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Apoptotic and Cytotoxic Effects of Lisinopril and Amlodipine in Human Hepatocellular Carcinoma Cell Model

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ABSTRACT

Objective: This study aimed to evaluate the cytotoxic and apoptosis-inducing effects of lisinopril and amlodipine in a human hepatocellular carcinoma (Hep3B) cell model. **Materials and Methods:** The cytotoxic effects of Hep3B cells treated with lisinopril and amlodipine were assessed using the MTT assay. Cell viability was analyzed at 24, 48, and 72 hours, and IC_{50} values were calculated. Apoptosis-related gene expression levels, including Bcl-2, Bcl-xL, Bax, and Bak, were determined using RT-qPCR. Statistical analyses were performed using one-way ANOVA, and differences with $p<0.05$ were considered significant. **Results:** Lisinopril reduced Hep3B cell viability in a concentration-dependent manner with an IC_{50} value of 74.04 µg/ml ($p<0.05$). Similarly, amlodipine decreased cell viability with an IC_{50} value of 38.04 µg/ml ($p<0.05$). Gene expression analysis revealed that lisinopril decreased the expression of the anti-apoptotic genes Bcl-2 and Bcl-xL while slightly increasing the pro-apoptotic genes Bax and Bak, whereas amlodipine strongly suppressed anti-apoptotic gene expression and enhanced pro-apoptotic gene expression ($p<0.05$). **Conclusion:** These findings suggest that lisinopril and amlodipine modulate apoptotic gene expression and proliferation in Hep3B cells, enhancing apoptosis. The observed cytotoxic and molecular effects indicate a potential anti-cancer activity, which may hold clinical relevance for hepatocellular carcinoma therapy.

Keywords: Amlodipine, Apoptosis, Cell Proliferation, Hepatocellular Carcinoma, Lisinopril.

Lisinopril ve Amlodipinin İnsan Hepatosellüler Karsinom Hücre Modelinde Apoptotik ve Sitotoksik Etkileri

ÖZ

Amaç: Bu çalışmada, insan hepatosellüler karsinomu (Hep3B) hücre modelinde lisinopril ve amlodipinin sitotoksik etkileri ve apoptoz indüksiyonu üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntem:** Lisinopril ve amlodipin ile muamele edilen Hep3B hücrelerinin sitotoksik etkileri, MTT testi kullanılarak değerlendirilmiştir. Hücre canlılığı 24, 48 ve 72. saatlerde analiz edilmiş ve IC_{50} değerleri hesaplanmıştır. Apoptoz ile ilişkili gen ekspresyon düzeyleri, Bcl-2, Bcl-xL, Bax ve Bak belirteçleri kullanılarak RT-qPCR yöntemiyle değerlendirilmiştir. İstatistiksel analizler One-way ANOVA kullanılarak gerçekleştirilmiş ve $p<0.05$ olan farklar istatistiksel olarak anlamlı kabul edilmiştir. **Bulgular:** Lisinopril, Hep3B hücrelerinin viabilitesini konsantrasyon-bağımlı bir şekilde azaltmış ve IC_{50}: 74.04 µg/ml olarak sitotoksisite göstermiştir ($p<0.05$). Amlodipin uygulaması da benzer şekilde Hep3B hücrelerinin canlılığını konsantrasyon-bağımlı olarak azaltmış ve IC_{50} değeri 38,04 µg/ml olarak bulunmuştur ($p<0,05$). Gen ekspresyon analizleri, lisinoprilin Bcl-2 ve Bcl-xL gibi anti-apoptotik genlerin ekspresyonunu azalttığını, Bax ve Bak gibi pro-apoptotik genlerin ise zamanla arttırdığını gösterirken, amlodipin anti-apoptotik genleri belirgin şekilde baskılayıp pro-apoptotik genleri güçlü şekilde artırdığını ortaya koymuştur ($p<0.05$). **Sonuç:** Bu bulgular, lisinopril ve amlodipinin Hep3B hücrelerinde apoptotik gen ekspresyonunu ve proliferasyonu modüle ederek apoptozu artırabileceğini göstermektedir. Gözlemlenen sitotoksik ve moleküler etkiler, potansiyel bir anti-kanser aktiviteye işaret etmekte olup hepatoselüler karsinom tedavisi açısından klinik açıdan önem taşıyabilirler.

Anahtar Kelimeler: Amlodipin, Apoptozis, Hücre Proliferasyonu, Hepatosellüler Karsinom, Lisinopril.

