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The Harmful Effects of Restorative Dental Filling Materials on The Kidney and Liver Tissues*

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Abstract: The purpose of this study was to examine the potential effects of restorative dental materials on the kidneys and liver tissues of rats. The histological, immunohistochemical and biochemical analyzes were conducted using 32 adult female Wistar albino rats classified into 4 groups (n=8): Control group, Dental amalgam group, Resin composite group, and Glass-ionomer group. The dental materials mentioned were put on the backs of the rat heads and kept there for 8 weeks. After humanely euthanizing rats, kidney and liver tissue was removed by necropsy, and Hematoxylin&Eosin and NF-κB p65 staining methods were used. In addition, glutathione, superoxide dismutase, lipid peroxidation and catalase levels have been identified. Immune-positive cells were higher in all sample groups than in the control group. Renal and hepatic degeneration has also been found in dental filling content groups. All biochemical findings revealed oxidative stress in all groups with the exception for control group. The three dental filling products have significant cytotoxic effects on the kidney and liver tissues, and may have been linked with oxidative stress in the tissues of the kidney and liver.

Keywords: Dental amalgam, Glass ionomer, Kidney damage, Liver damage, Resin composite.

Diş Dolgu Materyallerinin Karaciğer ve Böbrek Dokuları Üzerine Olan Zararlı Etkileri

Öz: Bu çalışmanın amacı restoratif dental materyallerin, sıçan böbrek ve karaciğer dokuları üzerindeki olası etkilerinin incelenmesidir. 32 adet, yetişkin, dişi Wistar albino cinsi ratlar Kontrol grubu, Dental amalgam grubu, Rezin kompozit grubu, Cam iyonomer grubu olmak üzere 4 gruba ayrılmış (n=8) ve ense kısımlarına yapılan kesilerle dental materyaller yerleştirilip 8 hafta beklenmiştir. Ötenaziden sonra yapılan nekropsi ile karaciğer ve böbrek dokuları alınmıştır. Kullanılan boyama yöntemleri, NF-κB p65 ve Hematoksilen&Eozin boyamadır. Biyokimyasal analiz olarak ise glutatyon, süper oksit dismutaz, lipit peroksidasyon ve katalaz seviyeleri ölçülmüştür. İmmün-pozitif hücreler, kontrol grubunda deney gruplarına göre daha az yoğunluk göstermiştir. Bununla birlikte, tüm deney gruplarında renal ve hepatik dejenerasyonlar görülmüştür. Tüm biyokimyasal analiz sonuçları, kontrol grubu hariç deney gruplarında oksidatif stres oluştuğunu göstermektedir. Çalışmamızda elde ettiğimiz sonuçlar, deney gruplarında kullanılan tüm restoratif dental materyallerin böbrek ve karaciğer dokusunda sitotoksik etki yarattığını göstermektedir. Çalışmamızda kullanılan restoratif dental materyaller, tüm deney gruplarında böbrek ve karaciğer dokularında oksidatif stres oluşmasıyla ilişkilendirilebilir.

Anahtar Kelimeler: Böbrek hasarı, Cam iyonomer, Dental amalgam, Karaciğer hasarı, Rezin kompozit.

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INTRODUCTION

Dental disorders are some of the most severe, persistent and critical health conditions and can have a major negative effect on the quality of life. There are several prevention options for these dental diseases; for example, dental filling is the most common treatment method for dental decay. Amalgam, resin composites and glass ionomers are widely used for a dental filling; however, these products are xenobiotic agents that which are known to have significant iatrogenic effects (1).

Amalgam, which has been commonly used for dental restoration for the last 150 years, is still a common xenobiotic drug. This is composed of mercury (50 percent), silver (35 percent) and tin (15 percent) (2). It is generally accepted that these products contribute significantly to the accumulation of metals in human tissues (3).

The use of resin composites in the dental filling is generally popular and has been used as an alternative to amalgam over the last decades. Resin composites consist of non-polymerized monomers, including bisphenol-A-glycidylmethacrylate (BisGMA), hydroxyethylene methacrylate (HEMA), urethane dimethacrylate (UDMA), and triethylene glycoldimethacrylate (TEGDMA) (4).

Glass ionomers, which are water-soluble, can be used as temporary and/or permanent filler products, crack sealants and bridge bonding. A significant level of fluoride release is the most critical feature of glass ionomers. This also contains elevated amounts of aluminum ion (5).

Oxidative stress is considered to be an issue with oxygen-related disruption in biological systems; nevertheless, cells are shielded from oxidative stress by enzymatic or non-enzymatic compounds of antioxidant activity. Oxidative stress is also correlated with the stimulation of the nuclear factor-kappa B (NF-κB) pathway, which is one of the important transcription factors that control the expression of genes that lead to the inflammatory cycle. Many molecules, such as the reactive oxygen species (ROS), are free electrons-containing compounds normally bound to oxygen and have the ability to influence the activity of NF-κB. There is evidence in current literature that inhibition of NF-κB activation can minimize lung, liver, and renal injury (6).

Dental filling products can have certain harmful consequences on the body as a whole owing to their poisonous properties. They will quickly travel across the bloodstream for delivery of other body tissues (7,8). The goal of this research was therefore to explore the potential effects of dental restorative materials on the liver and kidney tissues of rats. We have carried out comprehensive immunohistochemistry, biochemical and histological analyses to accomplish this aim.

MATERIALS and METHODS

Animals

Test rats were bought from the Scientific Experimental Testing and Development Center and the project was authorized by the Local Ethics Committee for Animal Studies (B.30.2.ATA.0.01.02/1885). In this sample, 32 female Wistar albino rats, 180-200 g and 12 weeks of age, were housed at temperatures between 19ºC and 22ºC at normal 12-hour light-dark cycle. The animals were divided into 4 groups (n=8); Group 1: Control, Group 2: dental amalgam, Group 3: resin composite and Group 4: glass-ionomer group. The optimal doses of the filling materials were calculated according to the data from the current literature. The 20 mg/kg dosage was selected as the best dosage for all experimental groups (9).

Experimental Procedures

The dental amalgam (Avalloy; Cavex, Haarlem, The Netherlands), resin composite (Filtek Z550; 3M ESPE, Schaumburg, IL, USA), and glass ionomer (Fuji II LC Capsule; GC Europe, Leuven, Belgium) were prepared in the form of 20 mg small spheres.

The capsule-formed amalgam was mixed via amalgamator (RotoMix; 3M ESPE, Schaumburg, IL, USA). The final concentration of the mixed amalgam was separated into 20 mg pieces, and the specimens were set aside for 48 hours. The resin composite specimens were subjected to an LED light source (VALO LED, Ultradent, South Jordan, UT, USA) for 40 seconds. Furthermore, the glass ionomer cement (GIS) capsules were mixed via an amalgamator, separated into 20-mg small spheres, and then set aside for 24 hours.

Each group of rats was anesthetized via 20-mg/kg thiopental sodium (Pentothal sodium IV Ampul, Abbott Lab, Istanbul, Turkey), and 2-3 cm lateral incisions were made on the backs of their heads; then, the dental filling materials were fixed in this area. The incision line was sutured and, after the surgery, 25 mg/kg sodium metamizole was administered to rats for 2 days as an analgesic. Amoxicillin was given to rats at a dosage of 1.75 mg/kg once every day for 1 week against any infection caused by pathogenic microorganisms. The rats were kept alive in the same conditions for 8 weeks.

Histological Examinations

After a high dose of anesthetic euthanasia, the kidney and liver tissues were removed via necropsy, and quickly fixed for histological examination in 10% buffered formalin for 24 to 48 h. After the routine preparation of the samples according to conventional light microscopic techniques and the samples have been embedded in paraffin. Then, 5 μm sections were obtained with a microtome (Leica RM2125RT, Leica Biosystems GmbH, Nussloch, Germany) from a paraffin block. Sections have been stained with Hematoxylin&Eosin for examination in the light microscope (Nikon Eclipse E600, Nikon Instech Co., Kanagawa, Japan) and photographed with a light microscope camera system (Olympus DP72, Olympus Optical Co., Tokyo, Japan).

Immunohistochemical Examinations

The NF-κB protein (p65) was used as an immune marker in the liver and kidney, and poly-L lysine-coated slides were used for the immunohistochemistry testing. The primary antibodies (NF-κB p65 Antibody (F-6) Cat. No: sc-8008, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used in a 1:80 dilution for 30 min at 37°C. The sections were placed in an automatic immunohistochemical staining machine (Ventana BenchMark GX System, Roche Diagnostics, Arizona, USA), undergoing and nuclear factor kappa B (NF-κB) staining. The specimens were first incubated with the diluted antibody used as a chromogenic agent, followed by a universal detection kit (UltraView Universal DAB Cat. No: 05269806, Roche Diagnostics, Arizona, USA). Hematoxylin (Hematoxylin Cat No: 05266726, Roche Diagnostics, Arizona, USA) was used as a counterstaining agent.

Biochemical Examination

The rats' livers and kidneys were obtained and preserved at -80°C for 3 days. For the homogenate preparation, the tissues have been placed in liquid nitrogen. Then, 0.5 g of the ground tissues were mixed with a buffer (4.5 ml), and the samples were placed in a homogenizer (IKA T18 Ultra-Turrax, Merck KGaA, Darmstadt, Germany) and homogenized on ice for 15 minutes. The samples were processed and centrifuged at 15000 rpm for 15 min and the supernatants were used for the study. Moreover, all of the analyses were performed at room temperature in triplicate (10,11). Then, the catalase (CAT) and superoxide dismutase (SOD) activities and glutathione (GSH) and lipid peroxidation (LPO) levels were determined.

Statistical Analysis

To assess the significance of the observed differences, we used a one- variance analysis (ANOVA) followed by a multiple- Duncan test. All statistical calculations were made using the Windows

IBM SPSS 17.0 software (IBM Software, New York, USA) and the statistical significance was set at P<0.05.

RESULTS

Histopathological Results of Liver Tissue

The liver parts had a regular appearance in the control group. Nevertheless, hepatocyte degeneration in the central vein was visible at first sight in the resin matrix, glass ionomer, and amalgam groups added, while pyknotic and hyperchromatic nuclei were seen in some hepatocytes. In addition, sinusoidal dilation has been observed in the glass ionomer and amalgam groups. Inflammatory cell infiltration in the portal region was found in the resin composite and amalgam groups, and vascular obstruction in the portal veins was also observed in all groups except the control group (Figure 1). The NF-κB immune staining approach was used to show and validate proof of oxidative stress-inducing filling products. No NF-κB positive hepatocytes were found in the control group; however, NF-κB immune positivity was observed in the liver tissue of the resin matrix, glass ionomer and amalgam groups (Figure 2).
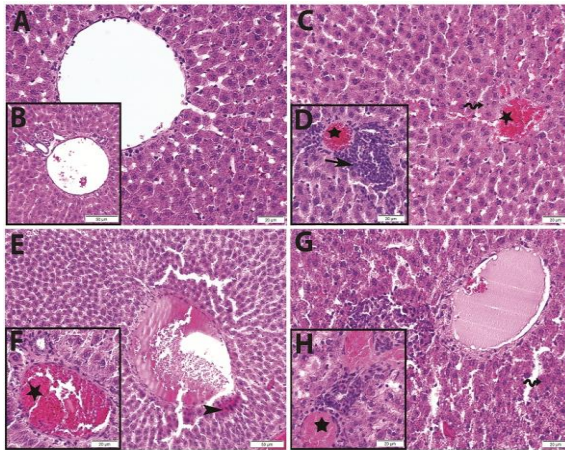

Figure 1. Light microscopic photomicrographs of liver sections (Haematoxylin&Eosin).
A, B: Control group, **C, D:** Resin composite group, **E, F:** Glass-ionomer group, **G, H:** Dental amalgam group. **star;** vascular congestion, **arrow;** inflammatory cells infiltration, **arrow head;** degenerative hepatocytes with dense eosinophilic cytoplasm, **curved arrow:** necrotic hepatocytes.

