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Original Article

Protective effects of the herbal formula Bovitacin Lipa against hydrogen peroxide- and alcohol-induced cytotoxicity in HepG2 cells

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ABSTRACT

Objective: Oxidative stress and alcohol-induced hepatocellular injury are major contributors to the development of liver disorders.

Material and Methods: The protective effects of Bovitacin Lipa were evaluated in hydrogen peroxide (H_2O_2)- and ethanol (EtOH)-induced cytotoxicity models using HepG2 cells.

Results: Exposure of HepG2 cells to H_2O_2 (1 mM) or EtOH (100 mM) significantly reduced cell viability while treatment with Bovitacin Lipa at concentrations ranging from 10 to 500 μg/mL markedly and dose-dependently restored cell viability under both stress conditions. At higher concentrations, cell viability approached levels comparable to normal control.

Conclusion: These results suggest that Bovitacin Lipa exerts a protective effect against oxidative stress- and ethanol-induced hepatocellular injury.

Keywords HepG2 cells, oxidative stress, alcohol injury, cell viability, Bovitacin Lipa

INTRODUCTION

The liver is a central organ essential for maintaining systemic homeostasis, functioning as a metabolic hub, detoxification system, and reservoir for key biomolecules. It orchestrates nutrient metabolism by processing carbohydrates, lipids, and proteins derived from the gastrointestinal tract, while synthesizing critical plasma proteins such as albumin and coagulation factors. Additionally, the liver produces bile acids necessary for lipid digestion and facilitates the clearance of xenobiotics and endogenous waste products. Its immunological role, mediated in part by Kupffer cells and other resident immune populations, further highlights its importance in host defense.[1,2] Owing to its remarkable regenerative capacity and cellular heterogeneity,[3] the liver is uniquely equipped to adapt to physiological demands; however, these same features also render it highly susceptible to metabolic and toxic insults.

Among the major etiological factors contributing to liver injury, excessive alcohol consumption and oxidative stress are particularly prominent. Ethanol metabolism, primarily via alcohol dehydrogenase and the microsomal ethanol-oxidizing system, generates toxic intermediates such as acetaldehyde and promotes the accumulation of reactive oxygen species (ROS).[4] This redox imbalance induces mitochondrial dysfunction, lipid peroxidation, and disruption of cellular membranes, ultimately leading to hepatocyte apoptosis or necrosis. Concurrently, exogenous oxidative stressors such as hydrogen peroxide (H_2O_2) exacerbate intracellular damage by impairing antioxidant defense systems.[5,6] These mechanisms collectively drive the progression of liver pathologies, including steatosis, alcoholic hepatitis, fibrosis, and cirrhosis, underscoring the need for effective strategies to mitigate oxidative and ethanol-induced hepatotoxicity.[7]

In response to these pathological challenges, substantial efforts have been directed toward the development of hepatoprotective agents targeting oxidative stress and ethanol-induced damage pathways. Conventional pharmacological approaches, as well as naturally derived compounds such as polyphenols, flavonoids, and bioactive peptides, have demonstrated varying degrees of efficacy in attenuating ROS production, enhancing antioxidant enzyme activity, and preserving mitochondrial integrity.[8–11] Nevertheless, despite promising preclinical findings, many candidates lack robust mechanistic validation under diverse cellular stress conditions, limiting their translational potential. Therefore, the identification and characterization of novel compounds with well-defined cytoprotective mechanisms remain a critical priority in hepatology research.

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Various bioactive peptides isolated from natural substance has recently emerged as a potential bioactive compound with hepatoprotective properties. Thus, the present study aimed to evaluate the cytoprotective effects and safety profile of Bovitacin Lipa (bioactive peptide isolated from liver of cow) using H_2O_2- and ethanol-induced HepG2 cell injury models. By elucidating its functional role under these pathophysiological conditions, this study seeks to provide foundational insights into its potential application as a therapeutic candidate for liver disease.

MATERIALS AND METHODS

Preparation of Bovitacin Lipa and analysis of amino acids

Bovitacin Lipa was provided by JBKLAB Co., Ltd (Gyeonggi-do, Korea) and used to analysis of amino acid and cytotoxicity assay. Briefly, Bovitacin Lipa was prepared from bovine liver through sequential washing, enzymatic hydrolysis, filtration, and spray drying. The cleaned liver was subjected to bioconversion using papain and bromelain at 65–75 °C for 48 h, followed by stepwise filtration (5 μm and 1 μm) at 100 °C to obtain a clarified hydrolysate. The final product was obtained as a spray-dried powder with a yield of approximately 23.8%. The process was presented in Figure 1. Sixten of constituent amino acids (tyrosine, glycine, serine, alanine, glutamic acid, lysine, leucine, methionine, valine, arginine, aspartic acid, isoleucine, threonine, phenylalanine, proline, histidine) were analyzed using Amino Acid Analyzer (LA8080, Hitachi, Tokyo, Japan) from Korea Health Functional Food.