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INTRODUCTION

Antihypertensive drugs play a vital role in regulating intravascular blood pressure and maintaining fluid-electrolyte balance. According to their mechanisms of action, antihypertensive drugs can be classified into four main groups: i) those affecting the renin-angiotensin-aldosterone system (RAAS), ii) those acting by inhibiting angiotensin-converting enzyme (ACE), blocking angiotensin type 1 receptors (AT1R), directly inhibiting renin, or antagonizing the binding of aldosterone to its receptor; iii) those acting by blocking calcium channels, either dihydropyridine or non-dihydropyridine calcium channels; and β-adrenergic receptor-blocking beta blockers; iv) diuretics that reduce the volume in the circulatory system (Laurent, 2017). In the literature, it has been reported that the mechanisms targeted by these drugs can affect cancer development either directly or indirectly, and it has been suggested that they may have the potential to be used as adjuvant therapy in cancer. In vitro studies, it has been shown that antihypertensive drugs can suppress growth in chemoresistant cells and increase chemosensitivity (Wong et al., 2020; Wegman-Ostrosky et al., 2015). These drugs are well tolerated, can be administered orally, and offer a more economical option compared to other cancer therapies (George et al., 2017).

The renin-angiotensin-aldosterone system (RAAS) comprises renin, angiotensin (Ang), angiotensin-converting enzyme (ACE), angiotensin I (Ang I), angiotensin II (Ang II), and angiotensin II type 1 (ATR1) and type 2 (ATR2) receptors (De Lange-Jacobs et al., 2020). Renin initiates the formation of angiotensin I from angiotensinogen. The conversion of angiotensin I to angiotensin II is primarily mediated by ACE, although it can also occur via non-ACE pathways (Maggioni et al., 2006). The local expression of RAAS in various tissues has been confirmed, indicating that this system can exert significant effects in cancer cells as well (Luan et al., 2019). Moreover, RAAS inhibitors have been shown to regulate proliferation and apoptosis in cancer cells, enhance autophagy-associated cell death, and reduce metastatic potential (Alvarenga et al., 2016). In this context, ACE inhibitors such as lisinopril reduce RAAS activity by blocking the conversion of Ang I to Ang II and exert regulatory effects on apoptosis and proliferation in cancer cells (Brunner-La Rocca et al., 1999). These findings suggest that RAAS inhibitors may serve as potential therapeutic agents for both antihypertensive treatment and cancer therapy (Iheanacho & Enechukwu, 2024).

Calcium channels play a critical role in signal transduction by controlling the entry of calcium ions into the cell and are classified into voltage-gated calcium channels (VGCC) and ligand-gated calcium channels (LGCC). These channels are considered important targets in cancer research due to their effects on fundamental cellular processes such as apoptosis, proliferation, differentiation, mitogenesis, and metastasis (Phan et al., 2017). Calcium channel blockers (CCBs), particularly by inhibiting L-type VGCCs, reduce intracellular calcium influx, thereby regulating cell proliferation and apoptosis (Alqudah et al., 2022). Dihydropyridine derivatives (amlodipine, nifedipine, nicardipine) and non-dihydropyridines (verapamil, diltiazem) have demonstrated antiproliferative and pro-apoptotic effects in cancer cells; in this context, amlodipine in particular exhibits multifaceted mechanisms that inhibit proliferation in cancer cells. It reduces intracellular Ca^{2+} concentration, thereby suppressing the activity of proteins necessary for cell cycle progression and increasing the expression of the negative cell cycle regulator $p21^{Waf1/Cip1}$ to support cell cycle arrest. Additionally, it decreases EGFR phosphorylation and inhibits the YAP/TAZ signalling pathway by modulating Ca^{2+} influx. These effects are considered key mechanisms underlying amlodipine's antiproliferative and anti-tumour potential in cancer cells (Fu et al., 2022; Carlos-Escalante et al., 2021).

Liver cancer is a growing health concern with increasing incidence and significant mortality worldwide (Villanueva et al., 2019). Liver cancer-related deaths rank fourth after deaths due to lung, colorectal, and gastric cancers (GBD 2015 Mortality and Causes of Death Collaborators, 2016). The incidence of the disease varies by sex and geographical regions due to differences in exposure to risk factors; Asia and Europe are the regions with the highest incidence of liver cancer (Chuang et al., 2009; Duan et al., 2019). Key factors that increase the risk of liver cancer include infections caused by hepatitis B and hepatitis C virus, liver parasites in endemic areas, lifestyle factors such as alcohol or tobacco use, metabolic disorders, and exposure to aflatoxins (Chahine et al., 2024; Akinyemiju et al., 2017). In the context of this high-incidence and high-mortality disease, investigating therapeutic approaches for liver cancer is of great importance. Although the effects of RAAS inhibitors and L-type calcium channel blockers have been studied in breast, prostate, and lung cancer cell lines, their impact on liver cancer remains largely unexplored, representing a gap in the literature.

