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Sesamin Protects Mouse Liver against Nickel-Induced Oxidative DNA Damage and Apoptosis by the PI3K-Akt Pathway

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ABSTRACT: Sesamin (Ses), one of the major lignans in sesame seeds and oil, has been reported to have many benefits and medicinal properties. However, its protective effects against nickel (Ni)-induced injury in liver have not been clarified. The aim of the present study was to investigate the effects of sesamin on hepatic oxidative DNA injury and apoptosis in mice exposed to nickel. Kunming mice were exposed to nickel sulfate with or without sesamin coadministration for 20 days. The data showed that sesamin significantly prevented nickel-induced hepatotoxicity in a dose-dependent manner, indicated by both diagnostic indicators of liver damage (serum aminotransferase activities) and histopathological analysis. Moreover, nickel-induced profound elevation of reactive oxygen species (ROS) production and oxidative stress, as evidenced by an increase of the lipid peroxidation level and depletion of the intracellular reduced glutathione (GSH) level in liver, were suppressed by treatment with sesamin. Sesamin also restored the activities of antioxidant enzymes (T-SOD, CAT, and GPx) and decreased 8-hydroxy-2-deoxyguanosine (8-OHdG) levels in nickel-treated mice. Furthermore, a TUNEL assay showed that nickel-induced apoptosis in mouse liver was significantly inhibited by sesamin. Exploration of the underlying mechanisms of sesamin action revealed that activities of caspase-3 were markedly inhibited by the treatment of sesamin in the liver of nickel-treated mice. Sesamin increased expression levels of phosphoinositide-3-kinase (PI3K) and phosphorylated protein kinase B (PBK/Akt) in liver, which in turn inactivated pro-apoptotic signaling events, restoring the balance between pro- and anti-apoptotic Bcl-2 proteins in the liver of nickel-treated mice. In conclusion, these results suggested that the inhibition of nickel-induced apoptosis by sesamin is due at least in part to its antioxidant activity and its ability to modulate the PI3K-Akt signaling pathway.

KEYWORDS: *sesamin, nickel, DNA injury, apoptosis, Akt, liver*

INTRODUCTION

Sesame seeds (*Sesamum indicum*) and their oil have been used in human diets for thousands of years and are believed to provide health benefits. Sesamin (Ses) is one of the major lignans in sesame seeds and oil. Several studies have shown that sesamin exerts antioxidative,[1,2] anti-inflammatory,[3−5] antihypertensive,[6−8] cholesterol-lowering,[9] anticarcinogen,[4,10] hepatoprotective,[11,12] renoprotective,[7,8] and neuroprotective effects.[5,13] Thus, sesamin seems to be one of the most reliable food factors, the physiological effects of which can be expected by individuals taking it as a supplement or remedy.[14] Increasing evidence shows that sesamin can protect the liver from injury induced by hepatotoxins.[12,15−17] Despite those pharmacological benefits, the molecular mechanisms by which sesamin elicits hepatoprotective effects are still unclear.

Nickel (Ni) is one of the most widely used metals in modern industry. The high consumption of nickel-containing products inevitably leads to environmental pollution by nickel and its byproducts at all stages of production, recycling, and disposal. Human exposure to nickel occurs primarily via inhalation and ingestion.[18] After entering the body, nickel penetrates all organs and accumulates primarily in bone and in the liver, heart, lungs, fat, peripheral nervous tissue, and brain,[19] which induces tissue damage. Although the precise mechanism of liver toxicity caused by nickel is not clear, there is evidence that Ni can cause generation of reactive oxygen metabolites and inhibit the activity of antioxidant enzymes in liver tissue.[19,20] Studies have demonstrated that Ni can induce DNA injury in the liver, and these effects were shown to be associated with reactive oxygen species (ROS) formation and apoptosis.[21−24]

The phosphoinositide-3-kinase (PI3K)-Akt signaling pathway plays a crucial role in cell growth and cell survival. The PI3K-Akt signaling pathway can be activated by many types of cellular stimuli or toxic insults.[25] Serine/threonine kinase Akt/PKB is the primary mediator of PI3K-initiated signaling. Activated Akt by PI3K regulates cell survival through phosphorylation of a variety of downstream targets such as pro-apoptotic protein, transcription factors, and another protein kinase.[26,27] The PI3K-Akt pathway can also mediate some of its survival signals through the Bcl-2 family.[27,29] Previous studies reported that Ni-induced apoptosis was associated with the PI3K-Akt pathway.[30−33] In the present study, we aimed to determine whether sesamin can protect mouse liver from Ni-induced DNA damage and apoptosis by modulating the PI3K-Akt pathway.

MATERIALS AND METHODS

Chemicals and Reagents. Sesamin (>95% purity) and nickel sulfate ($NiSO_4$) were obtained from Sigma Chemical Co. (St. Louis, MO, USA); anti-Bcl-2 antibody, anti-cleaved caspase-3, anti-Bax antibody, anti-PI3K p110 antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA); rabbit anti-Akt (total) antibody, rabbit anti-phospho-Akt (Ser473) antibody, and goat anti-rabbit IgG-HRP from

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Cell Signaling Technology (Beverly, MA, USA); and reagents and kits used in the assays of reduced glutathione (GSH) levels, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities in serum from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). A BCA assay kit was obtained from Pierce Biotechnology, Inc. (Rockford, IL, USA) and a TUNEL apoptosis detection kit from GenScript Corp. (Piscataway, NJ, USA). All other reagents unless indicated were obtained from Sigma Chemical Co.

Animals and Treatment. Kunming mice are the most widely used as a model for hepatic damage.[34] In this study, we used an experimental model of mice treated with nickel sulfate as a model of Ni-induced hepatic damage. Kunming mice (male, 4 weeks old, weighing approximately 18 g) were purchased from the Branch of National Breeder Center of Rodents (Beijing). The mice were maintained under constant conditions (23 ± 1 °C and 60% humidity) and had free access to rodent food and tap water under a 12 h light/dark schedule (lights on from 8:30 a.m. to 8:30 p.m.).[27]

After acclimatization to the laboratory conditions, the mice were randomly divided into six groups (10 mice in each). Group I served as control (treated intraperitoneally with isotonic saline for 20 days). Group II animals received intraperitoneally nickel sulfate (20 mg/kg/body weight, daily) following a daily oral gavage administration of 0.5% carboxymethylcellulose sodium for 20 days. Group III and IV animals received intraperitoneally nickel sulfate (20 mg/kg/body weight, daily) following a daily oral gavage administration of sesamin (60 and 120 mg/kg body weight, suspended in 0.5% carboxymethylcellulose sodium). Group V and VI animals were orally administered sesamin (60 and 120 mg/kg body weight, suspended in 0.5% carboxymethylcellulose sodium, daily).[8] The nickel sulfate dose is based on previous findings[35] and indicates the level of toxic intake of nickel in occupational exposure and some emergency situations.[19] The sesamin dose is based on previous findings, which showed that sesamin has protective effects on tissue damage.[8]

The experiment lasted for 20 days. At the end of treatment, mice were sacrificed and about 1 mL blood samples were drawn by cardiac puncture with heparinized tubes. The liver tissues were immediately collected for experiments and placed in ice-cold 0.9% NaCl solution, perfused with physiological saline solution to remove blood cells, and blotted on filter paper. Then the removed brain was immediately collected for experiments or stored at −70 °C for later use.

The present research reported in this paper was conducted in accordance with Chinese legislation and the NIH publication on the use and care of laboratory animals and was approved by the respective university committees for animal experiments.

Measurement of Serum Aminotransferase Activities. The activities of ALT and AST in serum were estimated spectrophotometrically using commercial diagnostic kits (Jiancheng Institute of Biotechnology).[36]

Histological Evaluations. The histological changes of liver were evaluated as described by us.[36,37] Cryosections were collected on 3-aminopropyltrimethoxysilane-coated slides (Sigma-Aldrich). The liver slices were stained with hematoxylin and eosin.

Deoxyribonucleotidyl Transferase (TdT)-Mediated dUTP-Fluorescein Isothiocyanate (FITC) Nick-End Labeling (TUNEL) Assay. For the TUNEL staining, the standard protocol for frozen sections was followed (BD ApoAlertTM DNA Fragmentation assay kit, BD Biosciences Clontech, Palo Alto, CA, USA). Apoptosis was assayed by TUNEL staining using our previous method.[36]

Assay of ROS Level. ROS was measured as described in our and others' previous papers, based on the oxidation of 2′7′-dichlorodihydrofluorescein diacetate to 2′7′-dichlorofluorescein.[37]

Assay of Thiobarbituric Acid Reactive Substances (TBARS) Levels in Liver. Tissue lipid peroxidation was measured according to our previous method.[36,37] Liver homogenate was incubated with 8.1% SDS (w/v) for 10 min followed by the addition of 20% acetic acid (pH 3.5). The reaction mixture was incubated with 0.6% TBA (w/v) for 1 h in a boiling water bath. Pink color chromogen was extracted in a butanol/pyridine solution (15:1) and read at 532 nm.

Assay of Liver Reduced Glutathione (GSH) Level. Liver GSH levels were measured using the commercial kits (Jiancheng Institute of Biotechnology).[37] GSH concentration was expressed as milligrams per gram protein.

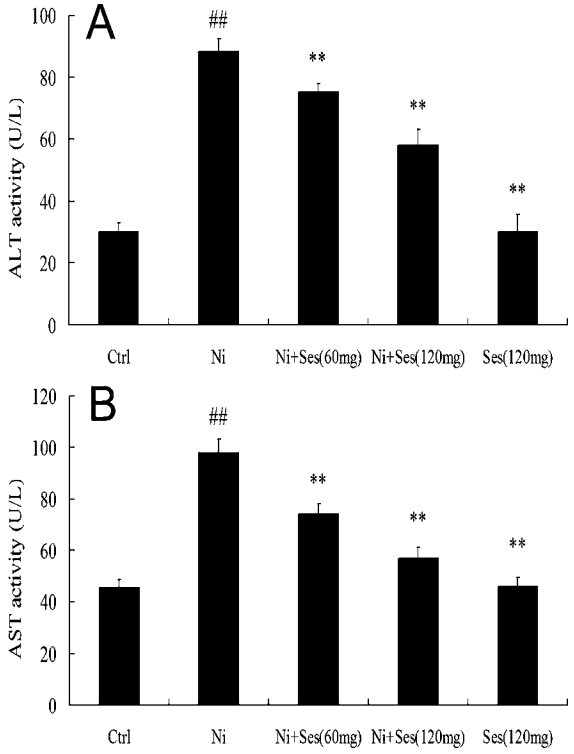

Figure 1. Effect of sesamin on nickel-induced changes in hepatic functional markers: (A) ALT activity; (B) AST activity. All values are expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the nickel-treated group.

Measurement of Liver Antioxidative Enzyme Activities. The activities of total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GPx) in liver were estimated spectrophotometrically using commercial diagnostic kits (Jiancheng Institute of Biotechnology).[36,37]

Assay of Nuclear 8-Hydroxy-2-deoxyguanosine (8-OHdG) Level. Nuclear 8-OHdG contents in liver were assayed as described in our previous paper.[36] Briefly, to prevent 8-OHdG formations as a byproduct during DNA isolation, nuclear DNA was extracted with a commercially available DNA extractor kit (Wako Pure Chemical Industries, Ltd.) containing an antioxidant NaI solution to dissolve cellular components. For further prevention of autoxidation in the cell lysis step, deferoxamine mesylate (Sigma Chemical Co.) was added to the lysis buffer.[38] The DNA was digested to deoxynucleotides with nuclease P1 and alkaline phosphatase, and levels of 8-OHdG (8-OHdG/10^{-5} deoxyguanosine) were assessed by high-performance liquid chromatography (HPLC) with an electrochemical detection system (Agilent Technologies, USA).