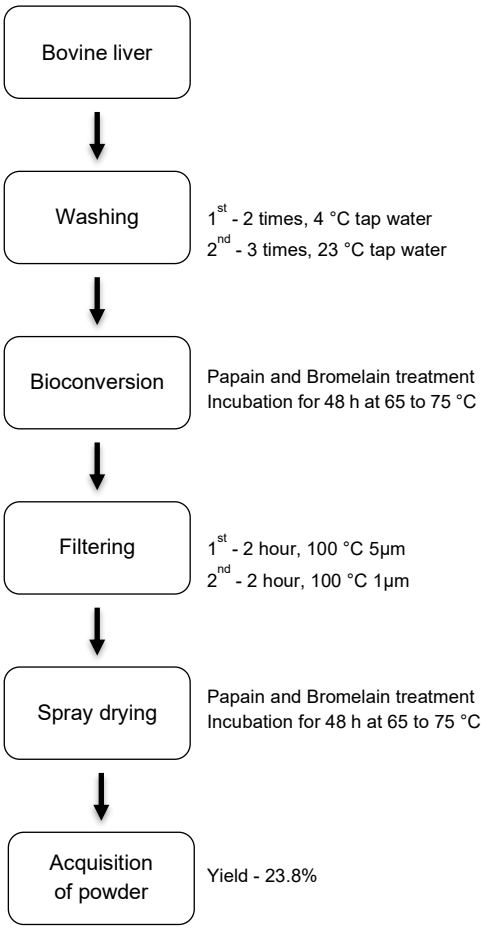

Fig. 1. Peptide extraction process.

Cell culture and treatment

Human hepatocellular carcinoma cells (HepG2) purchased from Korea Cell Line Bank (Seoul, South Korea) and were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Cytiva, MA, USA) supplemented with 10% fetal bovine serum (Cytiva, MA, USA) and 1% penicillin–streptomycin (Cytiva, MA, USA) under standard conditions (37°C, 5% CO_2).

Cell viability assay

HepG2 cells were seeded at a density of 2×10^4 cells/well in 96-well plates and incubated for 24 h to allow attachment. Subsequently, cells were treated with various concentrations of Bovitacin Lipa (1, 10, 25, 50, 100, 250, 500, and 1000 μg/mL) for an additional 24 h. After 48 h from initial seeding, cell viability was measured using a WST-8 colorimetric assay (BIOMAX, Gyeonggi-do, South Korea) according to the manufacturer's instructions. Cell viability was expressed as a percentage relative to the untreated control group.

To evaluate the protective effects of Bovitacin Lipa, oxidative and alcoholic injury were induced using hydrogen peroxide (H_2O_2) or ethanol (EtOH). HepG2 cells were seeded at 2×10^4 cells/well and incubated for 24 h. The cells were then exposed to either 1 mM H_2O_2 or 100 mM ethanol for 1 h to induce cellular damage. After stress induction, cells were treated with Bovitacin Lipa at concentrations of 10, 50, 100, and 500 μg/mL and further incubated for 24 h.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical significance was determined by comparison with the normal control or stress-treated groups. Significance levels are indicated as ###$p < 0.001$ vs. normal control and **$p < 0.01$, ***$p < 0.001$ vs. H_2O_2- or EtOH-treated groups.

RESULTS

Aanalysis of amino acids in Bovitacin Lipa

The total amino acid content was 764.19 mg/g, indicating that Bovitacin Lipa is a rich source of amino acids. Among the analyzed amino acids, glycine was the most abundant (215.75 mg/g), followed by proline (119.98 mg/g) and alanine (89.37 mg/g). These amino acids collectively accounted for a substantial proportion of the total amino acid composition, suggesting a predominance of non-essential amino acids. Moderate levels of glutamic acid (67.74 mg/g), arginine (66.73 mg/g), and aspartic acid (40.59 mg/g) were also observed. Essential amino acids such as lysine (37.61 mg/g), leucine (27.79 mg/g), valine (22.29 mg/g), phenylalanine (18.65 mg/g), threonine (13.13 mg/g), isoleucine (12.09 mg/g), methionine (6.01 mg/g), and histidine (5.90 mg/g) were present in relatively lower amounts compared to non-essential amino acids. Additionally, serine (15.38 mg/g) and tyrosine (5.18 mg/g) were detected at modest levels (Table 1).