This study was based on the hypothesis that lisinopril, an ACE inhibitor targeting the renin-angiotensin-aldosterone system (RAAS), and amlodipine, an L-type calcium channel blocker, could enhance the apoptotic response in human hepatocellular carcinoma (HCC) cells. These drugs were selected, because previous studies suggested their potential to modulate apoptotic pathways in cancer cells. Hep3B cells, derived from HCC, are suitable for investigating mitochondrial pathway–mediated apoptotic processes and drug-induced cellular stress responses due to their high proliferative capacity. These characteristics make Hep3B an advantageous model for evaluating pro-apoptotic effects and

cytotoxic mechanisms. Therefore, this cell line was chosen for the study, and the results demonstrated that both drugs could modulate apoptotic processes and exert potential anticancer effects (Lee & Kwon, 2019). The findings showed that lisinopril and amlodipine increased cellular apoptosis by regulating the expression balance of pro- and anti-apoptotic genes. These results support the potential of these molecules as therapeutic agents in liver cancer treatment and provide a scientific basis for further preclinical investigations. The present study is the first to simultaneously evaluate the effects of lisinopril and amlodipine on apoptotic processes in liver cancer, highlighting their potential role in HCC therapy and providing a novel contribution to the literature.

MATERIALS AND METHODS

Preparation of lisinopril and amlodipine stock solutions

Lisinopril (Cat. 83915-83-7) and amlodipine (Cat. 88150-42-9) were purchased from Sigma-Aldrich. Stock solutions prepared as 10 mg/ml with deionized water were sterilized through a membrane filter before use in cell culture experiments.

Cell culture

The human hepatocellular carcinoma cell line Hep3B (ATCC HB-8064) was maintained in Dulbecco's Modified Eagle Medium with L-glutamine (Cat. 41966-029; Gibco, USA), supplemented with 10% fetal bovine serum (Cat. A5256701; Gibco, USA) and 1% Penicillin-Streptomycin (Gibco, USA). Cells were cultured under a humidified atmosphere with 5% CO_2 at 37 °C. The cells were routinely screened for mycoplasma, and no mycoplasma contamination was detected in any of the tests performed.

Cytotoxicity analysis

For cytotoxicity, 0.5 mg/mL MTT solution was added to the cells and incubated at 37 °C for 4 hours. After that, formazan crystals were dissolved in isopropanol containing 0.008 M HCl and absorbance values at 570 nm were determined by spectrophotometric measurements. Each experiment was performed in at least three independent replicates ($n \geq 3$), and the data were compared with the control group.

RNA isolation and quantitative gene expression analysis

Based on the cytotoxicity findings, Hep3B cells were treated with lisinopril and amlodipine at concentrations corresponding to their respective IC_{50} values for 24, 48, and 72 hours. Following incubation, total RNA was isolated from trypsinized cell pellets using the GeneJET RNA Purification Kit (Cat. K0731; Thermo Scientific, USA). RNA purity and concentration were determined using a Thermo Scientific NanoDrop device (USA) through measurement of A260/A280 ratios, and samples with ratios ranging from 1.8 to 2.0 were considered suitable for subsequent experiments. cDNA was synthesized from 1 µg of total RNA per sample using the OneScript Plus cDNA Synthesis Kit (Cat. G236; Applied Biological Materials Inc., Canada). The synthesis protocol included primer annealing at 25 °C for 10 minutes, reverse transcription at 50 °C for 15 minutes, and reaction termination at 85 °C for 5 minutes.

RT-qPCR was carried out using BlasTaq 2X qPCR MasterMix (Cat. G891; Applied Biological Materials Inc., Canada) in 20 µL reaction volumes on a Bio-Rad CFX96 Touch Real-Time PCR Detection System. The amplification protocol consisted of an initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute for annealing.

All samples were analysed in technical triplicates, including positive and negative controls to ensure data reliability and comparability. The expression of target genes was adjusted relative to β-actin, and the relative fold changes were determined using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Study data were obtained from at least three independent technical replicates, and results are expressed as mean ± standard deviation (SD). One-way ANOVA analyses were performed, and the results indicated statistically significant differences among all groups. Tukey's post-hoc test was subsequently applied for pairwise comparisons, confirming that all group differences were statistically significant. P-values below 0.05 were considered statistically significant.