Western Blot Analyses. Tissues were homogenized in ice-cold lysis buffer (TBS, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 10 mg/mL aprotenin, 10 mg/mL leupeptin, 1 mM PMSF, 10 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mM NaF). Homogenates were centrifuged at 10000g for 10 min at 4 °C. The supernatants were collected and centrifuged again, and the final supernatants were collected. Nuclear and cytoplasmic extracts for Western blotting were obtained by using a nuclear/cytoplasmic isolation kit (Beyotime Institute of Biotechnology, Beijing, China). Protein levels were determined using the BCA assay kit (Pierce Biotechnology, Inc.). Samples (80 μg each) were separated by denaturing SDS-PAGE and transferred to a PVDF membrane (Roche Diagnostics Corp., Indianapolis, IN, USA) by electrophoretic transfer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membrane was preblocked with 5% BSA and 0.1% Tween-20 in Tris-buffered saline (TBST) and incubated overnight with the primary antibody (in TBST with 5% BSA). Each membrane was washed

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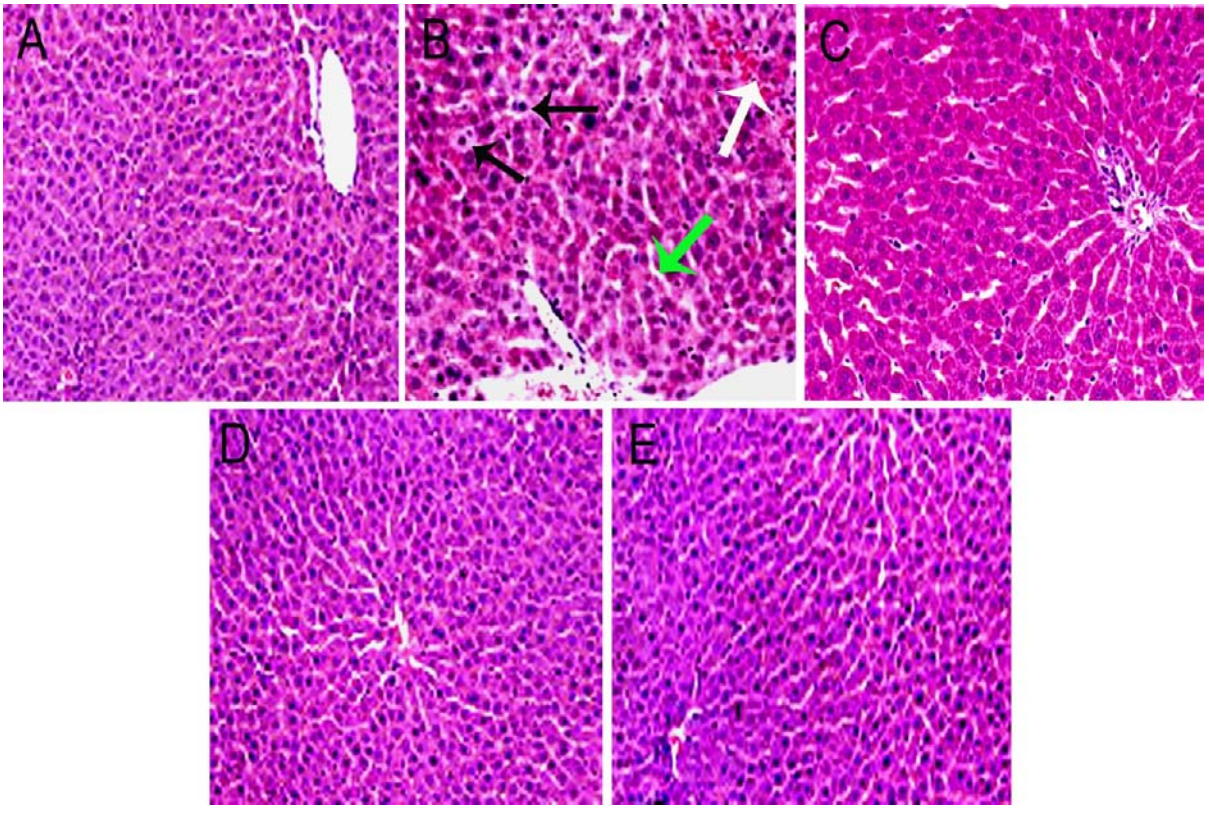

Figure 2. Morphological and histological evaluation of liver in mice: (A) control group; (B) mice treated with nickel sulfate (20 mg/kg); (C) mice treated with nickel sulfate (20 mg/kg) and fed sesamin (60 mg/kg); (D) mice treated with nickel sulfate (20 mg/kg) and fed sesamin (120 mg/kg); (E) mice fed sesamin (120 mg/kg). The white arrow indicates infiltrating leukocytes. The black arrow indicates hepatic cell necrosis. The green arrow indicates the enlarged sinusoids between the plates of hepatocytes. Original magnification, 10×10.

three times for 15 min and incubated with the secondary horseradish peroxidase-linked antibodies (Santa Cruz Biotechnology and Cell Signaling Technology, respectively). Quantitation of detected bands was performed with the Scion Image analysis software (Scion Corp., Frederick, MD, USA). To prove equal loading, the blots were analyzed for β-actin expression using an anti-β-actin antibody (Cell Signaling Technology). Each density was normalized using each corresponding β-actin density as an internal control and averaged from three samples, and we standardized the density of vehicle control for relative comparison as 1.0 to compare other groups.

Statistical Analysis. All statistical analyses were performed using the SPSS software, version 11.5. A one-way analysis of variance (ANOVA; $P < 0.05$) was used to determine significant differences between groups, and individual comparisons were obtained by Tukey's HSD post hoc test. Statistical significance was set at $P \leq 0.05$.

RESULTS

Sesamin Protects against Nickel-Induced Hepatic Dysfunction. ALT and AST are found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, red blood cells, and other bodily tissues, but are most commonly associated with the liver, so the activities of serum ALT and AST were considered to be serum biochemical markers of diseases hepatic damage.[36,37,39] To determine whether sesamin can attenuate the liver damage in the nickel-treated mouse, we measured the activities of serum ALT and AST (Figure 1). In nickel-treated mice, the activities of serum ALT and AST significantly increased by 194 and 114% as compared with these of the controls, respectively ($P < 0.01$). Interestingly, treatment with sesamin, on the other hand, showed dose-dependent inhibition in this elevation ($P < 0.01$) (Figure 1).

No significant difference of ALT and AST activities could be seen in the serum from the mice of the control group compared with either sesamin (60 or 120 mg/kg) group (data not shown).

Sesamin Alleviated Nickel-Induced Histology Changes in Liver. Liver histological study was used to determine the protective effect of sesamin on nickel-induced injury. As shown in Figure 2, the results of histopathological evaluation showed that sesamin exhibited a protective effect against nickel-induced liver injury. Nickel treatment caused visible histology changes including structure damage hepatocellular necrosis and leukocyte infiltration in mouse liver. In addition, the sinusoids between the plates of hepatocytes were markedly enlarged in the liver of nickel-treated mice (Figure 2B), whereas sesamin treatment significantly alleviated the nickel-induced damage in mouse liver. No visible histological changes in the liver could be observed between the control group and the sesamin group (Figure 2).

Sesamin Inhibited Nickel-Induced Apoptosis in Liver. We used the TUNEL assay to investigate the effect of sesamin on nickel-induced apoptosis (Figure 3). The number of TUNEL-positive cells in the liver of nickel-treated mice was significantly increased ($P < 0.01$), whereas sesamin markedly decreased the liver TUNEL-positive cells of mice treated with nickel in a dose-dependent manner (Figure 3). No significant difference in the number of TUNEL-positive cells in the liver could be seen in the livers from the mice treated with sesamin only as compared with vehicle controls.

Sesamin Inhibited Nickel-Induced Oxidative Stress in Liver. Many studies have suggested that the levels of ROS,

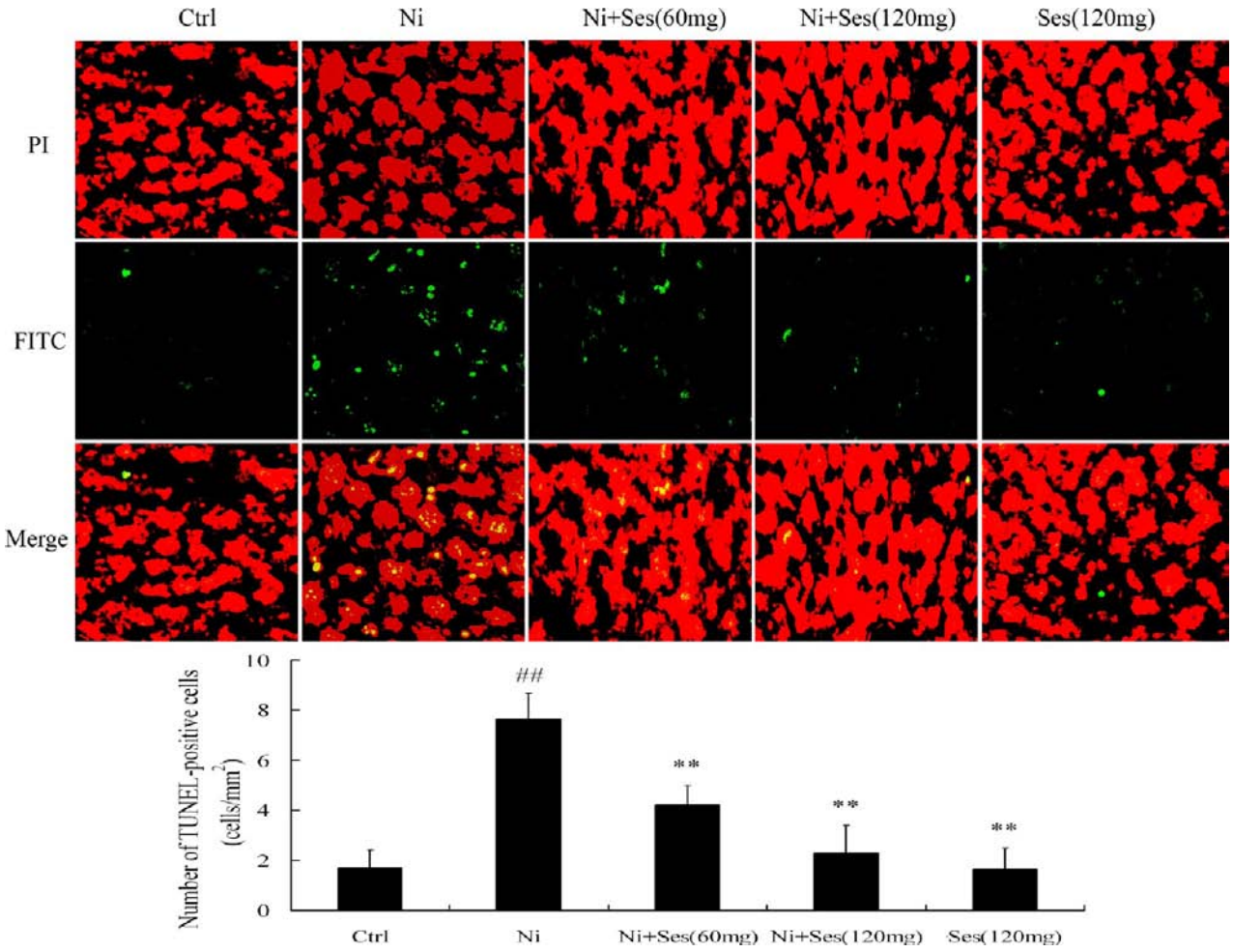

Figure 3. (Top) In situ detection of fragmented DNA [deoxyribonucleotidyl transferase-mediated dUTP-FITC nick-end labeling (TUNEL) assay] in the liver of mice. The liver tissues were processed for TUNEL and photographed using a fluorescence microscope with either a propidium iodide (PI) filter alone (up) or an FITC filter alone (middle). The merged images show that apoptotic cells appear yellow and nonapoptotic cells appear red (bottom). Scale bars = 100 mm. (Bottom) Histogram showing the relative proportion of TUNEL-positive cells in the liver of mice. All values are expressed as the mean ± SEM. (**) $P < 0.01$, compared with the control group; (##) $P < 0.01$, versus the nickel-treated group.

