Heme Oxygenase Inducers from Natural Products

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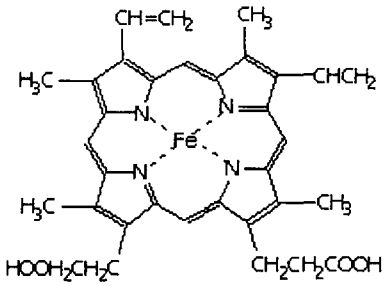
Abstract

Heme oxygenase (HO)-1 catabolizes heme into three products: carbon monoxide, bilirubin, and free iron. HO-1 serves as a protective gene by virtue of the anti-inflammatory, anti-apoptotic and anti-proliferative actions of one or more of these three products. HO-1 can be regarded as a potential therapeutic target in a variety of oxidant-mediated and inflammatory diseases. In this respect, it would be valuable to develop potent and selective inducers of HO-1 for therapeutic use. Here, we have shown that 1,2,3,4,6-penta-O-galloyl-beta-D-glucose, catalposide and dehydrocostus lactone are potent inducers of HO-1 and exert cytoprotective and anti-inflammatory activities *via* HO-1-dependent machanism.

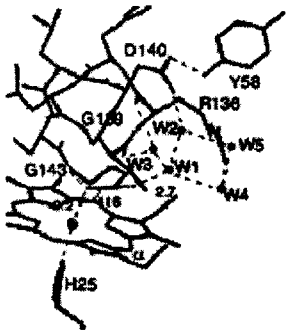
Keywords: Heme oxygenase-1, Cytoprotection, Anti-inflammation

Introduction

Irrespective of the source and mechanisms that lead to the generation of intracellular toxic mediators, mammalian cells have developed highly refined inducible systems against a variety of stressful conditions; upon stimulation, each one of these systems can be engaged concertedly to alleviate and hinder the manifestation of a distinctive metabolic disorder. Heme oxygenase-1 (HO-1), which is a redox-sensitive inducible protein, catabolizes heme into three products: carbon monoxide (CO), biliverdin, which is rapidly converted to bilirubin, and free iron. This enzyme has been shown to display potent anti-oxidant, anti-apoptotic and anti-inflammatory functions through the actions of one or more of these three products (see Fig.1). Although the key factors participating in signal transduction mechanisms required for transcriptional activation of HO-1 remain to be fully identified, this enzyme can be regarded as a potential therapeutic target in a variety of oxidant-mediated and inflammatory diseases. In this respect, it would be valuable to develop potent and selective inducers of HO-1 for therapeutic use. Here, we have shown that 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (PGG), catalposide and dehydrocostus lactone (DL) are potent inducers of HO-1 and exert cytoprotective and anti-inflammatory activities *via* HO-1-dependent machanism.