Ethical consideration

This study did not require ethical approval.

RESULTS

Lisinopril reduced the viability of Hep3B cells in a concentration-dependent manner. At 0.1 µg/ml, cell viability was 99.08%, 125.48%, and 77.44% at 24, 48, and 72 hours, respectively. At 0.5 µg/ml, viability was 115.28%, 106.27%, and 67.52%, indicating a transient proliferative effect at early time points. At 1.25 µg/ml, viability decreased to 89.89%, 73.01%, and 56.08%. At 6.25 µg/ml, values were 60.39%, 77.00%, and 44.36%, and at 31.25 µg/ml, 85.29%, 47.05%, and 41.25%. The highest concentration of 156.25 µg/ml caused the most pronounced cytotoxic effect, with cell viability dropping to 38.44%, 25.18%, and 33.91% at 24, 48, and 72 hours. The calculated IC_{50} for lisinopril was 74.04 µg/ml (Figure 1).

Likewise, amlodipine decreased Hep3B cell viability in a dose-dependent fashion. At 0.1 µg/ml, cell viability was 101.35%, 105.06%, and 140.76% at 24, 48, and 72 hours, indicating negligible cytotoxicity at low concentrations. At 0.5 µg/ml, viability was 96.93%, 93.18%, and 95.24%. At 1.25 µg/ml, it decreased to 67.77%, 94.86%, and 71.94%; at 6.25 µg/ml, 68.63%, 73.99%, and 54.75%; and at 31.25 µg/ml, 65.07%, 55.71%, and 42.28%. The highest concentration of 156.25 µg/ml caused the most

pronounced cytotoxic effect, with cell viability dropping to 51.57%, 40.95%, and 34.88%. The IC_{50} of amlodipine was calculated as 38.04 µg/ml (Figure 2).

Table 1. Primer sequences used for quantitative gene expression analysis.

Gene	Forward	Reverse
Bcl-2	5'-CTGCACCTGACGCCCTTCACC-3'	5'-CACATGACCCCACCGAACTCAAAGA-3'
Bcl-xL	5'-GATCCCCATGGCAGCAGTAAAGCAAG-3'	5'-CCCCATCCCGGAAGAGTTCATCTACT-3'
Bax	5'-TTTGCTTCAGGGTTTCATC-3'	5'-TCCTCTGCAGCTCCATGTTA-3'
Bak	5'-ACCAGCCTGTTTGAGAGTGG-3'	5'-AGTGATGCAGCATGAAGTCG-3'
β-actin	5'-CCAACCGCGAGAAGATGA-3'	5'- CCAGAGGCGTACAGGGATA-3'

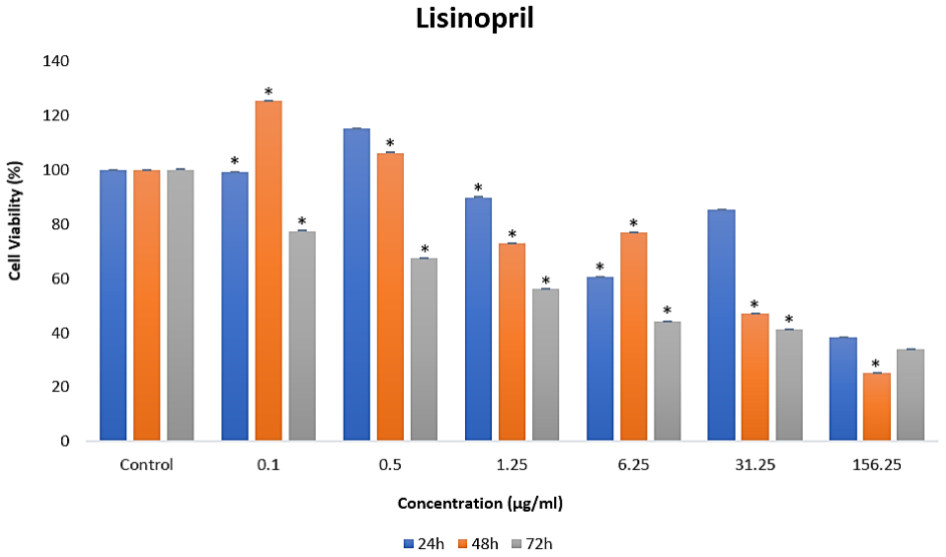

Figure 1. Lisinopril exhibited dose-dependent cytotoxic effects in Hep3B cells at 24, 48, and 72 hours, with an IC_{50} value of 7.404 µg/mL; cell survival significantly decreased in all treated groups versus the control (*p<0.05).

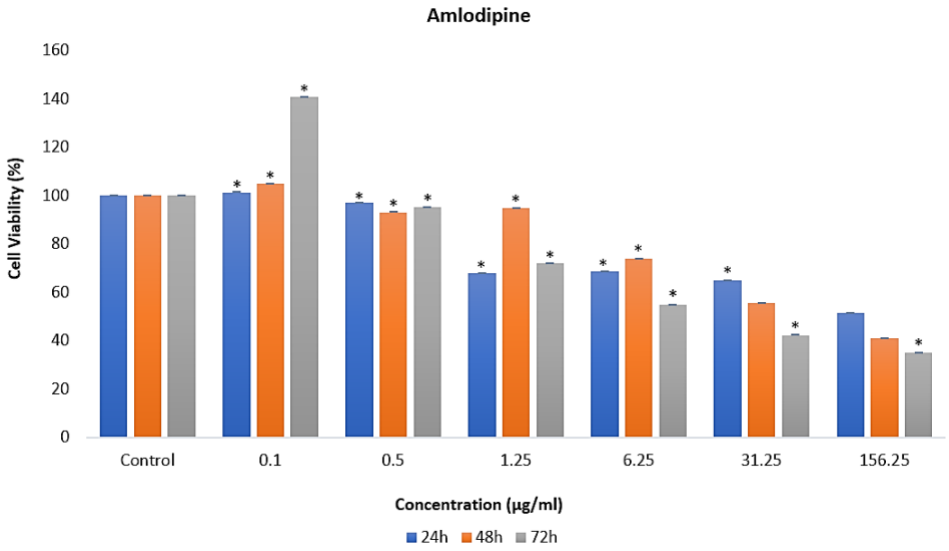

Figure 2. Amlodipine exhibited dose-dependent cytotoxicity in Hep3B cells at 24, 48, and 72 hours, with an IC_{50} value of 3,804 µg/mL; cell survival significantly decreased in all treated groups versus the control (*p<0.05).

Lisinopril and amlodipine influenced apoptotic gene expression in Hep3B cells in a time-dependent manner, with the primers used listed in Table 1. Lisinopril induced a modest but variable regulation of apoptotic genes. Bcl-2 expression slightly increased at 24 hours (1.05-fold) compared to the control but gradually decreased at later time points (0.95- and 0.73-fold at 48 and 72 hours, respectively). Similarly, BAX expression showed an early transient upregulation (1.96-fold at 24 hours) followed by normalization, while BAK expression increased at 48 hours before declining at 72 hours. These transient increases at early or intermediate time points may reflect short-term cellular stress or compensatory responses prior to the full activation of apoptotic pathways. In contrast, Bcl-xL expression remained consistently low (~0.30-fold) (Figure 3).

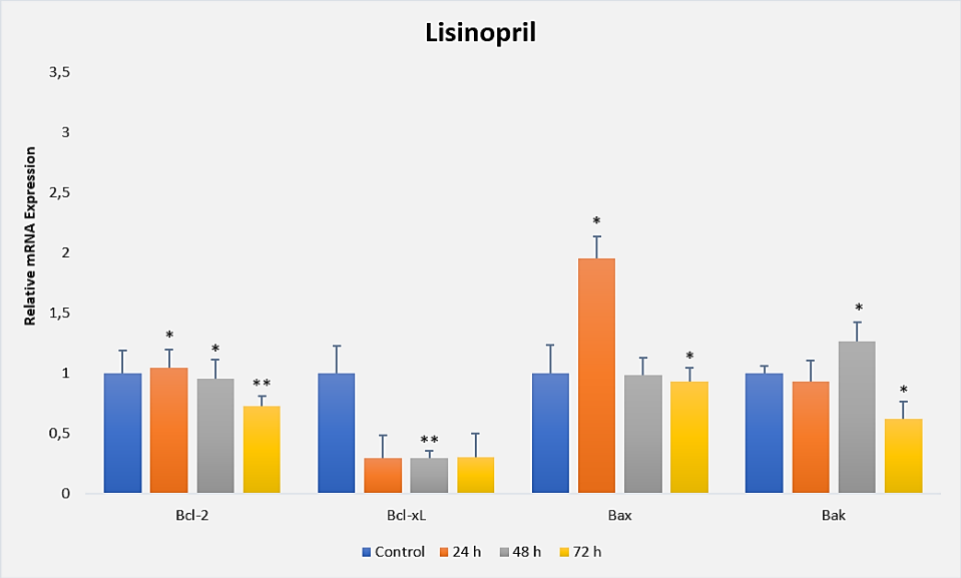

Figure 3. Effects of lisinopril on apoptosis gene expression in Hep3B cells at 24, 48, and 72 hours. Data are presented as the mean ± SD of three independent experiments, with *p<0.05 and **p<0.01 considered statistically significant.

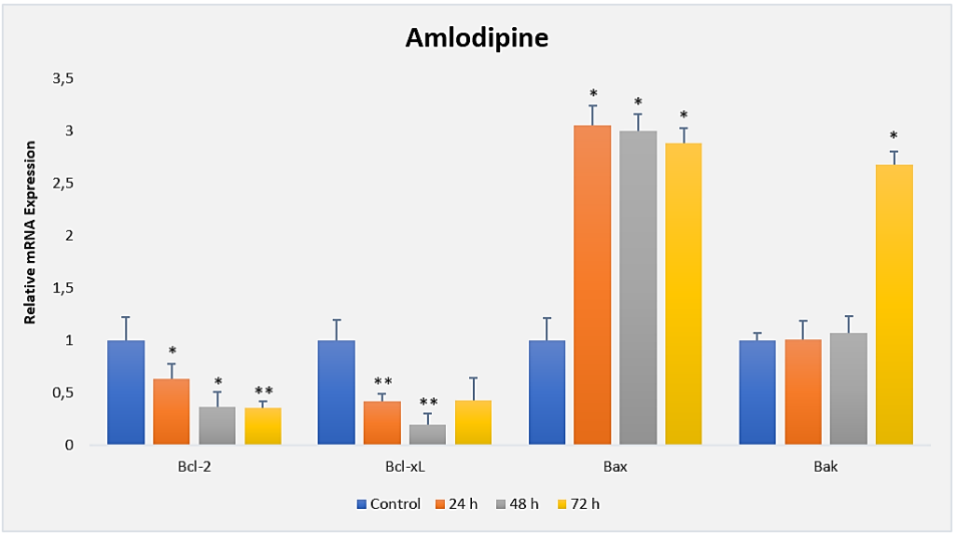

Figure 4. Effects of amlodipine on apoptosis gene expression in Hep3B cells at 24, 48, and 72 hours. Data are presented as the mean ± SD of three independent experiments, with *p<0.05 and **p<0.01 considered statistically significant.

Amlodipine exerted a more pronounced effect on apoptotic signalling, with Bcl-2 expression reduced to 0.63, 0.36, and 0.35-fold, and Bcl-xL expression approximately 0.41, 0.19, and 0.43-fold at 24, 48, and 72 hours, respectively. Although a slight increase in Bcl-xL was observed at 72 hours, the overall trend indicates sustained suppression of anti-apoptotic genes. Pro-apoptotic genes were markedly

upregulated, with Bax expression reaching 3.05, 2.99, and 2.88-fold, and Bak expression 1.00, 1.07, and 2.67-fold at the corresponding time points. Collectively, these findings suggest that both lisinopril and amlodipine dynamically and time-dependently modulate apoptotic gene expression, with amlodipine exerting a more pronounced pro-apoptotic effect (Figure 4).

DISCUSSION

Overexpression of the RAAS has been associated with serious pathologies such as diabetes, cardiovascular disease, hypertension, and renal failure (Hussain & Awan, 2018), and recent studies have also investigated the relationship between RAAS signalling and cell proliferation, inflammation, metastasis, and angiogenesis (Nehme et al., 2019). In this context, high RAAS activity has been reported to contribute to the development of cancers of the breast, pancreas, prostate, stomach, cervix, liver, ovary, brain, lung, skin, colorectal region, and hematopoietic cells (Haznedaroglu & Malkan, 2016).

Notably, one of the main effectors of RAAS, Ang II, activates the PI3K/Akt pathway upon binding to AT1R, which subsequently triggers NF-κB activation via IKKα/β and upregulates MMP-2 and MMP-9, thereby inhibiting apoptosis and promoting tumour progression, metastasis, and cell migration. This mechanism represents a key process explaining the role of RAAS activity in cancer development and has been particularly observed in human breast carcinoma models (Almutlaq et al., 2021; Hassani et al., 2023).