TBARS, and GSH might be indicators of oxidative stress. The results showed that sesamin could decrease nickel-induced ROS and TBARS levels (Figure 4). Nickel treatment markedly increased hepatic ROS and TBARS levels by 66 and 113% as compared with the controls, respectively ($P < 0.01$). However, treatment with sesamin, on the other hand, showed dose-dependent inhibition in this elevation ($P < 0.01$) (Figure 4).

As shown in Figure 4C, the GSH level in nickel-treated mice was markedly decreased by 54% as compared with the control ($P < 0.01$). However, sesamin significantly restored the liver GSH levels of mice in a dose-dependent manner (Figure 4C).

No significant difference of levels of liver ROS, TBARS, and GSH could be seen in the livers from the mice treated with sesamin only as compared with vehicle controls (Figure 4).

Sesamin Inhibited Nickel-Induced Oxidative DNA Damage in Liver. 8-OHdG is one important oxidative DNA lesion formed by the oxidation of the C-8 position of 2′-deoxyguanosine, which has commonly been used as a biomarker of oxidative DNA damage.[35,40] To determine whether sesamin can attenuate liver DNA damage in the nickel-treated mice, we measured the level of 8-OHdG. As shown in Figure 4D, the level of 8-OHdG in nickel-treated mice was significantly increased by 51% as compared with vehicle controls ($P < 0.01$). Interestingly, treatment with sesamin, on the other hand, inhibited this elevation in a dose-dependent manner ($P < 0.01$). There was no significant difference in levels of 8-OHdG between the sesamin group and the control group (Figure 4D).

Sesamin Restored the Activities of Antioxidant Enzymes in Nickel-Treated Mouse Liver. SOD, CAT, and GPx play important roles in maintaining the intracellular redox balance. As shown in Figure 5, hepatic T-SOD, CAT, and GPx activities were significantly decreased in Ni-treated mice by 55, 49, and 53%, respectively ($P < 0.001$). However, treatment with sesamin reversed the hepatic T-SOD, CAT, and GPx activities in a dose-dependent manner ($P < 0.01$) (Figure 5). Interestingly, there were no significant differences in hepatic activities of T-SOD, CAT, and GPx between the sesamin group and the control group (Figure 5).

Sesamin-Mediated Protective Action Involves PI3K-Akt Activation. Activation of PI3K-Akt is known to suppress apoptosis and promote cell survival.[30,41] To investigate whether PI3K-Akt signaling was involved in the action of sesamin, we determined the effects of sesamin on the PI3K-Akt pathway in mouse liver. The results showed that sesamin significantly enhanced the PI3K-Akt pathway in the liver of nickel-treated mice (Figure 6).

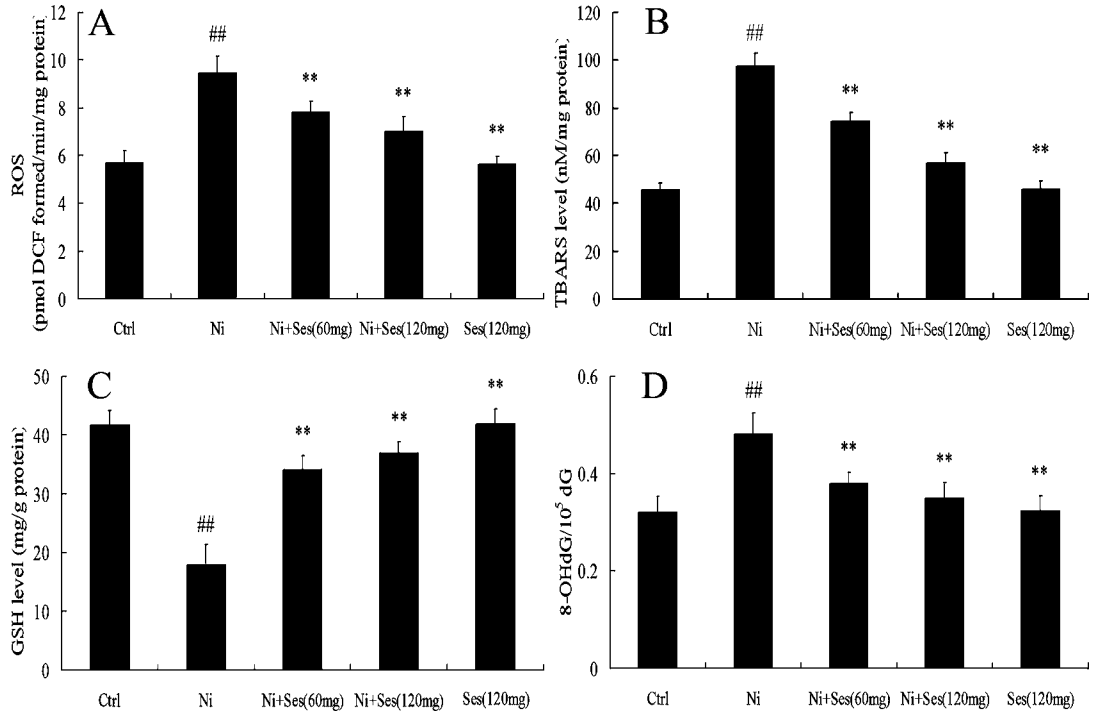

Figure 4. Effect of sesamin on oxidative stress and DNA damage in liver of nickel-treated mice: (A) level of ROS; (B) level of TBARS; (C) level of GSH; (D) level of 8-OHdG. Each value is expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the nickel-treated group.

The levels of PI3K p110 and phospho-Akt (Ser 473) were markedly decreased in the livers of nickel-treated mice as compared with the vehicle controls ($P < 0.01$). However, the down-regulation of PI3K p110 and phospho-Akt (Ser 473) was markedly suppressed in the sesamin and nickel cotreated mice ($P < 0.01$). No signicant changes of PI3K p110 and phospho-Akt (Ser 473) expression were seen between the sesamin-treated mice and the control mice (Figure 6).

