Original Article

Cytotoxicity of Silver Solder Employed in Orthodontics

Maria Perpétua Mota Freitas[a]; Hugo Mitsuo S. Oshima[b]; Luciane M. Menezes[c]; Denise C. Machado[d]; Christian Viezzer[e]

ABSTRACT

Objective: To test the null hypothesis that the silver soldering employed in orthodontics is not cytotoxic for fibroblasts.

Materials and Methods: This in vitro study was performed using a culture of mice fibroblasts (lineage NIH/3T3), divided into four groups (n = 10 each): control, negative control (stainless steel archwire), positive control (amalgam disks), and test group (silver soldering). After cell culture in complete Dulbecco modified eagle medium and achievement of confluence in 80%, the suspension was added to the plates of 24 wells containing the specimens and incubated in an oven at 37°C for 24 hours. The plates were analyzed on an inverted light microscope, photomicrographs were obtained, and the results were recorded as response rates based on modifications of the parameters of Stanford according to the size of the diffusion halo of the toxic substance and quantity of cell lysis.

Results: The results revealed a maximum response rate for the silver soldering group, as well as severe inhibition of cell proliferation and growth, more round cells with mostly darkened and granular aspects, suggesting lysis with cell death. A similar response was seen in the positive control group.

Conclusion: The hypothesis is rejected. The silver soldering used in orthodontics represents a highly cytotoxic material for the cells analyzed. (*Angle Orthod.* 2009;79:939–944.)

KEY WORDS: Cytotoxicity; Silver soldering; Orthodontics

INTRODUCTION

Many materials and brackets are used in orthodontics, and several scientific studies have been conducted to investigate these materials. Despite the technical and scientific advancements, the biocompatibility of some routinely used materials, which may cause adverse effects in clinical situations, is still questionable. Among these, the most common are resins and metals.

With regard to the metals, their influence on cellular proliferation and viability has been the subject of evaluation to determine their in vitro toxicity.[1–3] This is because when metal ions are released into tissue sites, the sites may show symptoms similar to allergic reactions, ie, inflammation in the skin or mucous membranes. The reactions can range from simple erythema to necrosis, depending on the toxicity of the primary irritant, concentration, and exposure time.[4]

Recent studies have demonstrated the relationship between cells and metallic cations released from dental alloys.[5–7] The metal parts that remain in the oral cavity are subject to corrosion because this environment's physical properties, ie, the chemical and microbiological properties, stimulate the dissolution of metals.[8–9] Among the cations present in the alloys, gold and palladium (noble elements) may not be detected, whereas silver, copper, zinc, and gallium are always found.[3,8] According to the International Register of Potentially Toxic Chemicals of the United Nations Environment Program, metallic ions such as cadmium, copper, silver, and zinc are present in silver soldering

[a] Professor, Department of Orthodontics, The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul, Brazil.

[b] Associate Professor, Department of Dental Materials Engineering, The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul, Brazil.

[c] Associate Professor, Department of Orthodontics, The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul, Brazil.

[d] Professor and Chair, Department of Immunology, The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul, Brazil.

[e] Graduate student, Department of Immunology, The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul, Brazil.

Corresponding author: Dr Maria Perpétua Mota Freitas, Department of Orthodontics, Av. Ipiranga, 6681 – Sala 209, Porto Alegre, RS 90619-900 Brazil
(e-mail: perpetuamf@hotmail.com)

Accepted: December 2008. Submitted: October 2008.

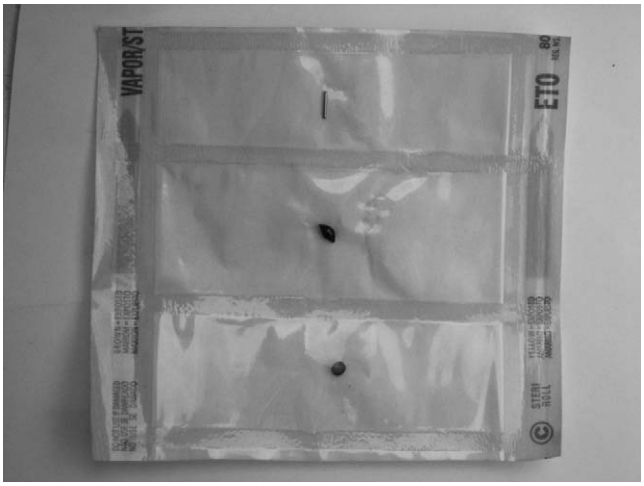

Figure 1. Specimens: stainless steel archwire, silver soldering, and silver amalgam.

