a single dose of melatonin to animals before irradiation lowers total chromosomal aberration from 46% to 32% (Badr et al., 1999).

Melatonin, a known scavenger of peroxynitrite. Melatonin depletion significantly increases peroxynitrite formation, enhances DNA damage and causes the decrease in mitochondrial respiration (Cuzzocrea et al., 1999).

In addition, melatonin is more effective in scavenging hydroxyl radicals than glutathione (Hardeland et al., 1993). The efficacy of melatonin in cadmium induced cytotoxicity is mainly attributed to its high lipophilicity (Reiter, 2000). It is well documented that the lipophilic agents are found to be very effective in reducing toxic effects of heavy metals and their elimination from various organ systems (Flora et al., 1997; El-Sokkary et al; 2005). Due to its small size and lipophilicity, melatonin crosses biological membranes easily, thus reaching all compartments of the cell. Melatonin has also been shown to be an efficient protector of DNA (Lopez-Burillo et al., 2003).

Table (4.1) : (Mean±SE) For the protective effects of five weeks exposure of melatonin and/ or selenium against Cadmium Chloride induced chromosomal aberrations in male albino mice. (P<0.05).

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Note : Similar letters in each column refer to non significant difference while different letters refer to significant difference between them.
REFERENCES:


