Metallic lead has a bluish tarnishes and their extracts may modulate cell proliferation was reflected when they become mature sperm, and most spermatogonia in the testis do not undergo spermiogenesis to become mature sperm. During normal spermatogenesis, not all spermatogonia in the testis undergo spermiogenesis to become mature sperm, and most of them are eliminated through spontaneous germ cell apoptosis (Brinkworth et al., 1995). Germ cell apoptosis can be induced by various environmental or physiological stresses (Furuchi et al., 1996). Two different mechanisms of cell apoptosis have been described: the extrinsic receptor-mediated pathway and the intrinsic mitochondria-dependent pathway. The extrinsic pathway is initiated by the binding of extracellular death ligands, such as Fas ligand (FasL) to their corresponding cell-surface receptors such as Fas. By contrast, the intrinsic pathway is mainly regulated by Bcl-2 family members including Bax, Bak, Bcl-2, and Bcl-xL which positively or negatively regulate mitochondrial outer membrane permeability to promote the release of cytochrome c and other apoptotic molecules (Print and Loveland, 2000).

Epidemiological studies have shown correlation between heavy metals concentrations in the body and human health. The body absorbs these toxic substances which are distributed into body systems and lead to different diseases. Lead is a chemical element in the carbon group. Lead is a soft and malleable metal, which is regarded as a heavy metal and poor metal. Metallic lead has a bluish-white color after being freshly cut, but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. Lead poisoning (also known as plumbism, colica pictorum, saturnism, Devon colic, or painter’s colic) is a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death. Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. Occupational exposure is a common cause of lead poisoning in adults. According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than 3 million workers in the United States are potentially exposed to lead in the workplace (Staudinger and Roth, 1998). One of the largest threats to children is lead paint that exists in many homes, especially older ones; thus children in older housing with chipping paint or lead dust from moveable window frames with lead paint are at greater risk. Prevention of lead exposure can range from individual efforts (e.g. removing lead-containing items such as piping or blinds from the home) to nationwide policies (e.g. laws that ban lead in products, reduce allowable levels in water or soil, or provide for cleanup and mitigation of contaminated soil, etc.). Elevated lead in the body can be detected by the presence of changes in blood cells visible with a microscope and dense lines in the bones of children seen on X-ray, but the main tool for diagnosis is measurement of the blood lead level. When blood lead levels are recorded, the results indicate how much lead is circulating within the blood stream, not the amount being stored in the body (Staudinger and Roth, 1998).

The therapeutic use of plants and their extracts may be a promising approach for the treatment of different diseases. Ocimum basilicum (basil) is an annual herb of the Lamiaceae family and is widely cultivated in different regions of the world. O. basilicum is widely used in folk medicine to treat a wide range of diseases and has numerous

**Ocimum basilicum** extract Ameliorate Lead-induced Testicular Apoptosis in rats

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**ABSTRACT**

The present study examined the efficacy of Ocimum basilicum extract, a natural herb, with antioxidant properties, against testicular toxicity induced by Lead, which is one of the most important toxic heavy metals. The intoxicated rats showed decreased cell proliferation was reflected by a decrease in Ki-67 expression, whereas the increase in apoptotic rate was associated with a decrease in the Bcl/Bax ratio. Concomitant treatment with aqueous basil extract led to an improvement in histological, morphometrical and immunohistochemical changes induced by lead. The beneficial effects of basil extract could be attributed to its antioxidant properties.

**KEYWORDS:** Apoptosis, Lead, Ocimum basilicum, Ki-67, Rats, Testis.

**INTRODUCTION**

Spermatogenesis is a well-organized complex process, in which diploid spermatogonia proliferate and differentiate into terminally haploid mature functional sperm. During normal spermatogenesis not all spermatogonia in the testis undergo spermiogenesis to become mature sperm, and most of them are eliminated through spontaneous germ cell apoptosis (Brinkworth et al., 1995). Germ cell apoptosis can be induced by various environmental or physiological stresses (Furuchi et al., 1996). Two different mechanisms of cell apoptosis have been described: the extrinsic receptor-mediated pathway and the intrinsic mitochondria-dependent pathway. The extrinsic pathway is initiated by the binding of extracellular death ligands, such as Fas ligand (FasL) to their corresponding cell-surface receptors such as Fas. By contrast, the intrinsic pathway is mainly regulated by Bcl-2 family members including Bax, Bak, Bcl-2, and Bcl-xL which positively or negatively regulate mitochondrial outer membrane permeability to promote the release of cytochrome c and other apoptotic molecules (Print and Loveland, 2000).

Epidemiological studies have shown correlation between heavy metals concentrations in the body and human health. The body absorbs these toxic substances which are distributed into body systems and lead to different diseases. Lead is a chemical element in the carbon group. Lead is a soft and malleable metal, which is regarded as a heavy metal and poor metal. Metallic lead has a bluish-white color after being freshly cut, but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. Lead poisoning (also known as plumbism, colica pictorum, saturnism, Devon colic, or painter’s colic) is a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death. Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. Occupational exposure is a common cause of lead poisoning in adults. According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than 3 million workers in the United States are potentially exposed to lead in the workplace (Staudinger and Roth, 1998). One of the largest threats to children is lead paint that exists in many homes, especially older ones; thus children in older housing with chipping paint or lead dust from moveable window frames with lead paint are at greater risk. Prevention of lead exposure can range from individual efforts (e.g. removing lead-containing items such as piping or blinds from the home) to nationwide policies (e.g. laws that ban lead in products, reduce allowable levels in water or soil, or provide for cleanup and mitigation of contaminated soil, etc.). Elevated lead in the body can be detected by the presence of changes in blood cells visible with a microscope and dense lines in the bones of children seen on X-ray, but the main tool for diagnosis is measurement of the blood lead level. When blood lead levels are recorded, the results indicate how much lead is circulating within the blood stream, not the amount being stored in the body (Staudinger and Roth, 1998).

The therapeutic use of plants and their extracts may be a promising approach for the treatment of different diseases. Ocimum basilicum (basil) is an annual herb of the Lamiaceae family and is widely cultivated in different regions of the world. O. basilicum is widely used in folk medicine to treat a wide range of diseases and has numerous
pharmacological activities. Many studies have reported that basil leaf extracts have potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties (Manosroi et al., 2006; Fathiazad et al., 2013). Sethi et al., (2003) reported that the leaves of O. sanctum possess good antioxidant and antistress potentials in experimental animals. Consumption of basil or basil oil has been associated with a reduction in total cholesterol, low-density lipoprotein and triglyceride levels (Harnafi et al., 2009). Supplementation with O. sanctum leaf extract reduced the severity of hydropericardium, hepatitis, myocarditis accompanied with hemorrhages, lung edema, lymphocytic depletion in lymphoid organs and focal interstitial nephritis (Batra and Gupta, 2006). Ocimum leaf extracts were found to protect the liver from heavy metals (Sharma et al., 2002) and prevent isoproterenol-induced myocardial necrosis in rats (Sood et al., 2005). Basil or basil oil is useful in the prevention and treatment of cardiovascular disease (Rupert, 2009). Sakr et al., (2011) reported that O. basilicum extract improved hepatotoxicity and apoptosis induced by CCl4 in rats. In the present study, we examined the effect of O. basilicum extract on Lead-induced testicular apoptosis in rats.

MATERIALS AND METHODS

Ocimum extract

Fresh leaves of O. basilicum were purchased from the local market, Tabriz, Iran. The leaves were rinsed with clean water to remove any foreign matter. Leaves were dried in the shade and ground to a fine powder using a laboratory mixer. One hundred grams of leaf powder was refluxed with 750 ml of double distilled water for 1 hour and concentrated using a rotary evaporator. The extract was stored at -20°C until used for experiments. The aqueous extract was used at a dose level of 20 mg/kg O. basilicum (Offiah and Chikwendu, 1999).

Animals

Male Wistar rats weighting 140±6 g were kept in an animal house under constant temperature conditions (24±2°C) for at least 1 week before and through the experimental work, being maintained on a standard diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture, and 5% vitamins. Water was available ad-libitum. All the experiments were done in compliance with the guide for the care and use of laboratory animals. Animals were divided into 4 groups (n=10 each) as follows:

Group 1, Rats were fed on the standard diet and served as a control group; group 2, Rats were treated with oral aqueous O. basilicum extract at a dose level of 20 mg/kg 5 days/wk for 8 weeks; group 3, Rats were treated with oral administration of lead at a dose level of 30 mg/kg b.w. 5 days/wk for 8 weeks (Ohta et al., 2000); group 4, Rats were treated with lead (30 mg/kg b.w) followed by oral administration of aqueous O. basilicum extract (20 mg/kg) 5 days/wk for 8 weeks.

