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MORALITY BEFORE KNOWLEDGE

The impact of valproic acid administration: Effects on the growth of tongue cancer cells

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ABSTRACT

Background: Tongue cancer represents the predominant malignancy within the oral cavity (25 – 40% of squamous cell carcinoma), necessitating treatment modalities such as surgery, radiotherapy, and chemotherapy. Valproic acid, an antiepileptic medication, functions as a histone deacetylase inhibitor or activator of anti-tumor signaling pathways. **Objective:** To deepen our understanding of the effects of valproic acid on the viability, cytotoxicity, proliferation, and migration capabilities of HSC-3 cells. **Method:** This study employed an in vitro laboratory approach, exposing HSC-3 cells to valproic acid. The experimental groups included a negative control with culture media devoid of valproic acid, and treatment groups exposed to valproic acid at concentrations of 145 ppm, 180 ppm, and 355 ppm, respectively. **Results:** Significant differences (p-value < 0.05) were observed between HSC-3 cells treated with valproic acid (145 ppm, 180 ppm, and 355 ppm) and the control group in terms of viability, cytotoxicity, proliferation, and migration. Reduced cell viability, increased cytotoxicity, and decreased proliferation were noted. Migration assays indicated suppressed migration of HSC-3 cells. **Conclusion:** In summary, this study reveals that valproic acid exerts substantial effects on various aspects of HSC-3 cell behavior. It decreases cell viability, enhances cytotoxicity, suppresses proliferation, and inhibits cell migration. These findings highlight the potential of valproic acid as a therapeutic agent for tongue cancer by targeting crucial cellular processes involved in cancer progression. Further research and clinical trials are essential to confirm these effects and explore their application in cancer treatment strategies. **Novelty/Originality of this article:**: This study shows valproic acid has potential as a therapeutic agent for tongue cancer by decreasing cell viability, increasing cytotoxicity, suppressing proliferation, and inhibiting migration of HSC-3 cells. These findings introduce a new application of valproic acid as an anticancer agent, expanding the use of antiepileptic drugs. This study opens up opportunities for developing more effective tongue cancer therapies and encourages further research and clinical trials to validate these findings.

KEYWORDS: cytotoxicity; HSC-3 cell; migration; proliferation valproic acid; viability.

1. Introduction

Cancer is one of the diseases that can cause death, and generally, it occurs frequently in developing countries (Kemenkes, 2015). According to the International Agency for Research on Cancer (2012), there were approximately 14.1 million new cases of cancer, 8.2 million cancer-related deaths, and 32.6 million people living with cancer worldwide (WHO, 2012). Oral cavity cancer is of particular concern, with nearly 75% of cases occurring in developing countries like Indonesia, contributing to 3-4% of all cancer cases (Sirait, 2013). Oral cavity cancer is characterized by the growth of cancer cells in the oral cavity, including the lips and lip mucosa, tongue, palate, gingiva, mouth floor, and buccal mucosa (Vasconcelos et al., 2014). Approximately 90% of oral cavity cancers are squamous cell

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carcinomas, malignant tumors originating from stratified squamous epithelium (Vasconcelos et al., 2014). These tumors have the ability to invade surrounding tissues and metastasize to distant sites, resulting in a poor prognosis and significantly reducing survival rates by 50% (Vasconcelos et al., 2014). Therefore, the discovery of anticancer drugs for treating oral cavity cancer remains highly necessary.

Valproic acid is an anticonvulsant drug that plays a role in epigenetic processes through histone modification during cell proliferation, differentiation, and apoptosis (Komariah et al., 2018). Epigenetic processes involve the modulation of gene expression without altering the nucleotide sequence of the gene (Harahap, 2014). Dysregulation of epigenetic regulation leads to aberrant gene expression and cellular transformation towards cancer (Lee, et al., 2015). Epigenetic regulation includes histone modification, DNA methylation, and non-coding RNA (Juliandi et al., 2010).

Histone modification is influenced by two main enzymes: histone acetyltransferases (HAT) and histone deacetylases (HDAC) (Chen et al., 2015). The balance between histone acetylation and deacetylation determines whether a gene is active or inactive, while imbalance can lead to changes in gene expression that contribute to pathological conditions such as cancer (Mottamal et al., 2015). The anticancer mechanism of HDAC inhibitors (HDACi) in cancer cells operates through two main pathways: extrinsic and intrinsic pathways (Mottamal et al., 2015). HDACi anticancer mechanisms vary depending on the type of cancer. HDACi are classified into five classes based on their chemical compounds, one of which is short-chain fatty acids like valproic acid (Eckschlager et al., 2017). Valproic acid is an anticonvulsant drug that affects histone modification by inhibiting HDAC enzyme activity (Komariah et al., 2017).