Sesamin Modulates Nickel-Induced Expression of Pro-apoptotic Proteins. PI3K-AKT has been shown to regulate the expression of pro-apoptotic and anti-apoptotic members of the Bcl-2 family such as Bcl-2 and Bax, suggesting the involvement of the mitochondrial intrinsic pathway of apoptosis.[41] Therefore, we examined the effects of sesamin on Akt regulated intrinsic pro-apoptotic proteins. As shown in Figure 7, nickel treatment increased the mitochondrial translocation of Bax ($P < 0.01$) and caused a reduction in the expression of the anti-apoptotic protein Bcl-2 ($P < 0.01$). However, sesamin treatment abolished the nickel-evoked pro-apoptotic signaling events in the liver of mice ($P < 0.01$). No signicant changes of Bcl-2 and Bax levels were seen between the sesamin group and the control (Figure 7).

Sesamin Reduced Nickel-Induced Caspase-3 Activation. Caspase-3 is one of the key executioners of apoptosis, capable of cleaving or degrading many key proteins such as nuclear lamins, fodrin, and the nuclear enzyme poly(ADPribose) polymerase (PARP).[42] To determine whether sesamin can attenuate apoptosis in the liver of nickel-treated mice, the activity of caspase-3 was also examined. As shown in Figure 7D, cleaved caspase-3 levels were significantly elevated by 138% as compared with the controls in the nickel-treated mouse liver ($P < 0.01$). Interestingly, treatment with sesamin, on the other hand, inhibited this elevation in a dose-dependent manner ($P < 0.01$). There were no significant differences in the caspase-3 levels between the sesamin group and the control (Figure 7C).

■ DISCUSSION

The liver is a primary site for xenobiotic metabolism and is the most common target organ for chemically induced injuries.[35,36] Liver is susceptible to Ni toxicity because it plays a major role in detoxification and also has the ability to produce metallothionin, a low molecular weight protein having a high affinity for nickel.[43] Previous studies have shown that nickel exposure caused liver injuries and dysfunction, which is followed by elevated levels of serum enzymes indicating cellular leakage and loss of functional integrity of the hepatic membrane.[33,44,46] The present investigation also reveals that the activities of AST and ALT in the serum of nickel-treated mice were markedly increased (Figure 1). Moreover, histological changes of the liver, such as structure damage, hepatocellular necrosis, leukocyte infiltration, and massive hemorrhage, had been observed in lead-treated animals (Figure 2). In this study, treatment with sesamin effectively protected mice against nickel-induced liver damage by reducing elevated serum ALT and AST activities (Figure 1) and alleviating hepatic histological changes (Figure 2). These results suggest that sesamin could protect mice against nickel-induced hepatic dysfunction and histopathologic damage.

Many studies suggest that one possible molecular mechanism involved in nickel hepatotoxicity is the disruption of delicate oxidant/antioxidant balance, which can lead to liver injury via oxidative damage.[36] Accumulating evidence has also shown that nickel causes oxidative stress by inducing the generation of ROS. The participation of free radicals in nickel toxicity may occur at different levels: (i) reactivity of Ni(II) with oxygen derivatives; (ii) incubation of Ni(II) with cysteine in an aerobic environment, generating the hydroxyl radical and the carbon-centered alkyl radical; (iii) reaction of Ni(II)−thiol complexes

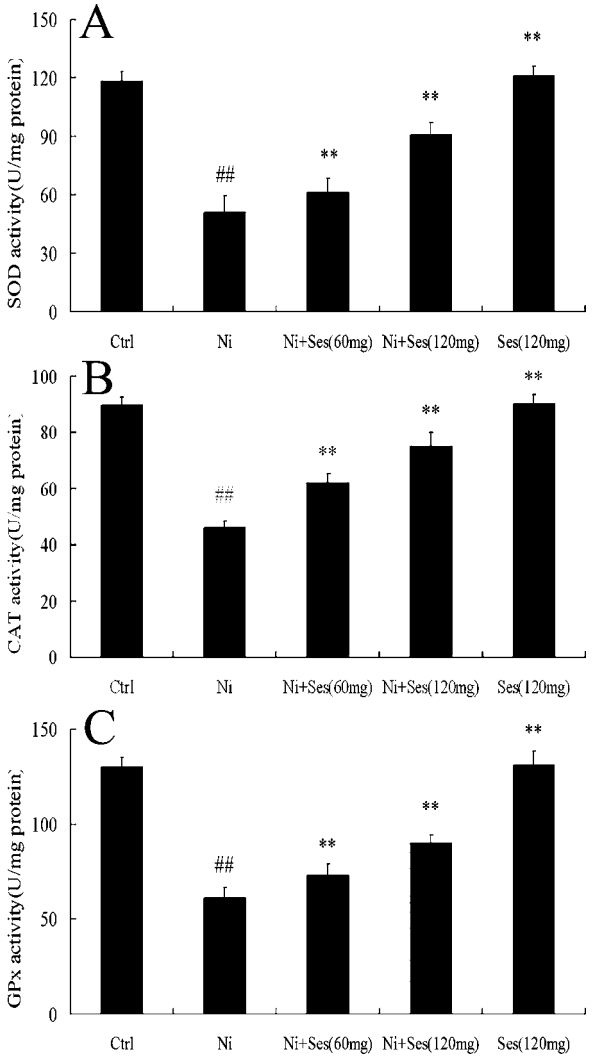

Figure 5. Effect of sesamin on the activities of antioxidant enzymes in liver of nickel-treated mice: (A) T-SOD; (B) CAT; (C) GPx. Each value is expressed as the mean ± SEM (n = 7). (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the nickel-treated group.