Şekil 1. Karaciğer kesitlerinin ışık mikroskobik fotomikrografları (Hematoksilin &Eozin).
A, B: Kontrol grubu, **C, D:** Resin kompozit grubu, **E, F:** Cam iyonomer grubu, **G, H:** Dental amalgam grubu. **yıldız;** vasküler tıkanıklık, **ok;** inflamatuar hücre infiltrasyonu, **ok ucu;** yoğun eozinofilik sitoplazmalı dejeneratif hepatositler, **kıvrımlı ok;** nekrotik hepatositler.
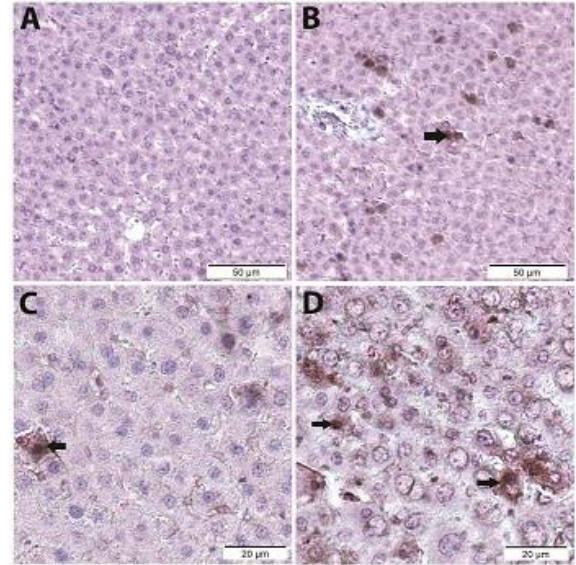

Figure 2. NF-κB positivity of liver tissues of all experimental groups (NF-κB p65).
A: Control group, **B:** Resin composite group, **C:** Glass-ionomer group, **D:** Dental amalgam group. **arrow;** NF-κB positive hepatocytes.
Şekil 2. Tüm deney gruplarının karaciğer dokularının NF-κB pozitifliği (NF-κB p65).
A: Kontrol grubu, **B:** Resin kompozit grubu, **C:** Cam iyonomer grubu, **D:** Dental amalgam grubu. **ok;** NF-κB pozitif hepatositler.

Biochemical Results of Liver Tissue

In this study, we measured LPO levels as an indicator of oxidative stress, as well as CAT and SOD enzyme activity and GSH levels to understand the action of defense mechanisms. The LPO level was significantly lower in the control group relative to the other experimental groups (P<0.05). When the CAT activity was analyzed, it was observed that the CAT activities of the amalgam and glass ionomer groups were different from the control group (P<0.05); however, no significant differences were established between the control and resin composite groups (P>0.05). There have been significant differences in

CAT activity between amalgam and glass ionomer groups (P<0.05). In addition, the findings also revealed that the SOD activity increased while the GSH level decreased in all the filling content groups added relative to the control group (P<0.05). All of the related data are presented in Table 1.

Table 1. Biochemical results of liver tissues in all experimental groups (Mean±Standard Deviation).
Tablo 1. Tüm deney gruplarının karaciğer dokularının biyokimyasal sonuçları (Ortalama±Standart Sapma).

Groups	LPO Level (nmol MDA/g tissue)	SOD Activity (mmol/min/mg tissue)	CAT Activity (µmol/min/mg tissue)	GSH Level (mmol/mg tissue)
Control	12.178±0.40[c]	0.326±0.07[c]	0.708±0.04[c]	2.192±0.11[a]
Dental Amalgam	13.822±0.22[b]	0.351±0.07[b]	0.979±0.10[a]	1.115±0.09[c]
Glass ionomer	15.334±0.27[a]	0.386±0.02[a]	0.852±0.04[b]	1.228±0.11[c]
Resin composite	14.889±0.11[a,b]	0.353±0.04[b]	0.799±0.03[a,c]	1.033±0.06[c]

[a,b,c] Superscripts show statistical differences
LPO: Lipid peroxidation, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione.