Gene expression is influenced by epigenetic regulations necessary for normal cell development (Ma et al., 2017). Valproic acid, through the inhibition of HDAC enzyme activity, has been widely used in breast cancer therapy (Liu et al., 2017), bladder cancer (Mologni et al., 2009), colon cancer (Saha et al., 2017), liver cancer (Sang et al., 2016), and CAL27 cell line (Hoffman, 2014). However, it has not been studied in HSC-3 cell lines. Given these issues, research on the effect of valproic acid inhibiting HDAC enzyme activity to influence HSC-3 cell growth is needed.

1.1 Tongue Cancer

Squamous cell carcinoma (oral cancer) is a type of disease that primarily originates from epithelial tissue, with the tongue being the most commonly affected location (Fazlipur & Masomi, 2013). This cancer often occurs on the tongue, especially in the anterolateral area. Major risk factors for oral cancer include alcohol and tobacco use (Norton, 2007). Tongue cancer itself is a malignancy originating from the mucosal epithelial tissue of the tongue, characterized by multi-layered flat epithelial cells (Marliana, 2015). Patients with tongue cancer usually experience symptoms such as pain, difficulty in speech, dysphagia, and weight loss (Cha, 2007).

Symptoms of tongue cancer are similar to other types of oral cancer (Cancer Treatment of America, 2018). Some symptoms of tongue cancer include: (1) Persistent pain on the tongue and/or jaw; (2) Lump or thickening inside the mouth; (3) White or red patches on the gums, tongue, tonsils, or mouth lining; (4) Sore throat or feeling like something is stuck in the throat; (5) Difficulty swallowing or chewing; (6) Difficulty moving the jaw or tongue.

1.2 Valproic Acid

Valproic acid (VPA), a short-chain chemical compound ($C_8H_{16}O_2$), is utilized as an anticonvulsant drug and has been found to inhibit the development of cancer cells (Sun & Coy, 2014). Specifically, VPA acts by inhibiting histone deacetylase (HDAC) or activating signaling pathways that suppress tumor growth (Sun & Coy, 2014). Histone acetylation, a process dynamically regulated by histone acetyltransferase (HAT) and HDAC enzymes, plays a crucial role in chromatin structure and gene transcription regulation (Ma et al.,

2017). HAT enzymes promote chromatin relaxation and gene activation, while HDAC enzymes are associated with chromatin condensation and transcriptional repression (Ma et al., 2017). Alterations in eukaryotic chromatin structure due to N-terminal histone acetylation are pivotal in regulating gene expression (Ma et al., 2017). In response to DNA damage, HDAC inhibitors induce hyperacetylation, activating the p53 protein, which in turn halts the G1 and S phases of the cell cycle, leading to cell cycle arrest, inhibited proliferation, enhanced apoptosis, and reduced cell differentiation (Komariah et al., 2017; Komariah et al., 2018).

Recent studies have emphasized the significant impact of histone acetylation and deacetylation dysregulation in the development and progression of various cancers (Sun & Coy, 2014). This imbalance in histone modification disrupts chromatin structure and gene expression, creating a conducive environment for cancer to initiate and advance. Notably, valproic acid has demonstrated considerable potential in cancer therapy due to its ability to inhibit histone deacetylase (HDAC) activity. By interfering with HDAC-mediated mechanisms, valproic acid promotes the hyperacetylation of histones, which subsequently alters gene transcription in a manner that can inhibit tumor growth (Sun & Coy, 2014).

The antiproliferative effects of valproic acid resulting from HDAC inhibition have been extensively documented across a wide spectrum of cancers. These include cervical, prostate, neuroblastoma, Medullary Thyroid Cancer (MTC), myeloma, colon, glioma, leukemia, breast, lung, bladder, melanoma, glioblastoma, Renal Cell Carcinoma (RCC), esophageal squamous cell carcinoma, endometrial stromal sarcoma, osteosarcoma, Hepatocellular Carcinoma (HCC), gastrointestinal carcinoid, pheochromocytoma, mesothelioma, pancreatic, head/neck squamous cell carcinoma, ovarian, myeloma, and cholangiocarcinoma (Sun & Coy, 2014). This broad spectrum of effectiveness underscores valproic acid's potential as a versatile therapeutic agent in oncology.

Valproic acid holds significant promise in cancer therapy, attributed to its well-documented mechanism of action, cost-effectiveness, and favorable clinical tolerability (Bordie & Brandes, 2014). This compound not only exerts direct effects on tumor cells but also plays a crucial role in modulating various aspects of cancer biology. It is involved in regulating cell growth, inducing apoptosis (programmed cell death), and inhibiting the invasive properties of cancer cells (Papi et al., 2010). These diverse and powerful effects underscore its potential as a valuable candidate for extensive research and possible inclusion in comprehensive cancer treatment strategies. Ongoing research is imperative to fully understand its therapeutic advantages and to refine its clinical applications across different types of cancer.

The profound impact of valproic acid on cancer therapy lies in its ability to modulate essential cellular processes, demonstrating efficacy across a wide spectrum of cancer types. Its role as an HDAC inhibitor highlights its therapeutic potential by influencing gene expression through chromatin modification, thereby affecting cell proliferation, apoptosis, and metastasis. Moreover, the favorable clinical profile and cost-effectiveness of valproic acid enhance its attractiveness as a candidate for further investigation and potential integration into cancer treatment strategies.