Immunohistochemical study

For immunohistochemical localization of Ki-67, Bax, and Bcl-2, fixed wax sections were stained using the avidin-biotin peroxidase method. Formalin-fixed paraffin-embedded tissue sections were deparaffinized, endogenous peroxidase activity was blocked with H2O2 in methanol and the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 minutes. The slides were allowed to cool to room temperature, and nonspecific binding was blocked with normal horse serum for 20 minutes at room temperature. The MIB-1 monoclonal antibody was used for detection of nuclear Ki-67, a marker of proliferating cells (1:40, code No. M7187, Dako, Cambridge, UK). Anti-Bcl-2 and anti-Bax (Dako) monoclonal antibodies were used for detection of bcl-2 and bax, respectively. Counterstaining was performed using Mayer's hematoxylin (Cat. No. 94585, BioGenex, Menarini Diagnostics, Antony, France). For evaluation of each marker, the percentage of positively stained cells in the total number of cells under ×40 magnification was calculated. Assessment for Bax, Bcl-2, and Ki-67 was performed according to the following semi-quantitative scale: (-), negative; (+), equivocally positive; (++), weakly positive; (+++), positive; and (++++), strongly positive (Al-Azemi et al., 2010).

Statistical analysis

The results were expressed as mean±SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student’s t-test using Minitab 12 computer program (Minitab Inc., State Collage, PA, USA).

RESULTS

Table 1 shows the expression of Bax and Bcl-2 in the testes of the different experimental groups. Testicular tissue obtained from lead-treated rats showed weak expression of Bcl-2 in comparison with the control group (Fig. 1A, B). Treatment of animals with lead+Ocimum extract upregulated the expression of Bcl-2 (Fig. 1). Increase expression of Bax was observed in animals treated with lead (Fig. 2A). Bax expression decreased in rats treated with lead+Ocimum (Fig. 2B). The Bcl/Bax ratio decreased in animals receiving lead (Table 1). In the seminiferous epithelium Ki-67 is expressed in the nuclei of spermatogonia. We observed an increase in expression of Ki-67 in control rats (Fig. 3A), whereas animals treated with lead showed decreased expression of Ki-67 (Fig. 3B). Animals treated with lead+Ocimum extract showed an increase in the expression of Ki-67 (Fig. 3C).
Figure 1: (A) Weak expression of Bcl-2 in germ cells of a rat treated with lead. (B) An increase in expression of Bcl-2 after treatment with lead+Ocimum (A and B, ×400).

Figure 2: (A) Section in testis of a rat treated with lead showing increase of Bax expression in Leydig cells. (B) Section in testis of a rat treated with lead+Ocimum showing decrease of Bax expression (A and B, ×400).

Figure 3: (A) Section in testis of a control rat showing marked expression of Ki-67. (B) Section in testis of a rat treated with lead showing decrease of Ki-67 expression (C). An increase in expression of Ki-67 after treatment with lead+Ocimum (A-C, ×400).

Table 1: Expression of Bax, bcl-2, and Ki-67 in testes of different animal groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Bax</th>
<th>Bcl-2</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Plant</td>
<td>++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Lead</td>
<td>++++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Lead+plant</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

DISCUSSION

Lead is one of the main environmental and occupational pollutants in industrialized countries. Exposure to lead is associated with serious health hazards. In present study, Testicular tissue obtained from lead-treated rats showed weak expression of Bcl-2 in comparison with the control group. In the present study, prolonged treatment with lead showed that some seminiferous tubules were virtually depleted of germ cells. This finding might be due to loss of those populations via apoptosis or differentiation failure. Similar explanation was reported in mutant mice (Meng et al., 2000). These changes might be explained by the reduced expression of Sertoli cell growth factor (Glial cell line-derived neurotrophic factor (GDNF) as well as retraction of the Sertoli cells cytoplasmic processes that are normally supporting germ cells, that might depress the spermatogonial differentiations (Buása et al., 2004). Lead induced mutation in the stem cell population of the embryonic gonad might be a cause of this germ cell depletion (Al Hakkak et al., 1988). Another explanation was reported by some authors based on the lead induced reduction of testosterone that might be implicated in disruption of sertoli junctions (Liu et al., 2003).