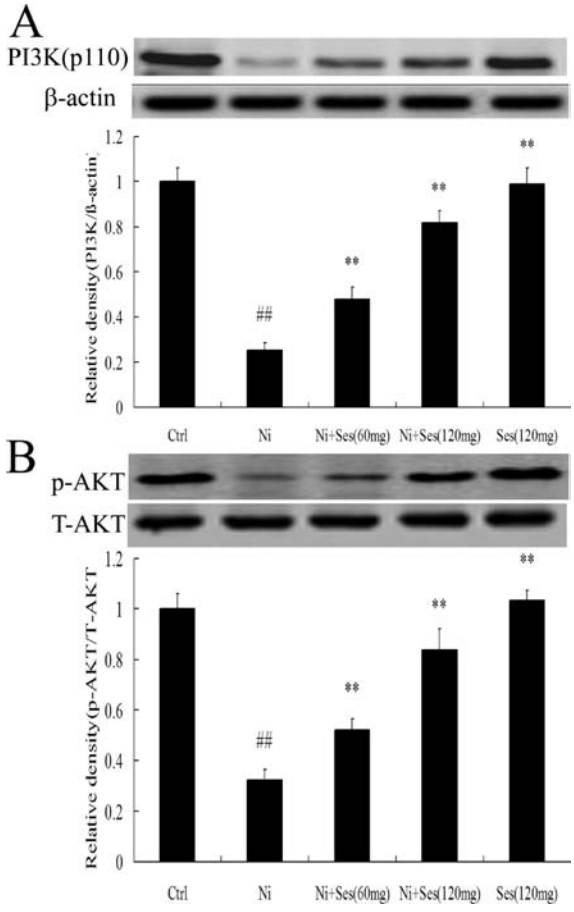

Figure 6. Protective effect of sesamin against nickel-induced apoptosis depends on the PI3K-Akt pathway: (A) effect of sesamin on the expression of the proteins in association with the PI3K-Akt pathway in mouse liver; (B) relative density analysis of the PI3K P110 protein bands; (C) relative density analysis of the P-Akt protein bands; β-actin was probed as an internal control in relative density analysis of the PI3K and Akt protein bands. The relative density is expressed as the ratio (PI3K P110/β-actin, phospho-Akt/Total-Akt). The vehicle control is set as 1.0. Values are averages from seven independent experiments. Each value is expressed as the mean ± SEM. (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the nickel-treated group.

and molecular oxygen and/or lipid hydroperoxides.[19] In the present study, we demonstrated nickel exposure induced overproduction of ROS in the mouse and led to hepatic oxidative damage. Moreover, the concentration of TBARS is considered to be a major oxidative stress marker. Numerous studies have suggested that nickel exposure could induce the increase of TBARS level in liver.[19,33,45] In the present research, levels of ROS and TBARS were remarkably increased in nickel-treated mouse liver as compared with the control, indicating that nickel exposure induced oxidative stress. Sesamin, one of the major lignans in sesame seed and oil, has been reported to have many benefits and medicinal properties. Even at high doses, sesamin has safety and tolerability profiles to animals.[46] In this study, sesamin decreased the ROS and TBARS productions in the nickel-treated mouse liver (Figure 5A,B), which may be due to the powerful antioxidant and free radical scavenging activities.[5,7] GSH can act as a nonenzymatic antioxidant by direct interaction of the SH group with ROS, or it can be involved in the enzymatic detoxification reaction for ROS, as a cofactor or a coenzyme, because it is a tripeptide containing cysteine that has a reactive SH group with reductive potency. Therefore, GSH is also considered to be an oxidative stress marker.[19,29,36] In this study, we observed a significant decrease in GSH level in the livers of nickel-treated mice, which is in agreement with several previous studies.[45,47,48] This may be due to the high ability of nickel to bind with the SH group of GSH and nickel-induced ROS increase.[19] Interestingly, we found that sesamin markedly increased the GSH level in nickel-treated mouse liver (Figure 4C). Our finding suggests that sesamin could at least partly attenuate oxidative stress by decreasing levels of ROS and lipid peroxide and increasing levels of GSH in nickel-treated mouse liver.

ROS can also induce DNA damage via causing single-strand breaks, DNA−protein cross-links, and modification of base residues such as the introduction of a hydroxyl group (−OH) into the C-8 position of guanosine and guanine residues, forming 8-OHdG and 8-hydroxyguanine, which are widely used as sensitive biomarkers of DNA oxidation.[20,49] It was reported nickel can induce an increase of 8-OHdG level in the liver.[21,47,50] In this study, the level of 8-OHdG was increased markedly in the

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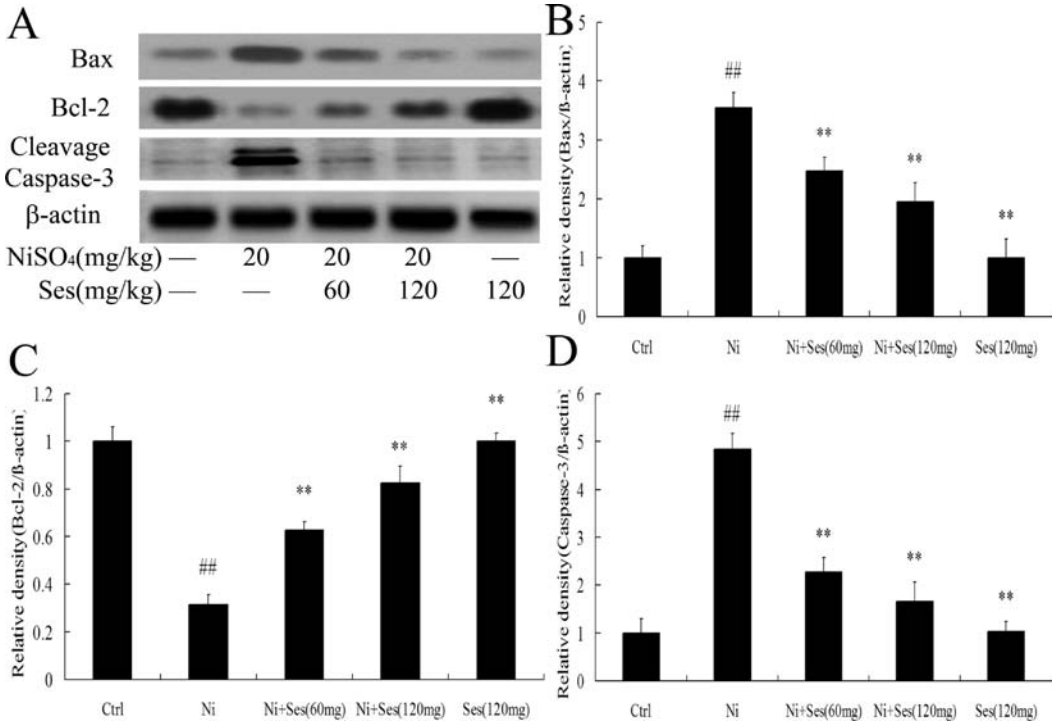