Moreover, excessive RAAS activation can be controlled using renin inhibitors, ACE inhibitors, ARBs, and aldosterone antagonists (Chen et al., 2018). For instance, the ACE inhibitor lisinopril reduces RAAS activity by blocking the conversion of Ang I to Ang II, thereby inhibiting Ang II–mediated anti-apoptotic signalling and regulating the expression of apoptosis-related genes (Brunner-La Rocca et al., 1999; Afsar et al., 2021).

Meta-analyses suggest that lisinopril and other ACE inhibitors may exert protective effects against various cancer types, including breast, lung, and colorectal cancers (Li et al., 2013). Population-based cohort studies further indicate that ACE inhibitor usage for hypertension treatment could lower the likelihood of developing lung cancer (Hicks et al., 2018).

Targeting RAAS represents a promising therapeutic strategy in cancer; in addition, voltage-gated calcium channels (VGCCs) are also being investigated as potential targets for future clinical cancer therapies. In HCT116 cells, inhibition of T-type calcium channels suppressed cell growth and triggered apoptotic processes (Dziegielewska et al., 2014). In another study showed that amlodipine dose-dependently inhibited the growth of human breast cancer cell lines and regulated apoptosis (Alqudah et al., 2022). In A549 lung cancer cells, amlodipine was observed to inhibit proliferation by arresting the tumour cell cycle. Mechanistically, this effect was shown to occur through the attenuation of the PI3K/Akt and Raf/MEK/ERK signalling pathways via EGFR and cell cycle–related proteins (cyclin D1, p-Rb, p27, p21) (Fu et al., 2022). Moreover, amlodipine has been reported to inhibit the proliferation of human epidermoid carcinoma cells by reducing BrdU incorporation into nucleic acids in A431 cells (Pasquier et al., 2016).

In this study, the effects of lisinopril and amlodipine on the viability and apoptotic gene expression of Hep3B hepatocellular carcinoma cells were investigated. Both drugs generally reduced cell viability in a concentration- and time-dependent manner; however, transient increases in viability were observed at certain concentrations compared to the control group. For example, lisinopril at 0.1 and 6.25 µg/ml showed increased cell viability at 48 hours, and at 156.25 µg/ml at 72 hours, while amlodipine at 0.1, 0.5, 1.25, and 6.25 µg/ml exhibited slight increases in viability at 48–72 hours. These fluctuations may reflect a hormetic response, where certain compounds stimulate cellular activity or metabolic adaptation at low or moderate doses, while higher doses exert cytotoxic effects. Such transient increases are unlikely to indicate true proliferative effects, but rather may represent cellular stress responses, compensatory mechanisms, or metabolic differences detected by the MTT assay (Bao et al., 2015). Overall, the general trend indicates suppression of cell proliferation at higher doses and longer exposure times, highlighting the complex dose- and time-dependent nature of drug–cell interactions. Lisinopril exhibited a modest and variable regulatory effect on apoptotic genes. Bcl-2 expression slightly increased at 24 hours compared to the control (1.05-fold) and gradually decreased at later time points (0.95- and 0.73-fold at 48 and 72 hours, respectively). In contrast, Bcl-xL expression remained low (~0.30-fold) at all time points. Bax expression showed an early transient increase (1.96-fold at 24 hours) followed by normalization, while Bak expression increased at 48 hours and decreased at 72 hours. These transient changes may reflect short-term cellular stress or adaptive responses preceding the activation of the apoptotic process (Zhang et al., 2025).

Amlodipine showed a more pronounced pro-apoptotic effect. Bcl-2 and Bcl-xL expression levels were significantly reduced at all time points, except for a slight increase in Bcl-xL at 72 hours, which may reflect a delayed feedback mechanism. Meanwhile, Bax and Bak genes were markedly upregulated, indicating activation of the mitochondrial apoptotic pathway. These findings are consistent with previous studies reporting that calcium channel blockers can induce apoptosis in cancer cells through mitochondrial dysfunction and suppression of pro-

survival signalling pathways such as PI3K/Akt and MAPK.

Consistent with the literature, our present study showed that treatment of Hep3B cells with both lisinopril and amlodipine led to a concentration- and time-dependent decrease in proliferation, accompanied by modulation of pro- and anti-apoptotic gene expression. Similarly, another study reported that ARBs and ACE inhibitors can induce autophagy-related cell death and exhibit anti-metastatic effects in prostate cancer cells, while L-type calcium channel blockers reduce intracellular Ca^{2+} levels, and suppress cell proliferation, androgen receptor–mediated gene expression, and growth (Iheanacho & Enechukwu, 2024). Taken together, these findings suggest that simultaneous targeting of the RAAS and VGCC pathways may synergistically inhibit tumour cell proliferation and enhance apoptotic responses.