Histopathological Results of Kidney Tissue

The histological structures of the kidney tissue were seen as usual in the control group; however, there were some pathological variations in the synthetic resin, glass ionomer and amalgam groups. For example, necrotic glomeruli are unusual in the resin composite and glass ionomer groups, and there were several degenerated tubules when the resin composite, glass ionomer and amalgam groups were evaluated. In addition, erythrocytes were collected in the interstitial region of the amalgam group. In our other experimental groups, abnormalities/damages have arisen in the kidneys; for example, the presence of pycnotic nucleus and eosinophilic cytoplasm in the glomerulus has been found in the resin composite and glass ionomer groups. Increased thickness of the basal membrane was exceptional in the intraglomerular capillary. In addition, mesangial cell proliferation was observed in the resin composite and glass ionomer groups, and selective lymphocyte infiltration and eosinophilic aggregation were also identified in the amalgam groups (Figure 3).
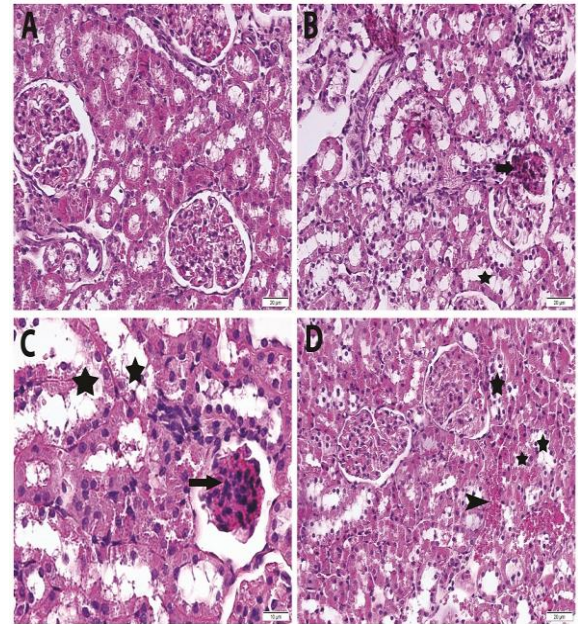

Figure 3. Light microscopic photomicrographs of kidney sections (Haematoxylin&Eosin).
A: Control group, **B:** Resin composite group, **C:** Glass-ionomer group, **D:** Dental amalgam group. **arrow;** necrotic glomerulus, **arrow head;** erythrocyte deposits in the renal interstitial space, **star;** degenerative tubules.
Şekil 3. Böbrek kesitlerinin ışık mikroskobik fotomikrografları (Hematoksilin&Eozin).
A: Kontrol grubu, **B:** Resin kompozit grubu, **C:** Cam iyonomer grubu, **D:** Dental amalgam grubu., **ok;** nekrotik glomerulus, **ok ucu;** renal interstisyal alanda eritrosit birikintisi, **yıldız;** dejeneratif tübüller.

The NF-κB immune staining procedure was also used for renal tissue, and the non-specific NF-κB immune positivity was calculated in the control group areas. There were several healthy NF-κB cells in the glomerulus; nevertheless, non-specific NF-κB positivity was found in the tubules of all the filling products used in the glomerulus (Figure 4).
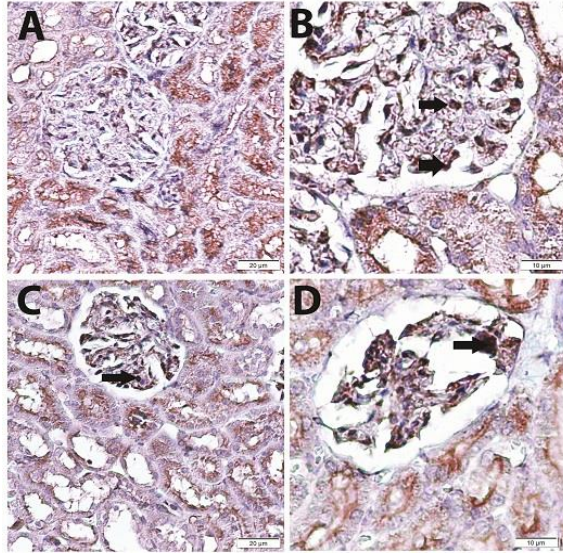

Figure 4. NF-κB positivity of kidney tissues of all experimental groups (NF-κB p65).