1.3 Cell Growth

The cell cycle is a recurring sequence of events that allows cell division into two daughter cells (Barberis et al., 2017). It plays a critical role in ensuring proper growth, development, and maintenance of tissues throughout an organism's life cycle. The cell cycle is typically divided into four phases: G1 (gap 1), S (synthesis), G2 (gap 2), and M (mitosis) (Barberis et al., 2017). Each phase is governed by specific checkpoints and regulatory mechanisms that ensure the orderly progression of events.

Cell cycle regulation is intricately controlled by molecular interactions within tissues. Key regulators include cyclins, which undergo periodic synthesis and degradation. Cyclins bind to and activate cyclin-dependent kinases (CDKs), enzymes that control the transition

between cell cycle phases (Barberis et al., 2017). This precise orchestration prevents uncontrolled cell division and maintains cellular homeostasis.

When cell cycle regulation is disrupted, uncontrolled cell growth can occur, leading to faster cell division than usual. This abnormal proliferation can trigger the development of cancer (Barberis et al., 2017). Cancer cells often exhibit dysregulated cell cycle checkpoints, allowing them to evade normal growth controls and proliferate without limit. Understanding the molecular mechanisms underlying cell cycle regulation and its disruption in cancer is crucial for developing targeted therapies that specifically inhibit cancer cell proliferation while sparing normal cells. Further research into these mechanisms holds the promise of uncovering new therapeutic targets and improving cancer treatment strategies. In normal cells, the number of cells is regulated by the balance between cell division and cell death (apoptosis) (Safitri et al., 2016). Cell growth can be observed through the cell's viability, the cytotoxicity of a substance applied to the cell, cell proliferation, inhibition rate, and migration ability.

Cell viability is a measure of the number of living cells within a given sample or cell culture medium, indicating the capacity of these cells to thrive and proliferate under specific conditions (Wyllie et al., 2000). This measure is essential for determining the optimal environmental conditions and nutrient requirements necessary for maintaining and supporting the growth of a cell population (Wyllie et al., 2000). Cytotoxicity, in contrast, refers to the detrimental effect of certain substances, such as chemical compounds, on cells, leading to cell death (Wyllie et al., 2000). This toxic effect can manifest as cellular damage that impairs the normal growth processes, potentially inhibiting or completely stopping cell proliferation (Kusumawardani, 2006). Furthermore, cell proliferation involves the expansion of the cell population through a process of cell growth followed by division, resulting in an increase in the number of cells. The dynamic relationship between these concepts is depicted in Fig. 1, which outlines the theoretical framework of this study. This framework illustrates the complex interactions between tongue cancer cells (HSC-3), various therapeutic interventions, and epigenetic treatments, and how these factors collectively influence key cellular processes such as apoptosis (programmed cell death), proliferation, differentiation, and migration. These processes are critical in understanding the progression of cancer and the effectiveness of different treatment strategies.