Heme



Heme Oxygenase

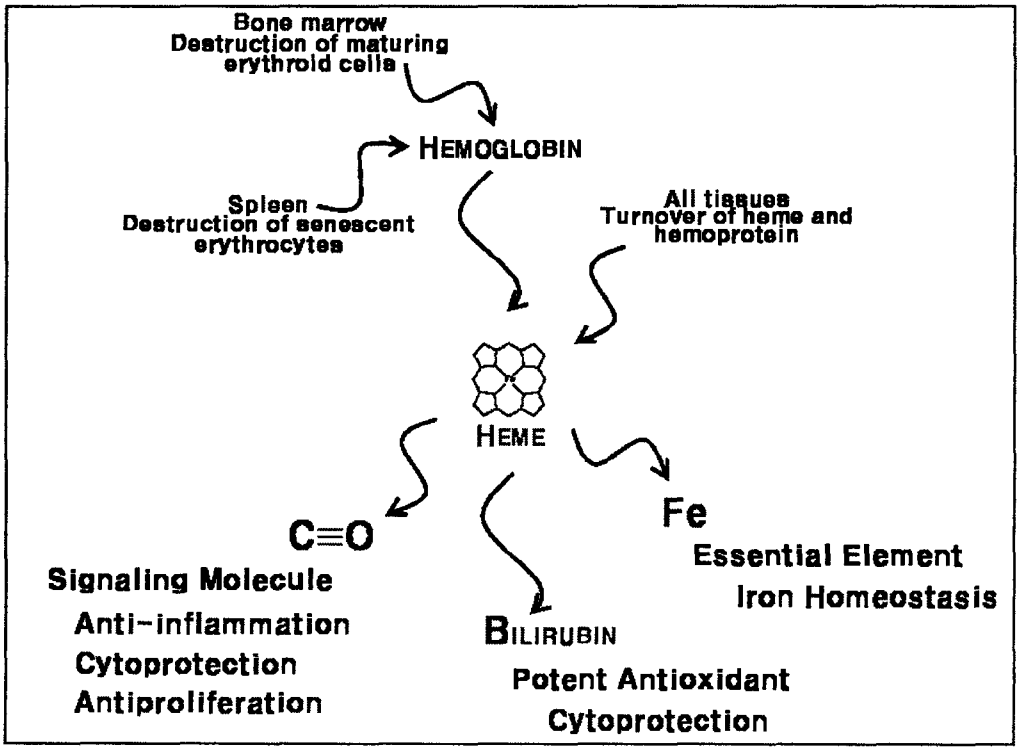


Fig. 1. Heme, heme oxygenase and roles of heme oxygenase.

Results and Discussion

PGG: The tannin PGG had been isolated from the root of *Paeonia suffruticosa* [1]. This compound has been reported to have anti-inflammatory and anti-proliferative activities via its inactivation of NF-κB [1,2]. To examine whether PGG could affect HO-1 gene expression, we treated the Neuro 2A cells for 18 h with various concentrations of PGG. The effects of various concentrations of PGG on heme oxygenase activity and HO-1 protein expression are shown in Fig. 2A and C. Western blot analysis revealed dose-dependent induction of HO-1 by PGG (Fig. 2C). Exposure of neuronal cells to PGG for 18 h resulted in a concentration-dependent increase in heme oxygenase activity (Fig. 2A). As shown in Fig. 2D, treatment of Neuro 2A cells with PGG resulted in a time-dependent increase in HO-1 expression. Using 30 μM of PGG, induction of HO-1 was evident as early as 6 h, and reached a maximum at 18 h after treatment of the cells with PGG. This effect correlated with a time-dependent increase in heme oxygenase activity as shown by heme oxygenase activity assay (Fig. 2B). RT-PCR was performed to examine the steady state levels of HO-1 mRNA in Neuro 2A cells after exposure to PGG. HO-1 mRNA was induced in time- and dose-dependent manners. (Fig. 2E and F).

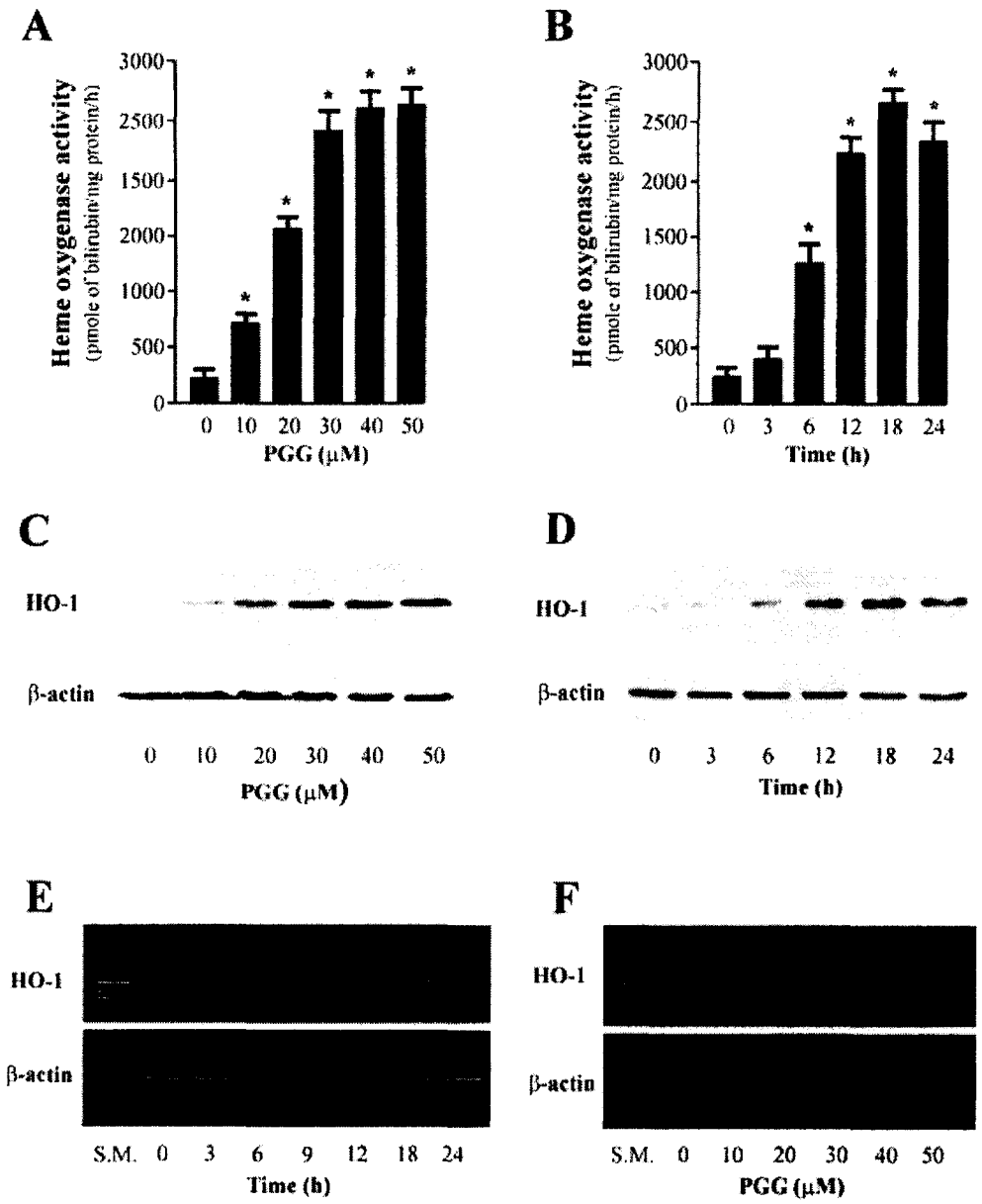


Fig. 2. Effect of PGG on heme oxygenase activity and HO-1 expression in Neuro 2A cells.

Catalposide: Catalposide and specioside (Fig. 3) were isolated from the stem bark of *Catalpa ovata* with high purity (more than 95%) as described previously [3,4]. Aglycone of catalposide was prepared by the enzyme reaction.

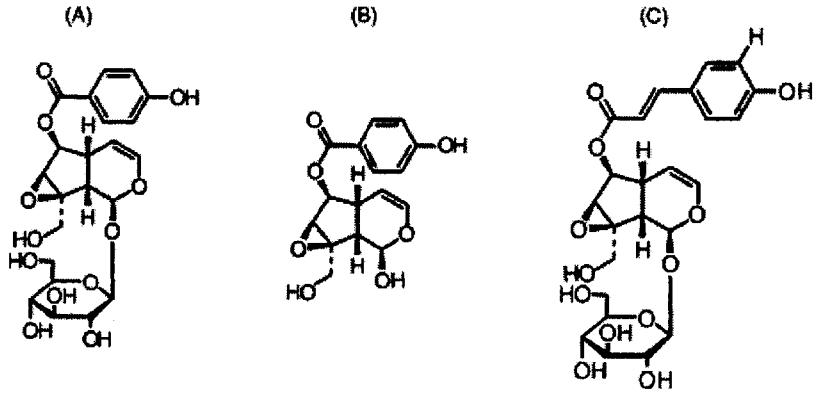


Fig. 3. Chemical structures of (A) catalposide; (B) aglycone of catalposide; and (C) specioside.

To examine whether catalposide could affect HO activity and HO-1 expression in Neuro 2A cells, we treated the cells for 24 h with various concentrations of catalposide. The effects of various concentrations of catalposide on HO activity and HO-1 protein expression in Neuro 2A cells are shown in Fig. 4. Exposure of neuronal cells to catalposide for 24 h resulted in a dose-dependent increase in HO activity (Fig. 4A). Western blot analysis revealed that enhanced HO activities by catalposide treatments

were directly correlated with HO-1 protein levels (Fig. 4B). We also investigated whether aglycone of catalposide and specioside, which is another compound isolated from *Catalpa ovata* with similar chemical structure of catalposide (Fig. 3), could induce HO activity. Unlike catalposide, the aglycone and specioside did not affect HO activity in Neuro 2A cells (Table 1).

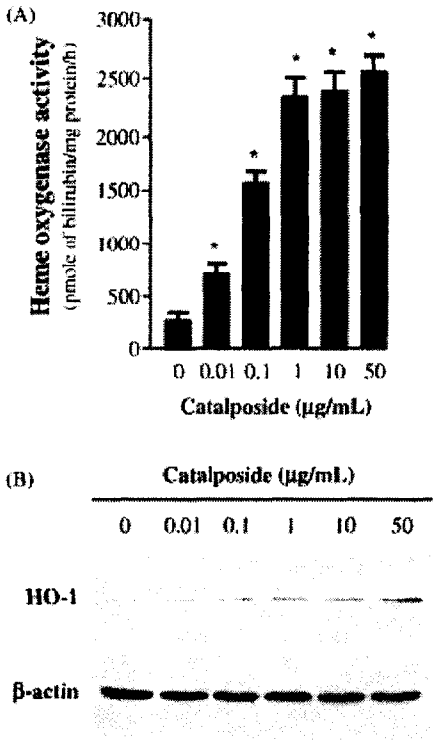


Fig. 4. Effects of catalposide on HO activity and HO-1 protein expression in Neuro 2A cells.

Table 1. Effects of catalposide, aglycone of catalposide, and specioside on HO activity in Neuro 2A cells.

Treatment	Heme oxygenase activity (pmol of bilirubin/mg protein h)
None	317 ± 58
Catalposide (1 μg/ml)	2146 ± 99*
Aglycone of catalposide (1 μg/ml)	498 ± 54
Specioside (1 μg/ml)	539 ± 76

Next, we examined the effects of catalposide pretreatment on hydrogen peroxide-induced cell death in Neuro 2A cells. The neuronal cells were pretreated 12 h with various concentrations of catalposide, and then exposed to hydrogen peroxide for 8 h. Catalposide was found to decrease hydrogen peroxide-induced cell death in a dose-dependent manner (Fig. 5A). The involvement of HO-1 in the cytoprotective effect of catalposide was confirmed using an inhibitor of HO activity, zinc protoporphyrin (ZnPP), which significantly blocked catalposide-mediated suppression of hydrogen peroxide-induced cell death (Fig. 5B). The increase in HO activity by catalposide was abrogated by ZnPP (Fig. 5C). These data show that the cytoprotective effect of catalposide might be due to the induction of HO-1.

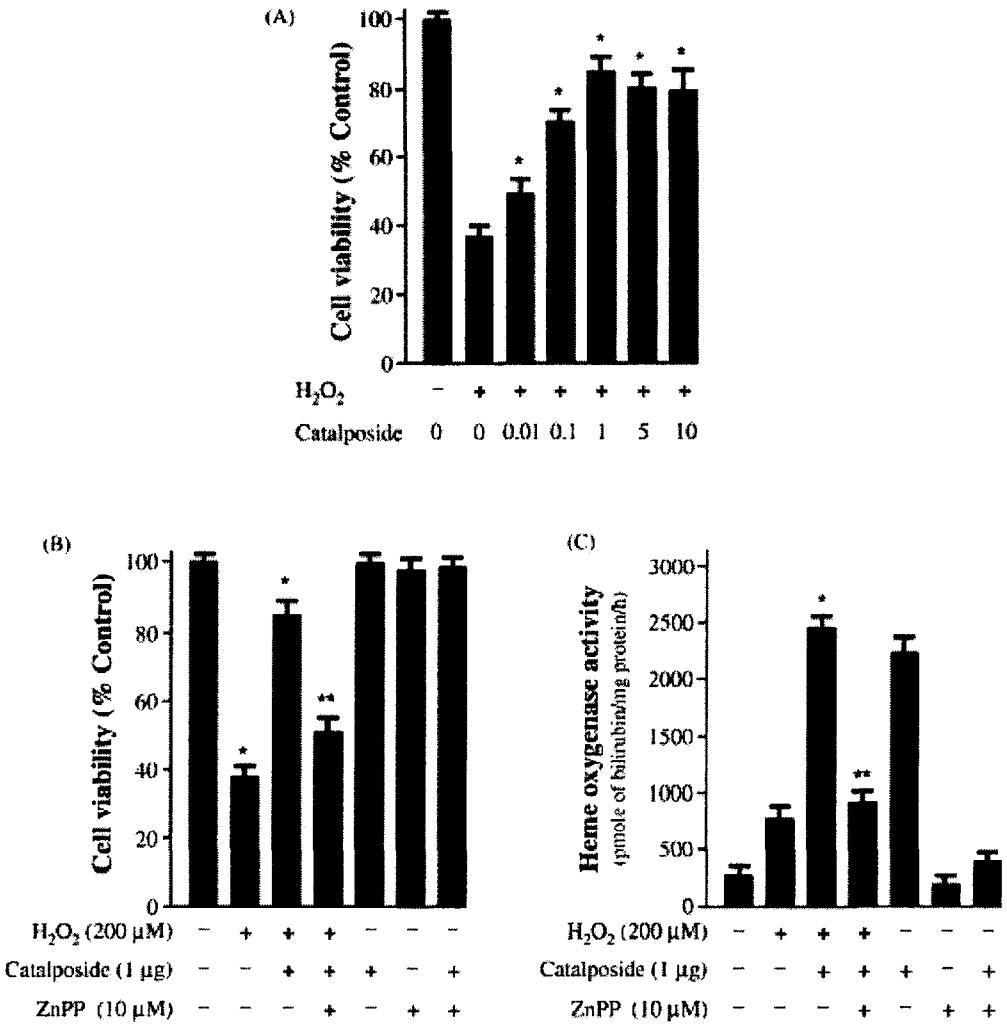
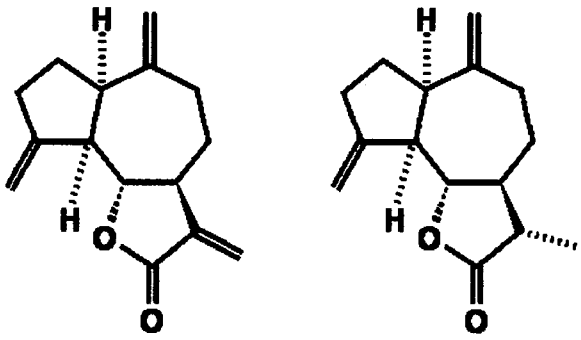


Fig. 5. Neuroprotective effect of catalposide against hydrogen peroxide-induced cell death. (A) Neuro 2A cells were pretreated with various concentrations of catalposide for 12 h and then incubated for 8 h with H_2O_2. (B) and (C) Cells were pretreated with catalposide for 12 h in the presence or absence of ZnPP. After this pretreatment, cells were incubated for 8 h with H_2O_2.

DL: DL was isolated from an extract of mokko (*Saussurea lappa*) as an inducer of HO-1. Mokko lactone (ML) was also isolated as an inactive compound from the extract. Their chemical structures are shown in Fig. 6.



Dehydrocostus lactone Mokko lactone

Fig. 6. Chemical structures of dehydrocostus lactone (DL) and mokko lactone (ML).

To examine whether DL and ML could affect HO activity and HO-1 expression in RAW 264.7 macrophages, we treated the cells for 24 h with either DL or ML. The effects of DL on HO activity and HO-1 protein expression are shown in Fig. 7. Exposure of the macrophages to DL resulted in dose-dependent increases in HO activity (Fig. 7A) and HO-1 expression (Fig. 7B). However, ML had no effect on both HO activity (Fig. 7A) and HO-1 expression (Fig. 7B).

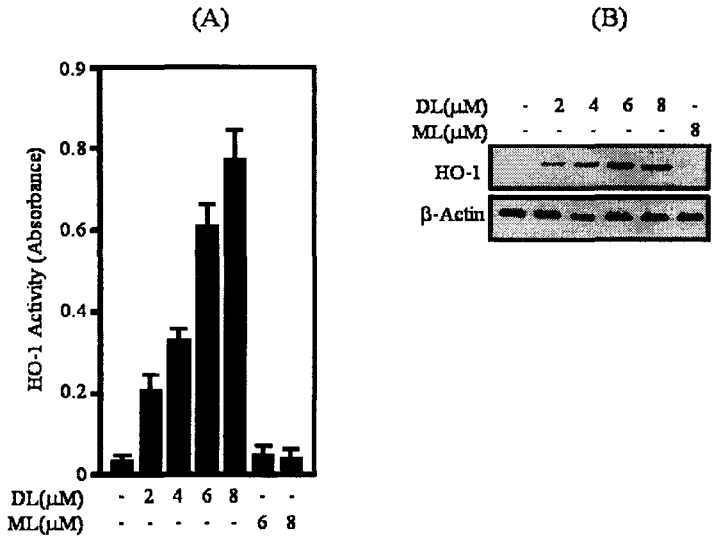


Fig. 7. Effects of DL on HO activity and HO-1 protein expression in RAW 264.7 cells.