A: Control group, **B:** Resin composite group, **C:** Glass-ionomer group, **D:** Dental amalgam group. **arrow;** NF-κB positive endothelial cells.
Şekil 4. Tüm deney gruplarının böbrek dokularının NF-κB pozitifliği (NF-κB p65).
A: Kontrol grubu, **B:** Resin kompozit grubu, **C:** Cam iyonomer grubu, **D:** Dental amalgam grubu. **ok;** NF-κB pozitif endotel hücreleri.

Biochemical Results of Kidney Tissue

Biochemical details from kidney tissue are provided in Table 2. Cellular consequences of oxidative stress LPO rates were statistically tested and were shown to be lower in the control group relative to amalgam, glass ionomer, and resin composite groups (P<0.05). In fact, anti-oxidant processes have played a part. Statistically significant differences were found between the control and filling content groups used in terms of GSH and CAT levels (P<0.05). In addition, differences between the groups could be found when the GSH and CAT levels were assessed. Interestingly, the SOD activity was not statistically different from the point of the control group (P>0.05).

Table 2. Biochemical results of kidney tissues in all experimental groups (Mean±Standard Deviation).
Tablo 2. Tüm deney gruplarının böbrek dokularının biyokimyasal sonuçları (Ortalama±Standart Sapma).

Groups	LPO Level (nmol MDA/g tissue)	SOD Activity (mmol/min/mg tissue)	CAT Activity (μmol/min/mg tissue)	GSH Level (mmol/mg tissue)
Control	10.400±0.22[c]	2.192±0.24[a,b]	0.94±0.06[c]	0.554±0.03[c]
Dental Amalgam	12.400±0.31[b]	2.112±0.12[b]	0.918±0.04[b]	0.628±0.06[b]
Glass ionomer	14.933±0.62[a]	2.202±0.18[a]	0.918±0.07[b]	1.188±0.02[a]
Resin composite	13.200±0.15[b]	2.208±0.21[a]	1.188±0.12[a]	0.607±0.04[b]

[a,b,c] Superscripts show statistical differences.
LPO: Lipid peroxidation, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione.

DISCUSSION and CONCLUSION

Oral disorders, such as decay, are one of the most significant health issues that the quality of life. Amalgam, resin composites, and glass ionomers are mostly used for a dental filling, and the specific properties of such products are the movement from the oral cavity to the bloodstream (9).

Amalgam is the primary cause of human access to mercury which contributes to the storage of this element in the tissues. Mercury is emitted by warmth in the oral cavity, chewing, washing, biological degradation owing to bacteria and electrochemical erosion. The average regular consumption of Hg amalgam vapor is approximately 9 μg (12), and the emitted Hg vapor is consumed by the lungs and gastrointestinal tissues. In-vivo and in-vitro research have also demonstrated that amalgams are the primary cause of mercury exposure, one of the well-known metals that are extremely harmful to human tissue (9). Horsted-Bindslev (13) has demonstrated that amalgam filling allows the movement of mercury to blood and urine. The adverse effects of

the key component of amalgam were observed using various approaches, and the findings of our amalgam group confirmed the above conclusions and information.

The biological reaction to dental restorative composites was mainly due to the release of monomers. Published monomers will quickly migrate to the pulp and gingival tissue of the teeth and enter the flowing blood (13). Co-monomers, such as HEMA and TEGDMA, may cause deformation and carcinogenic mutations in the nucleus; however, human gingival fibroblasts have been shown to trigger DNA damage (14). Bis-GMA contains a lot of synthetic content (15), and it is capable of growing DNA double-strand breaks in gingival fibroblast cells, whereas HGF, TEGDMA, and HEMA culture can bind to the cell membrane and easily influence membrane permeability (16). The histological, immunohistochemistry and biochemical findings of this study have demonstrated the toxic effects of resin composites in the associated experimental group.

Glass ionomer cement produce large levels of fluoride and high levels of aluminum ions (17), which corrupts the synthesis of RNA, DNA, and protein (18). In fact, studies have shown that AlF molecules of G protein receptors damage this enzyme and inhibit the signal transduction pathway (19).