and may be considered potentially dangerous chemical products. They are included in the list of substances and processes that may be of great risk to human life.[3]

In 1992, Grimsdottir et al[10] conducted an in vitro study to investigate the cytotoxicity of some metals employed in orthodontics and demonstrated the biocompatibility of stainless steel archwires. However, the orthodontic bands revealed cytotoxic effects that might be associated with the use of silver soldering and copper soldering in their fabrication. Accordingly, Gjerdet and Hero[11] stated that all soldered appliances undergo some degree of corrosion, which facilitates the release of metals that may cause adverse effects.

Considering the possible toxic effects of metals on the tissues, as previously reported in the literature, this study analyzed the hypothesis that the silver soldering employed in orthodontics is cytotoxic for fibroblasts.

MATERIALS AND METHODS

The cytotoxicity study was approved by the Institutional Review Board of The Pontifical Catholic University, Porto Alegre, Rio Grande Do Sul (protocol n. 1148/05-CEP). Tests were conducted in cell culture (lineage NIH/3T3, mice fibroblasts) to evaluate the response rate, determined from modifications of the parameters of Stanford.[12]

Table 2. Sequence of Fabrication of Amalgam Disks

Step	Procedure
1	Prepare the amalgam (GS80) in the amalgamator (SDI) for 7 seconds.
2	Place the mixture on a rectangular acrylic plate.
3	Press a second plate onto the mixture until a 1.5-mm space is established between the two plates (final thickness of the amalgam disk).
4	Use an amalgam carrier with a 2-mm-diameter opening to determine the disk width.
5	Polish with abrasive rubber point.
6	Rinse and dry.
7	Sterilize in autoclave.

Specimens

Four study groups (Figure 1) were established, as presented in Table 1. Group 1 was included only for the control of cell growth.

Fabrication of Specimens

The sequence for fabrication of specimens for amalgam disks (group 3) and silver soldering (group 4) is given in Tables 2 and 3, respectively.

Cell Culture

Mice fibroblasts (lineage NIH/3T3), purchased from American Type Culture Collection (Manassas, Va) and manipulated at the Laboratory of Cell Biology and Respiratory Diseases of the Institute of Biomedical Research of the São Lucas Hospital at The Pontifical Catholic University, were defrosted and cultured in Dulbecco modified eagle medium (DMEM) (Invitrogen, Carlsbad, Calif) supplemented with 10% of bovine fetal serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 μg/mL gentamicin (complete DMEM) in culture bottles (Techno Plastics Products, Trasadin-

Table 1. Groups and Characteristics of Specimens (n = 10 in Each Group)

	Group 1: Control	Group 2: Negative Control	Group 3: Positive Control	Group 4: Test
Material	—	Archwire segments	Amalgam disks (GS80)	3 mm of silver soldering wire
Composition	—	Austenitic stainless steel (Cr 17%–20%, Ni 8%–10.5%, Fe 65%–69.5%)	Ag (40%), Sn (31.3%), Cu (28.7%), Hg (47.9%)	Ag (55%–57%), Cu (21%–23%), Zn (15%–19%), Sn (4%–6%)
Manufacturer	—	Morelli, São Paulo, Brazil	SDI Ultramat, Victoria, Australia	Morelli, São Paulo, Brazil
Dimensions	—	0.018 inch	2 × 1.5 mm	—
Length	—	3 mm	—	3 mm
Cleaning	—	Rinsing/drying	Rinsing/drying	Rinsing/drying
Sterilization	—	Autoclave	Autoclave	Autoclave

Table 3. Sequence of Fabrication of Silver Soldering Specimens

Step	Procedure
1	Rectify and mark 0.06-mm stainless steel archwire (Morelli): 3 mm for placement of the soldering and 18 mm of spacing between them.
2	Place solder paste on the 3-mm mark on the archwire.
3	Mark off length of the silver soldering arch to be cast (3 mm).
4	Regulate the flame on a Blazer (Blazer, Farmingdale, New York) torch.
5	Fuse silver soldering on the stainless steel archwire.
6	Section and finish with abrasive rubber point.
7	Rinse and dry.
8	Sterilize in autoclave.