Effects of Bovitacin Lipa on basal cell viability in HepG2 cells

Bovitacin Lipa treatment (1 to 1000 μg/mL) did not exhibit cytotoxicity in HepG2 cells, even better it showed significantly increased cell proliferation compared to the non-treated cells. It was observed that the cell proliferation rate increased by approximately 130% for treatment with 1 μg/ml of Bovitacin Lipa, and this effect showed that cell proliferation increased by up to about 150% as the concentration of Bovitacin Lipa treatment increased ($p < 0.01$ or 0.001, Figure 2B).

Protective effect of Bovitacin Lipa against H_2O_2- and EtOH-induced cytotoxicity

Exposure of 1 mM of H_2O_2 or 100 mM of EtOH dramatically induced cytotoxicity in HepG2 cells compared to the normal group (approximately 53 or 67% for H_2O_2 or EtOH, respectively). However, treatment with Bovitacin Lipa (10–500 μg/mL) significantly restored cell viability under both stress conditions in a dose-dependent manner. In the H_2O_2-induced model, Bovitacin Lipa treatment increased cell viability from approximately 47% in control group to about 73 to 82%, with significant recovery observed at all tested concentrations ($p < 0.01$, Figure 3B). Similarly, in the EtOH-induced model, cell viability was reduced to around 33% in the control group, whereas Bovitacin Lipa treatment markedly improved viability up to 58 to 98%, with the highest concentration (500 μg/mL) nearly restoring viability to normal levels ($p < 0.001$, Figure 3C).

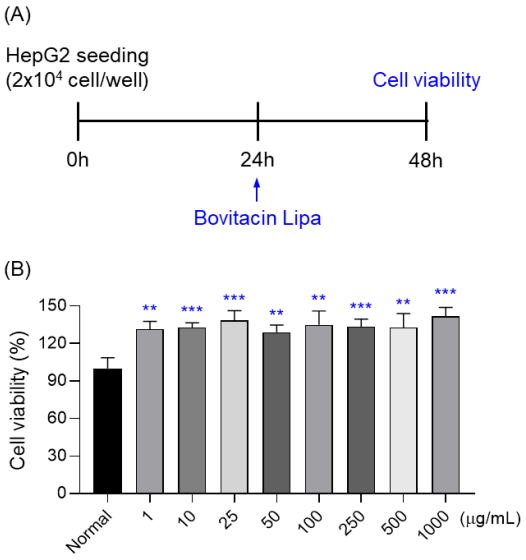

Fig. 2. Effect of Bovitacin Lipa on basal cell viability in HepG2 cells. (A) Experimental design. (B) Cells were treated with Bovitacin Lipa at the indicated concentrations (1 to 1000 μg/mL) and measured to cell viability. Data are expressed as mean ± SD. Statistical significance is indicated as **p < 0.01, ***p < 0.001 vs. normal control.

Table 1. Measurement of amino acids

Types of amino acids	Contents (mg/g)	Types of amino acids	Contents (mg/g)
Glycine	215.75	Valine	22.29
Proline	119.98	Phenylalanine	18.65
Alanine	89.37	Serine	15.38
Glutamic acid	67.74	Threonine	13.13
Arginine	66.73	Isoleucine	12.09
Aspartic acid	40.59	Methionine	6.01
Lysine	37.61	Histidine	5.9
Leucine	27.79	Tyrosine	5.18
Total amino acid		764.19 mg/g	