Oxidative stress is a very significant agent that leads to the pathophysiology of cytotoxicity. LPO is an oxidative stress measure and is dangerous due to unregulated, self-reinforcing processes that allow membrane lipids and other cell components to be disturbed. In other terms, it is a result of a cell injury. Responses to SOD and CAT, which are enzymatic antioxidants, as well as GSH, which are non-enzymatic antioxidants, have been identified when tissue damage has been detected in the kidney and liver (20). Dental filling materials include harmful substances that may induce significant toxicity of organs and cells. Throughout this study, we described and examined the cytotoxicity and potential involvement pathways of glass ionomers, amalgam and resin composites in the liver and kidney tissues.

The liver plays a vital role in the digestion of lipids, carbohydrates and proteins and performs tasks such as bile development, vitamin absorption, and drug and toxin detoxification. Mercury, resin composites, fluoride, and aluminum ions have extremely reactive effects, and when metabolized in the liver, they can quickly impact the liver in a reactive manner (9). Among this study, the LPO level, which is a measure of oxidative stress in the liver, was higher in the groups of restorative dental materials than in the control group.

Antioxidant enzymes are critical for the removal of reactive oxygen species produced under oxidative stress. In our study, the activity of CAT and SOD increased in exposed liver tissue amalgam, resin composite and glass ionomer relative to control liver tissue. This indicates that liver damage will contribute to a rise in oxidative stress-mediated enzyme activity. In addition, hepatocytes were harmed by DNA and protein degradation during the oxidative stress cycle.

Organisms use GSH to remove hydrogen peroxide (H_2O_2) and other peroxides, and the GSH level suggests the existence of a protection mechanism against ROS. In fact, the broken GSH cellular balance leads to the initiation of the apoptotic cascade (21). In our study, decreased GSH levels were calculated in the experimental community relative to the control group.

Our results for dental amalgam, resin composite, and glass ionomer showed that the liver was significantly affected by its toxic content. Histological and immunohistochemical data from the liver tissues of all experimental groups have shown that the nucleus of the hepatocytes is brittle and hyperchromatic. In fact, it is well recognized that NF-κB is a structural component of oxidative stress in the blood. In our study, many immune-positive cells have been detected in all groups of liver cells, except for control, which is one of the confirmations of the presence of oxidative stress in liver tissue.

The current knowledge about amalgam and its toxic effects has been confirmed in some scientific studies; for example, Jagadeesan et al. (22) showed mercury lead toxicity in the rat liver, when they examined the oxidative stress parameters. In this study, the LPO level, which is an indication of cell injury, was significantly increased in the HgCl$_2$-applied community. In addition, dental resin composite monomers have caused ROS production (23). Another study found that TEGDMA and HEMA, which are dental resin composite monomers, caused apoptosis and cell-cycle arrest (24).

The kidney tends to be a vital organ for amalgam, resin composite, and glass ionomer toxicity. First, we attempted to measure LPO levels in the kidney tissue and found elevated LPO rates in the kidneys in the amalgam, resin composite, and glass ionomer groups. In addition, LPO is an indication of oxidative stress, and the rising levels of LPO suggest membrane damage in the cell (6). For our study, we analyzed the SOD, CAT and GSH levels and noticed that the SOD activities in the amalgam, resin composite and glass ionomer groups were similar to those in control. For CAT activity, statistically significant differences in resin composite and amalgam groups were found relative to the control group. The CAT activity in the glass ionomer group was similar to the data of the control group. Nevertheless, the GSH level only increased in the glass ionomer group, as opposed to the CAT activity. This means that kidney damage can lead to improvements in oxidative stress-mediated enzyme function.

In our histopathological tests, there were many positive NF-κB cells in the dental filling test groups. During the oxidative stress cycle, the cells were harmed by the destruction of DNA and some proteins. In our histological analysis of the kidney, lymphocyte infiltration and eosinophilic aggregation in the amalgam groups is defined. Sharma et al. (25) have demonstrated that there has been substantial damage to the kidney tissue. High levels of LPO and antioxidant activity in the Hg induced kidney were established and histological damage was observed, especially in the glomerulus (25).

In conclusion, our results showed that all the restorative dental materials used in our study have a certain cytotoxic impact on the liver and kidney tissues. In comparison, oxidative stress can also occur in the liver and kidney tissue by the cytotoxic effects of dental filling products. Overall, close attention should be given to the use of all dental filling products.

Conflict of interest

The authors declare that they have no conflict of interest.

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