gen, Switzerland). This was incubated at a temperature of 37°C in a humidified oven containing 5% CO_2, which was changed twice a week until the cells reached 80% confluence. After confluence, the cells were removed by enzymatic action using 0.1% trypsin–ethylenediaminetetraacetic acid (Gibco, Grand Island, NY) and counted in a Neubauer chamber (Optik Labor, Friedrichsdorf, Germany). The suspension was added to plates of 24 wells (10 wells for the control of cell growth of the control group, 10 containing amalgam disks, 10 with orthodontic archwires, and 10 with silver soldering), in 500-μL increments, with a density of 4.5×10^5 cells per well. Finally, the cultures were again incubated for 24 hours.

Analysis of Cytotoxicity

After the 24-hour incubation period, the plates were analyzed on an inverted light microscope (Axiovent 25, Carl Zeiss SMT, Thornwood, NY) with a 10× objective, and photomicrographs were obtained. The results were recorded as response rates, according to the modified parameters of Stanford,[12] and are defined in Table 4. The rates were calculated in relation to the halos observed as two numbers separated by a bar: the first represented the size of the diffusion halo of the toxic substance, and the second indicated the quantity of cell lysis. Qualitative cell analysis was also performed based on the characteristics of cell proliferation, growth, morphology, and adhesion.

RESULTS

The response rates are presented in Table 5. The qualitative cell analysis revealed that groups 1 and 2 exhibited increases in the number of cells, confluent growth, and fusiform cells, typical of normal fibroblast development (Figure 2a,b). This was different from groups 3 and 4 (Figure 2c,d), which presented severe inhibition of cell proliferation and growth, with significant alterations indicated by the presence of more round cells, mostly with darkened and granular aspects, suggesting lysis with cell death.

DISCUSSION

The safety and health of orthodontic patients should be a constant concern for clinicians, and patients should not be placed at risk of adverse toxic effects by orthodontic appliances. Based on this assumption and considering the lack of studies on the biocompatibility of the silver soldering routinely employed in orthodontic appliances, the authors of the present a study sought to evaluate the cytotoxicity specifically of this material via an in vitro study of a fibroblast culture. Amalgam was used as a positive control (known to be highly cytotoxic) and stainless steel archwire (which is not cytotoxic) was used as a negative control; both materials have been demonstrated to be adequate for this purpose.[4] Other studies, eg, that conducted by Oh and Kim,[13] used copper and polyethylene alloys as positive and negative controls, respectively.

Investigations of the cytotoxicity of metals have taken different approaches in relation to cell characteristics and functions. Studies have evaluated the adhesion, proliferation, and metabolism of cells such as 3T3, Balb/c, L929, W138, and human fibroblasts and osteoblasts.[1,2] In the present study, the behavior of mice fibroblasts was assessed by modifications of the parameters of Stanford.[12] This is similar to the studies of Wigg et al[14] and Grill et al,[2] who advocated the investigation of proliferation by microscopic analysis of cell growth and division.

Concerning the results achieved, similar characteristics were observed for groups 1 (control) and 2 (orthodontic archwire) and between groups 3 (amalgam)

Table 4. Response Rates According to Modification of the Parameters of Stanford[12]

Index of Halo Size	Index of Quantity of Cell Lysis
0 = no halo detected around or under the specimen	0 = no lysis
1 = halo limited to the area under the specimen	1 = up to 20% of the halo with lysis
2 = halo not greater than 25% of the extent of the specimen	2 = 20% to 40% of the halo with lysis
3 = halo not greater than 50% of the extent of the specimen	3 = 40% to 60% of the halo with lysis
4 = halo greater than 50% of the extent of the specimen but not involving the entire plate	4 = 60% to 80% of the halo with lysis
5 = halo involving the entire plate	5 = more than 80% of cell with lysis in the halo