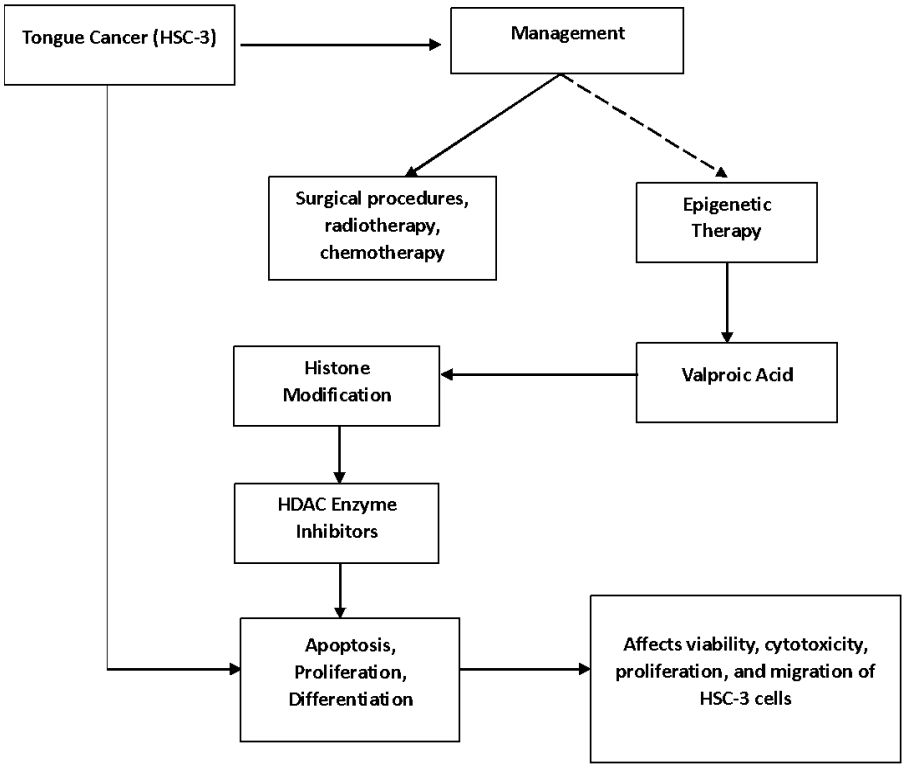

Fig. 1 Theoretical Framework Diagram

2. Methods

The type of research used was an in vitro laboratory experiment using valproic acid. This study was divided into two stages. The first stage involved the preparation of several concentrations of valproic acid. The second stage aimed to see the effect of valproic acid on the viability, cytotoxicity, proliferation ability, and migration of HSC-3 cells. The treatment groups in this study were divided into four. The first group was a negative control, consisting of a culture medium without valproic acid. The second, third, and fourth groups were given valproic acid at 145 ppm, 180 ppm, and 355 ppm in the culture medium.

The population of this study sample consists of HSC-3 cells that have been subcultured, originating from the Integrated Laboratory of the Faculty of Medicine, Yarsi University. HSC-3 cells are tongue cancer cells commonly used in oral cavity cancer-related research. They were selected for their characteristics representing tongue cancer typically encountered in clinical practice. These cells were cultivated under controlled laboratory conditions to ensure consistency in experimental outcomes between different trials.

2.1 Research variable and data analysis

The research variable is Valproic acid, which is the independent variable. The dependent variables are the viability, cytotoxicity, proliferation, and migration ability of HSC-3 cells. Valproic acid is a short-chain and branched fatty acid usually used as an anticonvulsant drug and is available in tablet form. This study diluted valproic acid with concentrations of 145, 180, and 355 ppm. Cell viability is the ability of cells to live after being exposed to a substance. HSC-3 cells that have been treated for 24 hours are measured by adding CCK-8 reagent, and the color changes that occur are measured using a spectrophotometer.

Cell proliferation is the phase of cells when they experience unhindered division. HSC-3 cells that have been treated for 24 and 48 hours are measured by adding a CCK-8 reagent, and the color changes that occur are measured using a spectrophotometer. Cell cytotoxicity is the ability of a substance to cause damage when it enters the cell. In this study, the number of cells that died from the number of cells that could still survive after being treated for 24 hours was measured by adding a CCK-8 reagent, and the color changes that occurred were measured using a spectrophotometer. Cell migration is the standard cell movement process in response to substance exposure. Cell migration in this study was observed through an increase in the acceleration of cell migration that was treated. Observations were made at 2, 21, and 23 hours and were viewed qualitatively. Data will be presented as mean ± standard deviation (SD). Normality tests were conducted using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used, and if significant differences were found ($p < 0.05$), post hoc tests were conducted.

3. Results and Discussion

3.1 Result

The assessment of valproic acid effectiveness at various concentrations on the viability, cytotoxicity, and proliferation of HSC-3 cells was based on color changes observed after treating the cells with the CCK-8 reagent. This was subsequently measured using a microplate reader at a wavelength of 450 nm. The darker the color, the greater the number of viable cells. The evaluation of HSC-3 cell migration rate was conducted using the T-scratch method. The cells are thawed in a T-25 flask, then harvested and counted. The cells are subsequently seeded into a 24-well multiplate, with 20,000 cells per well. The growth medium is replaced daily.

To create a scratch assay, gently scratch the monolayer with the tip of a 10 µL pipette. Ensure that the pipette tip remains perpendicular to the surface of the monolayer to achieve a consistent scratch distance from the initial scratch until the procedure is completed. After

performing the first scratch, create a second scratch perpendicular to the first to form a cross-shaped pattern or intersecting lines. The cells are then washed with PBS and treated with valproic acid. Observe cell growth at 2 hours, 21 hours, and 23 hours. Finally, capture images of the cells using a microscope and analyze any changes that have occurred.

3.1.1 Viability

Cells were seeded at a density of 15,000 cells per well in a 96-well plate, with a total volume of 0.2 ml per well. After incubating the cells for 24 hours, CCK-8 reagent (Cell Counting Kit-8) was added to the wells. Cell viability was then assessed by measuring the color change induced by the CCK-8 reagent using a microplate reader, with absorbance readings taken at a wavelength of 450 nm.

The average viability results of HSC-3 cells treated with valproic acid concentrations of 145 ppm, 180 ppm, and 355 ppm compared to those without valproic acid showed significant differences, with a respective decrease in HSC-3 cell viability of 0.67%, 3.51%, and 10.14%. This study aims to evaluate the potential of valproic acid in inhibiting the growth and migration of tongue cancer cells (HSC-3). The experimental methods used enable researchers to examine the effects of valproic acid under controlled laboratory conditions before potential clinical applications (Table 1).