Figure 7. Immunoblotting analysis of apoptosis-provoking proteins in response to nickel and sesamin: (A) effect of sesamin on the expression of the proteins in association with apoptosis in mouse liver; (B) relative density analysis of the Bax protein bands; (C) relative density analysis of the Bcl-2 protein bands; (D) relative density analysis of the cleaved caspase-3 protein bands. *β*-Actin was probed as an internal control in relative density analysis of the protein bands. The vehicle control is set as 1.0. Values are averages from seven independent experiments. Each value is expressed as the mean ± SEM. (##) $P < 0.01$, compared with the control group; (**) $P < 0.01$, versus the nickel-treated group.

liver of nickel-treated mice (Figure 4D), which may be because nickel may bind to DNA-repair enzymes and generate oxygen free radicals to cause protein degradation in situ. However, treatment with sesamin markedly decreased the hepatic 8-OHdG level in nickel-treated mice, which could be associated with decreased ROS level in the livers of mice (Figure 4D). Our findings indicate that sesamin may play an effective role in protecting liver function from nickel-induced oxidative DNA damage by decreasing the 8-OHdG level in the livers of nickel-treated mice.

Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which act as preventive antioxidants, play a major role in protecting cells against oxidative stress, and the levels of these antioxidants might provide a clear indication of the extent of cytotoxic damage that occurs in various tissues.[46] Many studies show nickel can decrease the activities of these antioxidant enzymes, which might be either due to direct binding of the metal to the active site of the enzyme or due to increased free radical production induced by the metal.[45,51] Numerous studies have also shown that sesamin can regulate antioxidant capacities by increasing the superoxide dismutase, catalase, and glutathione peroxidase activities.[4,5,7,8] In this study, sesamin markedly renewed the activities of these antioxidant enzymes in the liver of nickel-treated mice and significantly enhanced the antioxidant capacity of the body (Figure 5). This suggested that sesamin could at least partly attenuate oxidative stress by increasing the activities of the antioxidant enzymes in nickel-treated mouse liver.

PI3K-Akt signaling is known to protect a variety of cells from apoptosis. Many studies have shown that nickel can induce apoptosis through the PI3K-Akt signaling pathway.[30−33] In the present study, we also found that the expression levels of PI3K and Akt phosphorylation decreased. However, sesamin treatment significantly up-regulated the levels of PI3K and Akt phosphorylation in mouse liver (Figure 6), suggesting that sesamin could protect mouse liver by regulating the PI3K-Akt signaling pathway. Activated Akt can also regulate cellular survival and metabolism by binding and regulating many downstream effectors, such as Bcl-2 family proteins and many caspases.[28,29] Bcl-2 and Bax, two of the Bcl-2 family proteins, have been shown to play a major role in determining cell survival or death after apoptotic stimuli.[28,31] In this study, our results show that sesamin significantly reduced the expression of Bax and increased the expression of the antiapoptotic protein Bcl-2 in nickel-treated mice (Figure 7B,C). Caspase is a family of proteins that are some of the main executors of the apoptotic process. The caspase pathway is a well-identified downstream target for PI3K-Akt.[27,30,41] Caspase-3 is one of the key executioners of apoptosis, capable of cleaving or degrading many key proteins such as nuclear lamins, fodrin, and the nuclear enzyme PARP.[42] The present study showed that nickel increased the number of TUNEL-positive cells and the activity of caspase-3 in the livers of mice. However, we found that sesamin markedly decreased the number of TUNEL-positive cells and the activity of caspase-3 in mice treated with nickel (Figures 3 and 7D). Thus, our findings indicate that sesamin might protect the liver against nickel-induced apoptosis by up-regulating the Bcl-2/Bax ratio and by activating the PI3K-Akt pathway.

In summary, this study demonstrates for the first time that sesamin has potent protective effects against nickel-induced apoptosis by modulating the PI3K-Akt pathway in mouse liver. We propose a possible protective effect of sesamin (Figure 8). Here we demonstrate that sesamin administration attenuated nickel-induced hepatic dysfunction and histopathologic

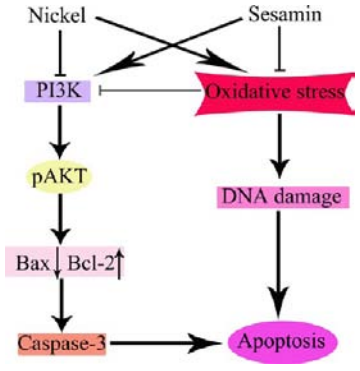

Figure 8. Schematic diagram showing protective signaling of sesamin in nickel-induced liver damage. "→" indicates activation or induction, and "|−" indicates inhibition or blockade.

changes. Sesamin attenuated nickel-induced hepatic oxidative damage by inhibiting ROS generation and increasing liver GSH level. Treatment with sesamin also increased the activities of antioxidant enzymes and decreased 8-OHdG levels in nickel-treated mouse liver. Sesamin treatment could effectively inhibit nickel-induced apoptosis in the liver by increasing expression levels of PI3K, phospho-Akt, and Bcl-2 and decreasing expression levels of Bax and caspase-3. Sesamin seems to be a potent hepatoprotective drug, and its use in maintaining a healthy liver and preventing toxic liver damage deserves consideration. However, there are fewer studies on the absorption of sesamin in humans. The question warrants further investigation and further examination.

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Notes

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ABBREVIATIONS USED

AKT, protein kinase B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; Ni, nickel; 8-OHdG, 8-hydroxy-2-deoxyguanosine; PI3K, phosphoinositide-3-kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TUNEL, deoxyribonucleotidyl transferase (TdT)-mediated dUTP-fluorescein isothiocyanate (FITC) nick-end labeling

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