The present study showed that lead induced testicular apoptosis as indicated by an increase in Bax and decrease in Bcl-2 in germ cells. In agreement with this result, Al-Azemi et al., (2010) reported that Cd-treatment upregulates Bax and downregulates Bcl-2 expression in the testis of rats. Zhang et al., (2010) found that Cd has obvious adverse effects on the proliferation of piglet Sertoli cells, resulting in DNA damage, cell apoptosis, and aberrant morphology. Induction of apoptosis by exposure to Cd has been reported by other investigators (Zhou et al., 1999). Ki-67 is a marker of cell proliferation. It is expressed in the nuclear matrix of cells during the late G1-, S-, G2- and M phases of the cell cycle, it peaks in the G2- and early M-phases (Sasaki et al., 1987). The expression of Ki-67 decreased in the testes of animals treated with lead. Similarly, Falana et al., (2013) reported that lead caused testicular alterations in the testes of rats and decreased the...
expression of Ki-67. Di-(n-butyl) phthalate exposure delays germ cell development in both the fetal and postnatal life of rats, and reduces Ki-67 expression (Ferrara et al., 2006).

Testicular oxidative stress induced by different pollutants leads to male infertility. Lead may increase oxidative stress by binding to the sulphydryl groups of proteins and by depleting glutathione (Valko et al., 2005). Oxidative stress may promote alteration in DNA repair mechanisms and induction of cell proliferation (Beyersmann and Hartwig, 2008). Sen Gupta et al., (2004) reported that the levels of the testicular antioxidants enzymes, superoxide dismutase, catalase and glutathione peroxidase are greatly diminished upon Cd exposure. Lead may induce germ cell apoptosis by enhancing the generation of ROS-like superoxide ion, hydroxyl radicals and hydrogen peroxide. Oxidative stress can result in peroxidation, mitochondrial dysfunction or DNA damage in germ cells and oxidative damage in Leydig cells (Szuster Ciesielska et al., 2000).

In the present work, we showed that O. basilicum extract protects the testis from lead toxicity as indicated by the restoration of the histological structure and increase in the number of germ cell layers. Moreover, testicular apoptosis decreased as indicated by a decrease of Bax and increase in Bcl 2 in germ cells. In accordance with these results, Khaki et al., (2011) reported that O. basilicum extract protected rats from testicular damage and reduced apoptosis after exposure to an electromagnetic field. Asuquo et al., (2010) found that O. gratissimum extract improved the testicular histopathological alterations in diabetic rats. The protective properties of O. basilicum have been extensively investigated. Sakr et al., (2011) reported that treating animals with CCl4 and aqueous leaf extracts of O. basilica led to an improvement, in both histopathological and biochemical alterations induced by CCl4. Furthermore, apoptosis was reduced in hepatic cells. Sharma et al., (2002) reported that O. basilicum has a nephroprotective effect against mercury toxicity. Basil or basil oil can be used in prevention and treatment of cardiovascular disease (Rupert, 2009) and in prevention of isoproterenol induced myocardial necrosis (Sood et al., 2005). Aqueous leaf extracts of O. basilicum protect rats against paracetamol-induced hepatotoxicity (Khuon, 2012).

The leaves of O. basilicum are a rich source of flavonoids which possess various biological properties related to antioxidant mechanisms (Zhang et al., 2009). Caffeic acid is another component in the leaf of the O. basilicum that has antioxidant, anti-inflammatory, and cancer chemopreventive activities (Neradil et al., 2003). Another constituent of O. basilicum is a p-coumaric acid possess radical scavenging and antioxidant activity at high concentration (Yeh and Yen, 2003). Dasgupta et al., (2004) reported that O. basilicum increased the activity of xenobiotic metabolizing phase I and phase II enzymes, promoting antioxidant-enzyme responses by significantly increasing activities of the hepatic glutathione reductase, superoxide dismutase, and catalase, increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice. The extract also showed significant anti lipid peroxidation effects in vitro, in addition to exhibiting significant activity in scavenging superoxide radical and nitric oxide radicals, indicating their potent antioxidant effects (Meera et al., 2009). The results of the present study indicate that the ameliorative effect of O. basilicum against testicular toxicity of lead may be attributed to its antioxidant properties due to phenolic compounds. So, authors suggest the further studies on this plant to isolate its active ingredients.

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