Table 1. Viability of HSC-3 Cells

Treatment	Concentration	N	Viability (%)	P-value
Control	-	5	94.01±0.47[a]	0.000
Valproic Acid	145 ppm	5	93.38±0.14[a]	
	180 ppm	5	90.71±0.71[b]	
	355 ppm	5	84.48±0.55[c]	

The average viability results of HSC-3 cells treated with valproic acid concentrations of 180 ppm and 355 ppm compared to 145 ppm showed a decrease in HSC-3 cell viability by 2.86% and 9.53%, respectively. Additionally, the average viability results of HSC-3 cells treated with valproic acid concentration of 355 ppm compared to 180 ppm showed a decrease in HSC-3 cell viability by 6.87% (Fig. 2).

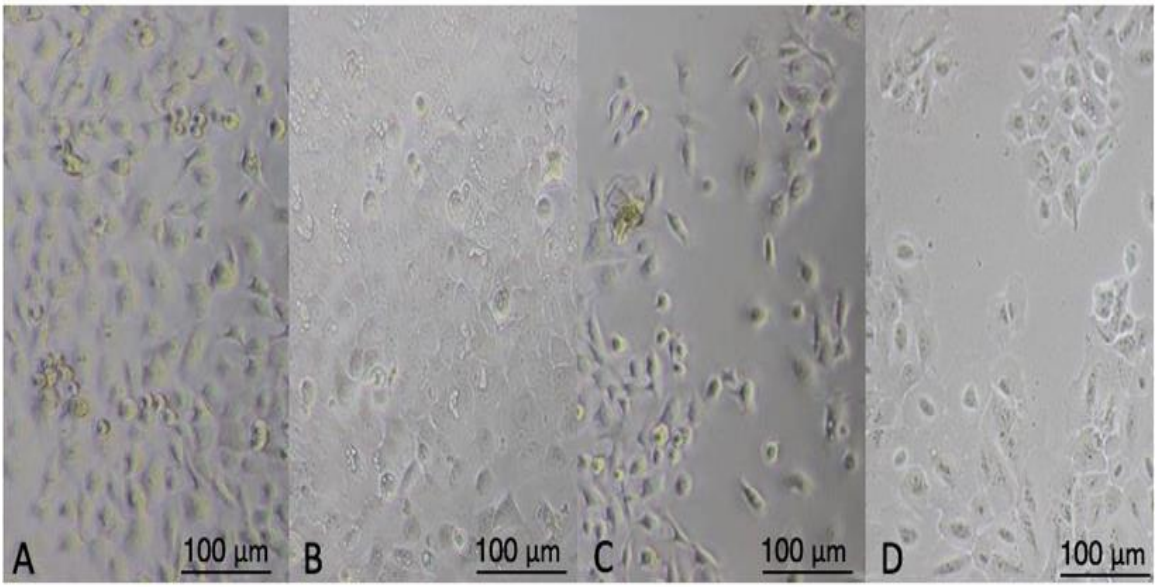

Fig. 2. HSC-3 cell viability assay after 24 hours A) control; B) valproic acid 145 ppm; C) valproic acid 180 ppm; D) valproic acid 355 ppm.

3.1.2 Cytotoxicity

The number of cells seeded is 15,000 cells per well in a volume of 0.2 mL, using a 96-well plate. After allowing the cells to incubate for 24 hours, the CCK-8 (Cell Counting Kit-8) reagent is added to the culture wells. Cytotoxicity is assessed by calculating the number of cells that have undergone death relative to those that remain viable. This is done by adding the CCK-8 reagent, and the resulting color change is measured using a microplate reader at a wavelength of 450 nm. The average cytotoxicity results of HSC-3 cells treated with valproic acid concentrations of 145 ppm, 180 ppm, and 355 ppm compared to untreated cells showed significant differences, with successive increases in HSC-3 cell cytotoxicity by 10.70%, 55.35%, and 159.53% (Table 2).

Table 2. Cytotoxicity of HSC-3 Cells

Treatment	Concentration	n	Cytotoxicity	P-value
Control	-	5	5.98 ± 0.47^{c}	0.000
Valproic Acid	145 ppm	5	6.62 ± 0.14^{c}	
	180 ppm	5	9.29 ± 0.71^{b}	
	355 ppm	5	15.52 ± 0.55^{a}	

The average cytotoxicity of HSC-3 cells exposed to valproic acid at concentrations of 185 ppm and 355 ppm, when compared to a lower concentration of 145 ppm, demonstrated a significant increase in toxicity, with HSC-3 cell death rising by 40.33% and 134.44%, respectively. Furthermore, when the concentration of valproic acid was elevated to 355 ppm from 180 ppm, the average toxicity of HSC-3 cells escalated dramatically, showing a 67.06% increase in cell death. These findings indicate a dose-dependent relationship, where higher concentrations of valproic acid substantially enhance the cytotoxic effects on HSC-3 cells.