Table 5. Response Rates Observed in the Study Groups

Group 1 (Control)		Group 2 (Negative Control)		Group 3 (Positive Control)		Group 4 (Test)	
No.	Response Rate	No.	Response Rate	No.	Response Rate	No.	Response Rate
1	0/0	1	0/0	1	5/5	1	5/5
2	0/0	2	0/0	2	5/5	2	5/5
3	0/0	3	0/0	3	5/5	3	5/5
4	0/0	4	0/0	4	5/5	4	5/5
5	0/0	5	0/0	5	5/5	5	5/5
6	0/0	6	0/0	6	5/5	6	5/5
7	0/0	7	0/0	7	5/5	7	5/5
8	0/0	8	0/0	8	5/5	8	5/5
9	0/0	9	0/0	9	5/5	9	5/5
10	0/0	10	0/0	10	5/5	10	5/5

and 4 (silver soldering). In groups 1 and 2, the cells exhibited a normal pattern with significant proliferation and confluent growth (Figure 2a,b), confirming the biocompatibility of orthodontic archwires that was previously reported by Grimsdottir et al.[10] Conversely, groups 3 and 4 presented severe halos of growth inhibition, as well as a high degree of cell lysis, indicating the cytotoxicity of amalgam and silver soldering (Figure 2c,d).

Considering that the normal sequence of the life cycle of fibroblasts is when these cells come into contact with a surface, they are attracted, determine adhesion, and then diffuse in the medium. The quality of adhesion consequently influences the morphology and capacity of proliferation and differentiation, as reported by Serrano et al.[15] It may be suggested that the ions released from the silver soldering initially prevented the cell adhesion and consequently caused the subsequent events. To confirm this assumption, Grill et al[2] considered that calculation of the percentage of cells in the ''S'' phase would be ideal by immunocytochemistry or by incorporation of thymidine. This was based on the fact that cell adhesion to the substrate may be fundamental for cell proliferation. This occurs because the cells become adherent when they initiate the process of division at the onset of the aforementioned ''S'' phase, determining a specific arrangement of fibronectins.

With regard to cell morphology, the reaction of fibroblasts in the presence of silver solder specimens (group 4) led to the observation of more round cells, isolated and mostly darkened, similar to the positive control and different from the control group and the negative control, in which the fibroblasts presented an elongated shape with confluent and dense growth. These findings suggest a high potential for toxicity of the material analyzed and agree with the report of Solmi et al,[16] who associated this fact with ion release, instability of the present ions, and/or corrosion of the metallic alloys involved. Solmi et al mentioned that such elements as copper and zinc, present in the composition of the soldering studied and considered toxic by the International Register of Potentially Toxic Chemicals of the United Nations Environment Program, are more unstable than other metals present at higher concentrations in soldering alloys, such as silver. Within this context, Syrjanen et al[6] stated that metallic alloys with low gold content (noble metal) present the probability of greater toxicity. They also noted that many metallic cations influence the viability of fibroblasts, with silver being the most powerful cation inhibiting the incorporation of thymidine.

The alloy used in this study presented the following composition: silver (55%–57%), copper (21%–23%), zinc (15%–19%), and tin (4%–6%). Considering the higher silver, copper, and zinc concentrations, and taking into account the greater instability of copper and zinc, a possible participation of these ions in the development of cell alterations may be suggested. As mentioned by Syverud et al,[17] alloys with higher copper content present more severe oxidation and greater toxicity compared to alloys with less copper, as indicated by the greater evidence of cell irritation. Moreover, in vitro studies have demonstrated the capacity of copper to initiate risky oxidizing events that may interfere with important cell activities. This occurs because the copper, when free, is able to catalyze the formation of highly toxic hydroxyl radicals, which may cause neurodegenerative alterations. In a laboratory study, Manzl et al[18] attempted to analyze the toxicity of copper and observed the hepatotoxicity of this ion by induction of calcium flow and consequent loss of cell viability.

With regard to the zinc and silver, studies[19] have shown that zinc plays an important role in skeletal growth and development as well as maintenance of bone health. It is a cofactor of alkaline phosphatase, which is responsible for formation and mineralization of the bone matrix. However, at excessive doses this ion may be toxic.[20] According to Barceloux,[20] the recommended daily ingestion of this ion for adult individuals is 15 mg. Daily doses higher than 1 to 2 g may