NF-κB is the transcription factor that binds to the κB sequence. Under normal conditions, NF-κB is present in the cytoplasm in its inactive state as a heterotrimer consisting of p50, p65, and I-κBα. When NF-κB is activated, I-κBα undergoes ubiquitination, phosphorylation, and degradation, and p50-p65 complex is released to be translocated to the nucleus where it stimulates the transcription of its target genes. The target genes whose transcription is regulated by NF-κB include various inflammatory cytokines and inflammatory enzymes. We next examined whether DL could affect NF-κB activity and I-κBα phosphorylation in RAW 264.7 macrophages stimulated with the endotoxin lyphopolysaccaride (LPS). As shown in Fig. 8, 12-h pretreatment with ML,

but not with DL, resulted in decreases in NF-κB activity and I-κBα phosphorylation.

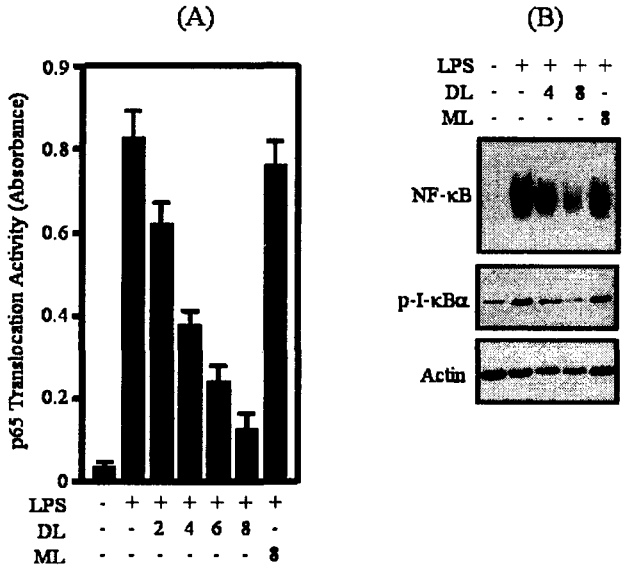


Fig. 8. Effects of DL on NF-κB activity and I-κBα phosphorylation in RAW 264.7 cells stimulated with LPS.

The involvement of HO-1 in DL-mediated NF-κB inactivation was confirmed using an inhibitor of HO activity, ZnPP, which significantly abolished DL-mediated suppressions of NF-κB activation and I-κBα phoshporylation (Fig. 9). In addition, the HO-1 inducer CoPP inhibited NF-κB activation, as DL did (Fig. 9). Togther, these data suggest that the inhibitory effect of DL on NF-κB might be due to the induction of HO-1.

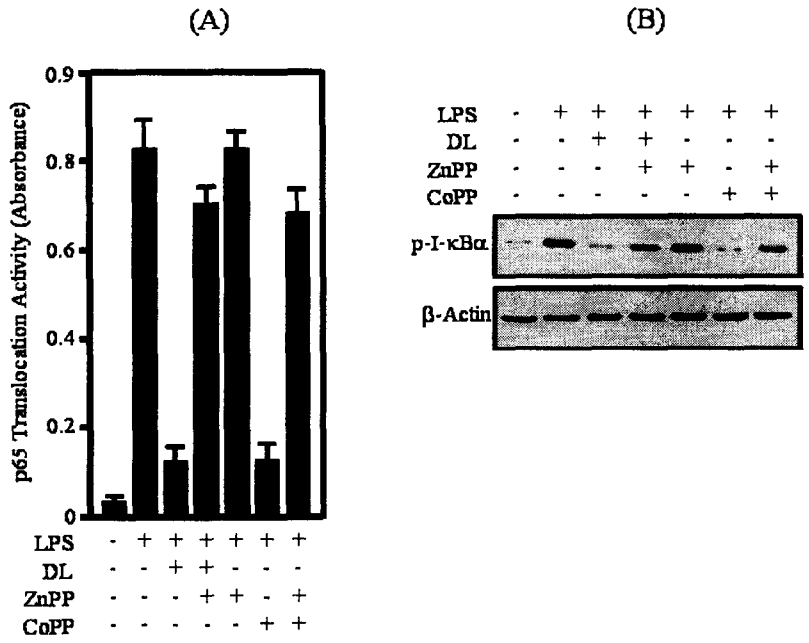


Fig. 9. Effects of ZnPP on ML-mediated NF-κB inactivation in RAW 264.7 macrophages stimulated with LPS.

It has been suggested that HO-1 gene induction is essential for restoring cellular homeostasis and that the beneficial effects of increased heme oxygenase activity may represent a promising therapeutic expedient to preclude tissue injury and, consequently, impede the progression of several diseases. This study identifies natural substances that could be used for therapeutic purposes as potent inducers of HO-1 in the protection of tissues against inflammatory and neurodegenerative conditions [5-9].

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