3.1.3 Proliferation and Inhibitor Rate

The average cell proliferation of HSC-3 cells over a 24-hour period, when treated with valproic acid at concentrations of 145 ppm, 180 ppm, and 355 ppm, demonstrated statistically significant differences ($p<0.05$) compared to untreated controls. Specifically, proliferation rates decreased by 17.05%, 28.80%, and 52.15%, respectively, with increasing concentrations of valproic acid. Furthermore, the average proliferation of HSC-3 cells treated with valproic acid at 180 ppm and 355 ppm showed reductions of 14.17% and 42.32% in cell proliferation, respectively, compared to the 145 ppm concentration. Additionally, the average proliferation of HSC-3 cells treated with valproic acid at 355 ppm, in comparison to the 185 ppm concentration, revealed a decrease in cell viability of 32.8%.

The average cell proliferation of HSC-3 cells over a 48-hour period, treated with valproic acid at concentrations of 145 ppm, 180 ppm, and 355 ppm, showed significant differences compared to untreated controls ($p < 0.05$). The proliferation of HSC-3 cells decreased by 30.68%, 39.52%, and 80.91% at these respective concentrations. When comparing the effects of valproic acid at concentrations of 180 ppm and 355 ppm to the 145 ppm concentration, the average cell proliferation decreased by 12.75% and 72.46%, respectively. Additionally, the average proliferation of HSC-3 cells treated with valproic acid at 355 ppm compared to 180 ppm showed a reduction in cell viability of 68.43%.

The average cell proliferation of HSC-3 cells over a 72-hour period, treated with valproic acid at concentrations of 145 ppm, 180 ppm, and 355 ppm, showed a decrease in proliferation by 40.12%, 56.16%, and 91.24%, respectively, compared to the control group with no valproic acid treatment. Additionally, the average proliferation of HSC-3 cells treated with valproic acid at 180 ppm and 355 ppm, when compared to the 145 ppm concentration, revealed a reduction in cell viability of 26.78% and 85.37%, respectively. Furthermore, the average viability of HSC-3 cells exposed to valproic acid at 355 ppm,

compared to the 180 ppm concentration, demonstrated a decline in cell viability of 80.02% (Fig. 3).

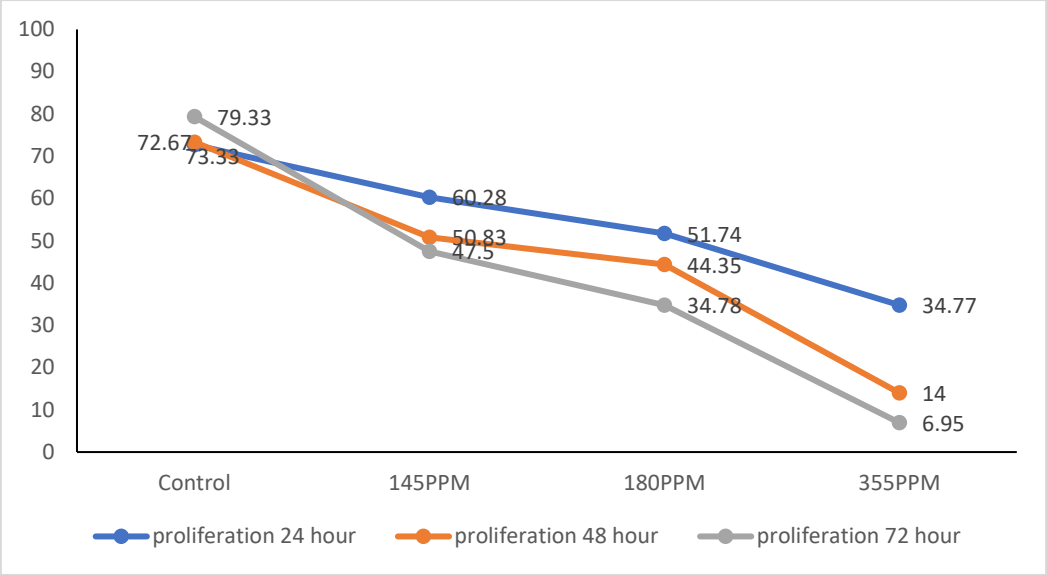

Fig. 3 Proliferation Graph

The average inhibition rate of HSC-3 cells across a 24-hour period, when exposed to valproic acid at concentrations of 145 ppm, 180 ppm, and 355 ppm, revealed marked and statistically significant differences when compared to the untreated control group. In the control group, the inhibition rate showed a gradual decline throughout the 24-hour, 48-hour, and 72-hour observation intervals, indicating a reduced effectiveness in inhibiting cell proliferation over time. In stark contrast, the groups treated with valproic acid at the aforementioned concentrations exhibited a notable increase in the inhibition rate, underscoring the compound's capacity to significantly curb the proliferation of HSC-3 cells. This data strongly suggests that valproic acid plays a crucial role in suppressing the growth rate of HSC-3 cells relative to untreated conditions. The detailed inhibition values for each group over the observation periods are illustrated in Fig. 4. Meanwhile, the results of the cell migration acceleration test using the T-scratch method on confluent cells involved scratching the middle part and observing at 2 hours, 21 hours, and 23 hours. The migration speed results were observed using a microscope (Fig. 5).