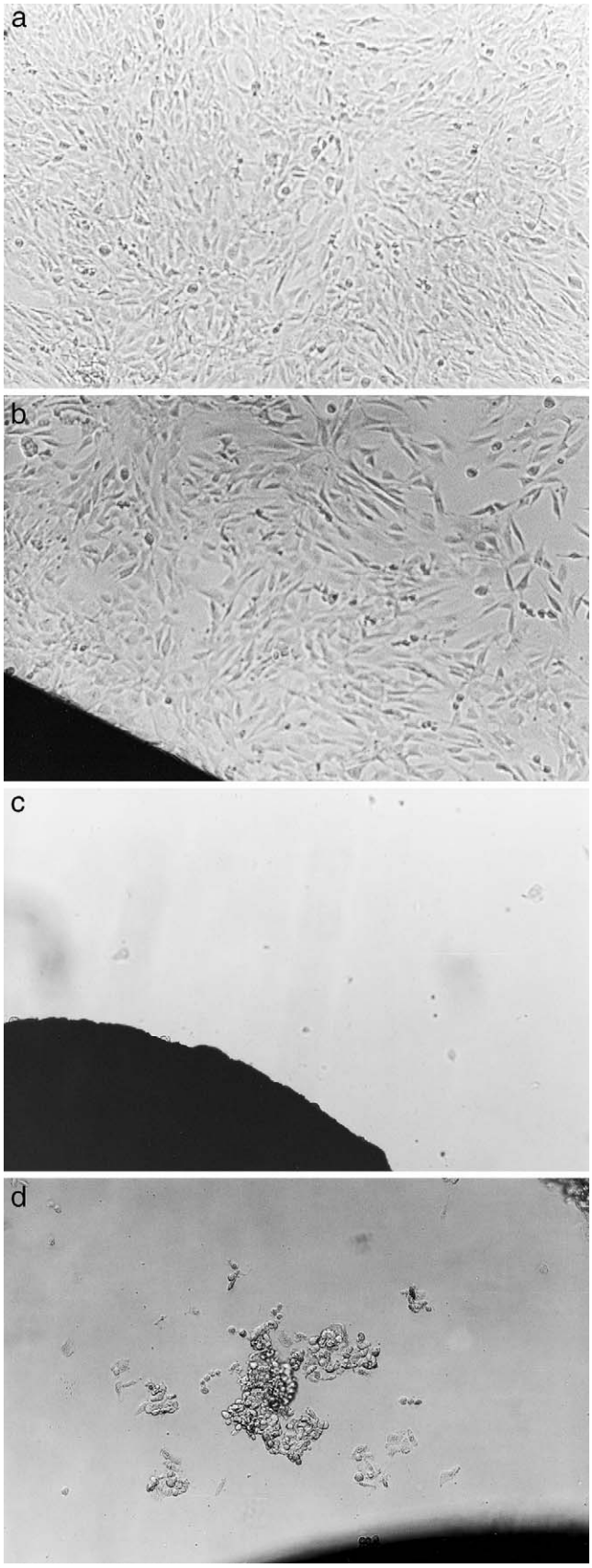

Figure 2. Cell aspect in (a) the control group, (b) negative control, (c) positive control, and (d) test group.

trigger such effects as irritation and corrosion of the gastrointestinal tract, renal tubular necrosis, and acute nephritis. Recent in vitro studies of silver have analyzed the biocompatibility of this ion using materials as orthopedic pins for external fixation, endodontic posts, or complete and/or partial dentures. In a study of orthopedic pins, Bosetti et al[21] demonstrated that silver does not present genotoxicity or cytotoxicity compared to stainless steel.

Although it is not cited in the composition by the manufacturer, cadmium may be present as a contaminant in silver soldering,[22] perhaps playing an important role in the aforementioned alterations that characterize cytotoxicity. This chemical substance may alter mitochondrial permeability, representing a progressive process up to cell death. In their study, Li et al[23] demonstrated that this ion may cause mitochondrial dysfunction, including inhibition of respiration, loss of potential of membrane transportation, and release of c-cytochromes. Çelik et al[24] observed that cadmium may induce alterations in the DNA of intact cells, as well as hinder their repair potential.

This study revealed alterations secondary to the presence of silver soldering on the cells analyzed, demonstrating its cytotoxicity. However, the methodology employed did not provide information on the mechanism of inhibition of cell proliferation, growth, adhesion, and lysis, as well as information about the role played by each ion on these processes, suggesting a need for further studies on this subject.

CONCLUSION

- The silver soldering used in orthodontics exhibits severe cell toxicity; in the present study it resulted in the inhibition of proliferation, growth, and development of the cells analyzed.