In in vivo studies, pretreatment of rats with amlodipine, lisinopril, or allopurinol demonstrated significant protective effects against acetaminophen-induced hepatotoxicity. In these studies, treated animals showed significantly decreased serum transaminase levels, hepatic malondialdehyde, myeloperoxidase, and nitrate/nitrite levels, while hepatic glutathione and catalase levels were significantly increased. Histopathological evaluations strongly supported the biochemical findings (Mohammed et al., 2016). These results indicate the mechanistic roles of calcium channels, angiotensin-converting enzyme, and xanthine oxidase in the pathogenesis of acetaminophen-induced hepatotoxicity.

In contrast, case reports in the literature have described hepatotoxicity associated with lisinopril and amlodipine use. For example, in a 59-year-old woman with type 2 diabetes, high body mass index, and hypertension, cholestatic liver injury developed after five weeks of lisinopril treatment, and the same study reported a total of six cases from different sources associated with lisinopril-induced liver injury (Al-Rifaie et al., 2020). Similarly, in an 88-year-old woman with a history of hypertension, marked elevations in liver enzymes and right upper quadrant pain were observed after approximately two weeks of amlodipine treatment. Following discontinuation of the drug, liver enzymes gradually returned to normal over approximately seven weeks (Yet Kwong Horman et al., 2022).

Taken together, these findings highlight a discrepancy between the protective effects observed in in vivo animal studies and the rare hepatotoxic effects reported in case studies. The very limited number of patients in the case reports restricts the generalizability of these observations; therefore, these data alone are insufficient to confirm the hepatotoxic potential of these drugs. Nevertheless, to validate the reliability of the hepatoprotective effects observed in rat models, comparative studies of lisinopril and amlodipine in human healthy liver cell lines are warranted. Such investigations could provide mechanistic insights into both the protective and potential toxic effects of these agents. Consequently, integrating preclinical and clinical data would allow a more accurate evaluation of the safety and therapeutic potential of these drugs.

In conclusion, the present study supports findings in the literature and demonstrates that targeting the RAAS and VGCC pathways may suppress proliferation and enhance apoptotic responses in tumour cells. While our findings are promising, further studies are needed to elucidate the mechanistic roles of the RAAS and VGCC pathways in tumour progression and to evaluate their clinical potential.

Study Limitations and Strengths

To more comprehensively evaluate the interactions of the RAAS and VGCC pathways across different types of liver cancer, various liver cancer cell lines such as HepG2 and SK-HEP-1 should be used, and the reliability of the findings should be tested using normal hepatocyte cell lines such as THLE-2, THLE-3, and LO2. Additionally, the inclusion of a standard chemotherapeutic positive control (e.g., doxorubicin or cisplatin) in cytotoxicity and apoptosis analyses would enhance the comparability and validity of the experiments. Current studies primarily focus on gene expression levels, and validation of these findings at the protein level is of critical importance; protein-level confirmation is indispensable for understanding the actual functional effects of signalling pathways and apoptotic mechanisms and for increasing the reliability of the findings. Furthermore, detailed investigation of the downstream effects and interaction mechanisms of signalling pathways involved in the apoptotic process, such as p53, MAPK, JAK-STAT, and members of the caspase family, remains limited. Moreover, in vivo studies are lacking, which limits the translation of these in vitro findings to physiologically relevant conditions. Comprehensive studies on the responses of these signalling pathways to combination therapies and their long-term effects, both in vitro and in vivo, will contribute to a more holistic understanding of apoptotic mechanisms and allow for a more accurate evaluation of the therapeutic potential of the RAAS and VGCC pathways as targets.

CONCLUSION

In conclusion, data from both the literature and our current study indicate that regulating cell proliferation and apoptotic processes through calcium channels could serve as a potential combination therapy along with approaches targeting the RAAS pathway. The crosstalk between RAAS and VGCC pathways seems to affect proliferation and apoptotic responses in cancer cells, and further insights into these mechanisms may support the development of more precise and effective therapeutic strategies in the future. Additionally, combining RAAS- and

VGCC-focused treatments could offer a promising approach to restrict tumour progression and enhance patient survival.

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Conflict of Interest
The author declares no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions
Plan, design: ASA; **Material, methods and data collection:** ASA; **Data analysis and comments:** SGT; **Writing and corrections:** ASA, SGT.

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Ethical Approval
This study did not require ethical approval.

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