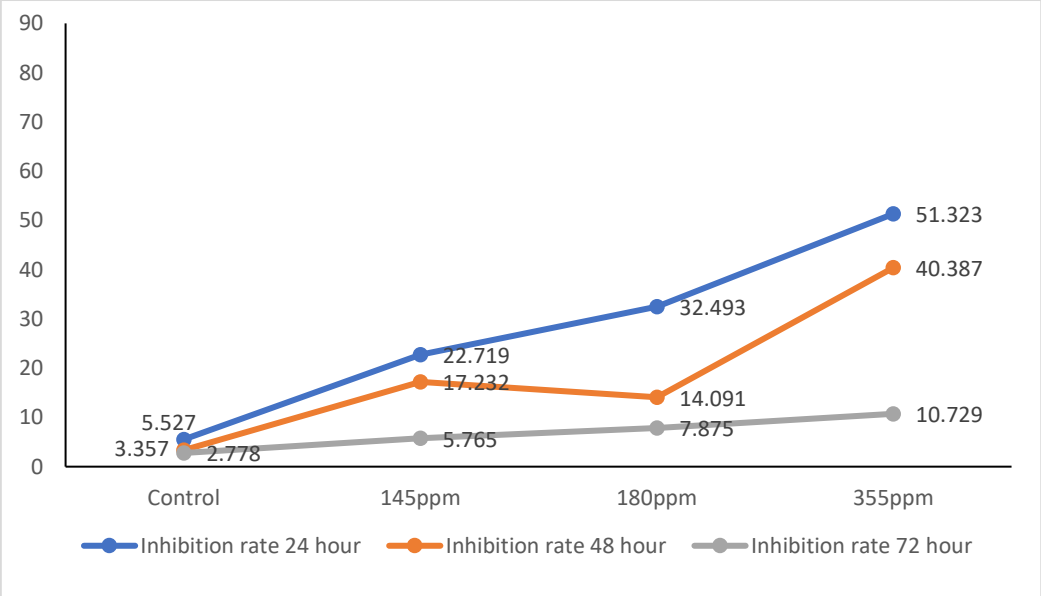

Fig. 4 Inhibition Rate Graph

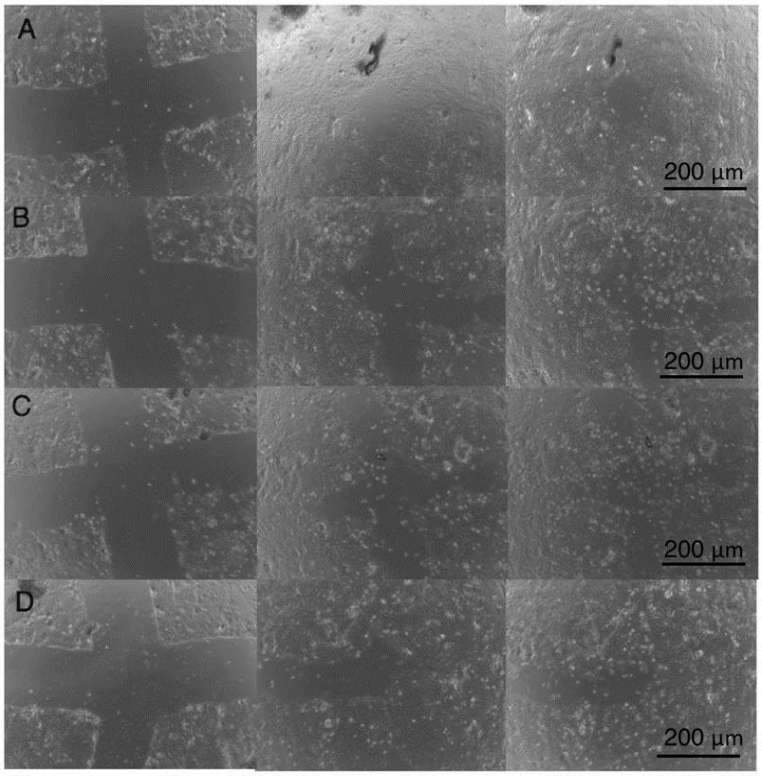

Fig. 5 Migration of HSC-3 cells observed at 2 hours, 21 hours, and 23 hours; A) control; B) valproic acid 145 ppm; C) valproic acid 180 ppm; D) valproic acid 355 ppm.