REFERENCES

1. Craig RG, Hanks CT. Cytotoxicity of experimental casting alloys evaluated by cell culture tests. *J Dent Res.* 1990;69: 1539–1542.
2. Grill V, Sandrucci MA, Basa M, et al. The influence of dental metal alloys on cell proliferation and fibronectin arrangement in human fibroblast cultures. *Arch Oral Biol.* 1997;42: 641–647.
3. Wataha JC, Hanks CT, Sun Z. In vitro reaction of macrophages to metal ions from dental biomaterials. *Dent Mater.* 1995;11:239–245.
4. Grimsdottir MR, Hensten-Pettersen H, Kullmann A. Cytotoxic effect of orthodontic appliances. *Eur J Orthod.* 1992; 14:47–53.
5. Brune D. Metal release from dental biomaterials. *Biomaterials.* 1986;7:163–175.
6. Syrjanen S, Hensten-Pettersen A, Kangasniemi K, Yli-Urpo A. In vitro and in vivo biological responses to some dental alloys tested separately and in combinations. *Biomaterials.* 1995;7:169–176.

7. Eliades T, Zinellis S, Eliades G, Athanasiou GE. Nickel content of as-received, retried end-recycled stainless steel brackets. *Am J Orthod Dentofacial Orthop.* 2002;122:217–220.
8. Sestini S, Notarantonio L, Cerboni B, et al. In vitro toxicity evaluation of silver soldering, electrical resistance, and laser welding of orthodontic wires. *Eur J Orthod.* 2006;28:567–572. Epub 2006 Oct 11.
9. Wataha JC, Craig RG, Hanks CT. The release of elements of dental casting alloys into cell-culture medium. *J Dent Res.* 1991;70:1014–1018.
10. Grimsdottir MR, Gjerdet NR, Hensten-Pettersen A. Composition and in vitro corrosion of orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 1992;101:525–532.
11. Gjerdet NR, Hero H. Metal release from heat-treated orthodontic archwires. *Acta Odontol Scand.* 1987;45:409–414.
12. Stanford JW. Recommended standard practices for biological evaluation of dental materials. *Int Dent J.* 1980;30:140–180.
13. Oh K, Kim K. Ion release and cytotoxicity of stainless steel wires. *Eur J Orthod.* 2005;27:533–540.
14. Wigg MD, Menezes LM, Quintão CCA, Moreira TC, Chevitarese O. Elásticos extra-orais: avaliação da citotoxicidade. *Ortodontia Gaúcha.* 1997;1:151–157.
15. Serrano MC, Pagani R, Peña J, Portolés MT. In vitro biocompatibility assessment of poly(ϵ-caprolactone) films using L929 mouse fibroblasts. *Biomaterials.* 2004;25:5603–5611.
16. Solmi R, Martini D, Zanarini M, et al. Interactions of fibroblasts with soldered and laser-welded joints. *Biomaterials.* 2004;25:735–740.
17. Syverud M, Dahl JE, Morisbak E. Corrosion and biocompatibility testing of palladium alloy castings. *Dent Mater.* 2000;17:7–13.
18. Manzl C, Enrich J, Ebner H, Dallinger R, Krumschnabel G. Copper-induced formation of reactive oxygen species causes cell death and disruption of calcium homeostasis in trout hepatocytes. *Toxicology.* 2004;196:57–64.
19. McClung JP, Stahl CH, Marchitelli LJ, et al. Effects of dietary phytase on body weight gain, body composition and bone strength in growing rats fed a low-zinc diet. *J Nutr Biochem.* 2006;17:190–196.
20. Barceloux DG. Copper. *Clin Toxicol.* 1999;37:217–237.
21. Bosetti M, Masse A, Tobin E, Cannas M. Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. *Biomaterials.* 2002;23:887–892.
22. Shimizu S, Narita MT, Sujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. *Nature.* 1999;399:483–487.
23. Li M, Xia T, Chun-Sun J, Li L, Fu J, Zou Z. Cadmium directly induced the opening of membrane permeability pore of mitochondria which possibly involved in cadmium-triggered apoptosis. *Toxicology.* 2003;194:19–33.
24. Çelik A, Çomelekoglu U, Yalin S. A study on the investigation of cadmium chloride genotoxicity in rat bone marrow using micronucleus test and chromosome aberration analysis. *Toxicol Ind Health.* 2005;21:243–248.