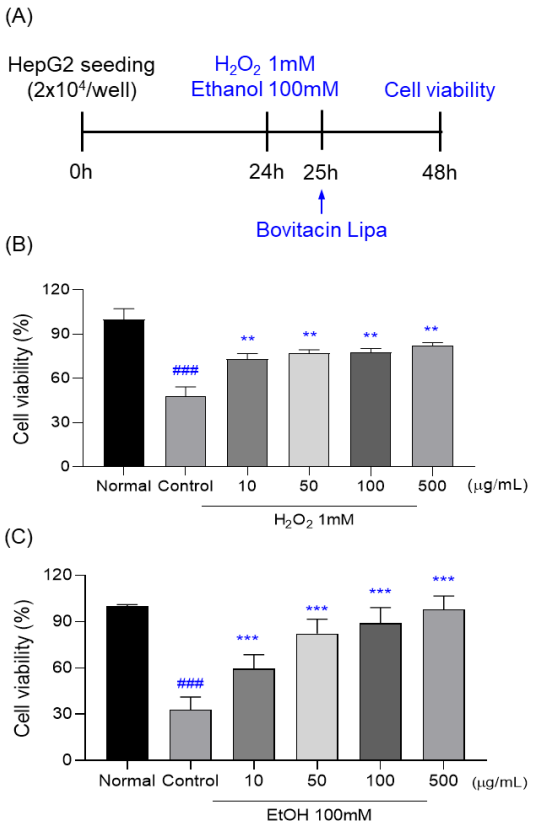

Fig. 3. Protective effect of Bovitacin Lipa against H_2O_2- and ethanol-induced cytotoxicity in HepG2 cells. (A) Experimental design. Effect of Bovitacin Lipa on (B) H_2O_2- and (C) EtOH-induced cytotoxicity. Cells were treated Bovitacin Lipa (10 to 500 μg/mL) with/without H_2O_2 (1 mM) and EtOH (100 mM) and measured to cell viability. Data are presented as mean ± SD. ###$p < 0.001$ vs. normal group; **$p < 0.01$, ***$p < 0.001$ vs. H_2O_2 or EtOH-treated control group.

DISCUSSION

In the present study, we employed H_2O_2 (1 mM) and ethanol (100 mM)-induced cytotoxicity models in HepG2 cells to evaluate the hepatoprotective effects of Bovitacin Lipa. These in vitro models are extensively validated systems for mimicking acute oxidative and alcohol-mediated liver injury. H_2O_2 at several millimolar concentrations is known to generate intracellular reactive oxygen species (ROS), leading to lipid peroxidation, mitochondrial dysfunction, and apoptosis.[12,13] Similarly, ethanol at concentrations of 50 to 200 mM is commonly used to reproduce alcohol-induced hepatocyte injury through mechanisms involving CYP2E1 activation, ROS accumulation, and acetaldehyde toxicity.[14] The selected concentrations (1 mM H_2O_2 and 100 mM ethanol) in our study induced a significant reduction in cell viability (47% and 33%, respectively), thereby providing a robust and reproducible injury window suitable for evaluating cytoprotective interventions. Therefore, our experimental model can be considered appropriate and physiologically relevant for assessing hepatoprotective efficacy.

Our results clearly demonstrate that Bovitacin Lipa significantly attenuates both oxidative stress- and ethanol-induced cytotoxicity in a dose-dependent manner. Notably, treatment with Bovitacin Lipa restored cell viability to near-normal levels at higher concentrations, suggesting a strong cytoprotective capacity. This protective effect may be attributed to multiple mechanisms, including antioxidant activity, ROS scavenging, and potential modulation of intracellular defense systems such as glutathione and antioxidant enzymes.[15,16] Previous studies have reported that bioactive peptides derived from animal exert hepatoprotective effects by reducing oxidative stress, inhibiting apoptosis, and regulating inflammatory signaling pathways.[17,18] The observed effects of Bovitacin Lipa are consistent with these mechanistic paradigms and suggest that they may function as a potent hepatoprotective agent.

On the one hand, Papain and bromelain, cysteine protease enzymes derived from *Carica papaya* and *Ananas comosus,* exhibit relatively low substrate specificity compared to animal-derived enzymes such as trypsin, allowing them to cleave a wide range of peptide bonds and are known to produce heterogeneous peptide mixtures with enhanced biological activities.[19,20] In our data, enhanced efficacy may be due to the unique and various composition of peptides generated during enzymatic hydrolysis using papain and bromelain.[21,22] The amino acid composition of Bovitacin Lipa further supports its hepatoprotective potential. Representatively, glycine, the most abundant amino acid, has been extensively reported to exert cytoprotective and anti-inflammatory effects in liver injury by stabilizing cell membranes and inhibiting Kupffer cell activation.[23] as well as others also well are known to hepatoprotective effects.[24–26] Therefore, the synergistic presence of these amino acids likely contributes to the overall hepatoprotective activity of Bovitacin Lipa.

Despite these findings, several limitations of this study should be acknowledged. First, the use of a single cell line (HepG2) may not fully recapitulate the complexity of in vivo liver physiology, particularly with respect to alcohol metabolism and immune responses. Second, the study primarily focused on cell viability as an endpoint, without directly measuring mechanistic biomarkers such as ROS levels, apoptosis markers, or antioxidant enzyme activities. Third, the precise bioactive peptide sequences responsible for the observed effects were not identified, limiting mechanistic interpretation. Finally, in vivo validation using animal models or clinical studies is required to confirm the translational relevance of these findings.

In conclusion, Bovitacin Lipa exhibits potent hepatoprotective effects against oxidative stress and ethanol-induced cytotoxicity in HepG2 cells. Its efficacy is likely attributable to a combination of bioactive peptides and amino acid composition, supporting its potential as a novel functional ingredient for liver health. Further studies are warranted to

elucidate its mechanisms of action and validate its efficacy in vivo.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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