3.2 Discussion

Valproic acid (VPA), a branched short-chain chemical molecule ($C_8H_{16}O_2$), is utilized as an anti-convulsant medication with additional properties in suppressing cancer cell development. Specifically, VPA acts as a histone deacetylase inhibitor or signaling activator to suppress tumor growth (Sun & Coy, 2014). Histone acetylation undergoes dynamic regulation by enzymes such as histone acetyltransferase (HAT) and histone deacetylase (HDAC). HAT promotes chromatin relaxation and gene transcription activation, whereas HDAC is involved in chromatin condensation and transcriptional silencing. Viability results of HSC_3 cells exposed to valproic acid show decreased viability compared to unexposed HSC_3 cells (control); higher concentrations of valproic acid demonstrate higher viability reduction. This research indicates valproic acid's ability to diminish cell adaptability. It is suggested that valproic acid has the capability to inhibit cancer cell growth. According to Naji et al., valproic acid significantly reduces MCF-7 cell survival by arresting the cell cycle in G1 phase, thereby halting cell proliferation (Qi et al., 2005). According to Qi et al., (2005) the ability of nano-chitosan to influence cell viability occurs due to interactions between nano-chitosan and cell membranes, resulting in cell leakage and necrosis (Qi et al., 2005). According to Loh et al., (2019) disruption of cell membrane integrity is evidenced by leakage of alanine transaminase into the extracellular environment, leading to cell necrosis. Additionally, cellular internalization of nano-chitosan also inhibits mitochondrial dehydrogenase enzyme activity, which is suspected to affect cell survival, thus decreasing cell viability.

Cytotoxicity results of HSC3 cells exposed to valproic acid indicate increased cytotoxicity compared to unexposed HSC3 cells (control); higher concentrations of valproic acid demonstrate higher cytotoxicity. This research shows valproic acid's capability to reduce cell survival. It is suggested that valproic acid is toxic to cancer cells. According to Zhao et al., valproic acid significantly inhibits SKOV3 cell growth by inducing cell cycle arrest and apoptosis. HDAC inhibitors show selective cytotoxicity against tumor cells with minimal toxicity to normal cells (Shan et al., 2012) According to Qi et al. (2005) exposure to nano-

chitosan causes plasma membrane damage, ultimately resulting in leakage and cell necrosis. Furthermore, nano-chitosan-induced necrosis may involve mitochondrial damage, which undergoes a drastic decrease in potential, leading to mitochondrial dysfunction almost concurrently with or shortly after loss of plasma membrane integrity, resulting in cell necrosis.

Cell proliferation results of HSC3 cells exposed to valproic acid indicate decreased proliferation compared to unexposed HSC_3 cells (control); higher concentrations of valproic acid demonstrate higher proliferation reduction. This research demonstrates valproic acid's ability to inhibit cell division. It is suggested that valproic acid has the capability to inhibit cancer cell growth. According to Zhijian et al., valproic acid can reduce CAL27 cell growth with increasing VPA concentration. According to Potdar et al. (2016), exposure to nano-chitosan on oral cancer cells can induce apoptosis and cause cell cycle arrest. Disruption of the cell cycle according to Qi et al. (2005) shows a significant decrease in G0 and G1 phases, which may lead to increased concentration-dependent apoptosis due to nano-chitosan. Proliferation assay results of HSC-3 cells with concentrations of 145 ppm, 180 ppm, and 355 ppm, and different exposure times of 24 hours, 48 hours, and 72 hours, illustrate that higher concentrations and longer exposures of valproic acid can lower HSC-3 cell viability and increase cytotoxicity against HSC-3 cells.

Research results show that valproic acid administration can affect cell viability testing, cytotoxicity, proliferation, and HSC3 cell inhibition rate. Valproic acid has the ability to influence changes in eukaryotic chromatin structure caused by N-terminal histone acetylation, which plays a central role in gene transcription regulation. Valproic acid works by inhibiting HDAC activity, leading to hyperacetylation as a response to DNA damage. When DNA damage is detected, protein p53 is activated, resulting in G1 and S phase arrest in the cell cycle, thereby preventing cell proliferation and differentiation, and increasing apoptosis. This theory supports research findings described by decreases in viability and proliferation, and increases in cytotoxicity. However, observations of migration acceleration show minimal inhibition compared to control. It is suggested that valproic acid, through HDACi activity, influences the expression of genes involved In migration processes, such as suppression of MMP-1 and MMP-9 gene expression. This was proposed by Ahrens et al. (2015), indicating that substances with HDACi activity can induce cell death and inhibit cell migration.49 It is suggested that in the study by Potdar & Shetti (2016), suppression of MMP-1 and MMP-9 genes involved in cancer cell migration may inhibit metastasis.

4. Conclusions

Valproic acid can reduce the ability of HSC-3 cells to survive; the higher the valproic acid concentration, the lower the viability of HSC-3 cells. Valproic acid has high cytotoxicity against HSC-3 cells, with a concentration of 355 ppm showing higher cytotoxicity than concentrations of 145 ppm and 180 ppm. In addition, valproic acid can inhibit the proliferation of HSC-3 cells, where the higher the concentration, the lower the proliferation ability of HSC-3 cells. Valproic acid can also inhibit the acceleration of HSC-3 cell migration, although the inhibition is not visible. Further research is recommended to use higher concentrations with more significant amounts to determine the starting point of the effect of valproic acid on HSC-3 cells more precisely. In addition, cell migration should be observed using T-scratch software to see the percentage of open areas that occur.

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Author Contribution

S. R. conceived and designed the study, performed the experiments, analyzed and interpreted the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper and approved the final draft.

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The authors declare no conflict of interest.

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