Cytotoxicity of Turmeric Extract (*Curcuma longa linn.*) on BHK21 Fibroblast Cells

Nadya Fithrie Azzahra¹, Retno Indrawati², Indeswati Diyatri²
¹,²,³Airlangga University, Surabaya, Indonesia
Email: nadya.fithrie.azzahra-2020@fkg.unair.ac.id

Abstract

Wound healing is the process of recovery from injury to stop bleeding, and fibroblasts play a crucial role in the healing process and contribute to the regeneration of periodontal tissue. Turmeric extract (*Curcuma longa linn.*) is an alternative treatment that uses natural ingredients with antibacterial, antioxidant, and anti-inflammatory properties. The aim of this study is to demonstrate the toxicity of turmeric extract (*Curcuma longa linn.*) on BHK21 fibroblast cells. Turmeric extract (*Curcuma longa linn.*) was administered at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78% to BHK21 fibroblast cells, and its cytotoxicity was tested using the MTT Assay. The research results show that the percentage viability of fibroblast cells decreases successively with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78%, with the highest percentage at a turmeric extract concentration of 0.78% and the lowest at a concentration of 100%. The conclusion of this study is that turmeric extract (*Curcuma longa linn.*) is not cytotoxic to BHK-21 fibroblast cells at concentrations of 0.78%, 1.56%, and 3.12%.

Keywords: Cytotoxicity, *Curcuma longa linn.*, BHK-21 Fibroblasts.

A. INTRODUCTION

Wound healing is the process of the organism's response to injury to stop bleeding and restore the protective barrier that prevents infection by maintaining internal homeostasis, allowing the organism to survive. The overall wound healing process occurs in several phases that work together. The phases of wound healing include hemostasis, inflammation, proliferation, and remodeling (Cialdai et al., 2022). Fibroblasts play a crucial role in the wound healing process, such as stopping fibrin clot formation, creating the extracellular matrix (ECM), and forming collagen structures as well as the contraction process in the wound. The wound healing process can be disrupted if fibroblasts do not form (Dick et al., 2023). The homeostasis phase can lead to the oral cavity harboring various microbial flora. Imbalances in flora or colonization by microorganisms such as bacteria can infect the oral cavity and its mucosa (Saini et al., 2019).

Periodontitis is an inflammatory disease characterized by infection in the supporting tissues around the teeth, known as the periodontium. Additionally, genetics, environmental factors, and behavioral factors also play a role in the development of the disease, affecting an individual's vulnerability to attacks and the speed of its progression. The periodontium consists of various structures, including the gingiva, the connective tissue beneath it, the cementum covering the root surface of the tooth, alveolar bone, and the periodontal ligament between the cementum and alveolar bone. The junctional epithelium of the gingiva is a structure located at the
base of the gingival sulcus, regulating the presence of bacteria in this area. The most prominent feature of periodontitis is the activation of osteoclastogenesis and subsequent destruction of alveolar bone, which is an irreversible process leading to the loss of tooth-supporting structures (Könönen et al., 2019). The prevalence of periodontitis in Indonesia is still relatively high. RISKESDAS 2018 data shows a 74.1% prevalence of periodontitis cases in Indonesia (KEMENKES, 2018).

Fibroblasts are the main cells in the fibrous connective tissue of the periodontium. They play a crucial role in the synthesis and remodeling of the extracellular matrix, including collagen, elastin, and nonfibrillar glycoproteins. Fibroblasts also have contractile and motile abilities that assist in the structural organization of tissues, particularly in the development of the periodontal ligament. Additionally, fibroblasts play a role in the turnover, repair, and regeneration of periodontal tissues. They can synthesize and repair collagen as well as other components of the extracellular matrix. Fibroblasts can also produce cytokines that mediate tissue damage and stimulate bone resorption by osteoclasts. For example, matrix metalloproteinase-1 enzyme produced by fibroblasts and macrophages can degrade extracellular matrix collagen under physiological conditions. Fibroblasts and other mesodermal tissues can produce tissue inhibitors of metalloproteinases. These inhibitors play a role in regulating the degradation of connective tissue and are found in high concentrations in healthy periodontal areas. This highlights the central role of fibroblasts in the process of remodeling periodontal tissues, including the periodontal ligament (Srivastava et al., 2017).

Fibroblast cells used in the Baby Hamster Kidney 21 cell culture technique. BHK-21 cells are commonly used to test the toxicity of a substance, especially in dentistry. Toxicity is assessed by examining the viability percentage of the cells under investigation (Yuliati, 2005). Cytotoxicity tests are conducted to observe the viability of fibroblast cells, assessing the influence of substance concentration and exposure time, including its toxic effects (Wyllie, 2000). Cytotoxicity tests are necessary before a drug substance is applied to humans. The MTT method is the most frequently used test for evaluating the cytotoxicity of a substance by assessing cell viability (Vajrabhaya, 2018). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a soluble yellow compound used to evaluate cellular enzymatic activity and the number of live cells. Living cells with metabolic activity can reduce MTT salt to form a blue-purple formazan. This formazan can be measured spectrophotometrically to determine the level of cell survival. The formation of the blue-purple color indicates a high level of cell survival, while low optical absorbance indicates cytotoxicity or cell damage. The use of MTT provides information about the cytotoxic effects or therapeutic potential of a substance on living cells in cell culture (Meizarini et al., 2005).

Alternative treatment is non-medical treatment where the equipment and materials used are not part of standard medical treatment. The National Institute of Health states that alternative therapy is a collection of treatment and healthcare
systems, practices, and products generally not part of conventional treatment. One form of alternative treatment is the use of herbal medicines (Ayu et al., 2020).

Alternative treatment using natural ingredients such as turmeric or Curcuma longa Linn., which has antibacterial properties, is becoming increasingly popular. The use of turmeric has been practiced in many countries in South Asia, where it is used as an antiseptic and antibacterial to treat burns and bruises. In Ayurvedic medicine in India, turmeric is used to treat various respiratory diseases such as asthma, hyperactive bronchial, allergies, as well as liver diseases, anorexia, rheumatism, diabetic wounds, colds, coughs, and sinus infections. Turmeric has also been used to treat sprains and swelling (Ramadhani et al., 2018).

Turmeric (Curcuma longa Linn.) contains compounds such as flavonoids, alkaloids, essential oils, curcumin, tannins, terpenoids, and saponins (Wijaya et al., 2022). Previous research has proven that turmeric extract has benefits as an antioxidant, anti-inflammatory, and antibacterial (Khatun et al., 2021). Curcumin, the active compound in turmeric, acts as an antibacterial agent by damaging the cytoplasmic membrane, disrupting bacterial metabolism through denaturation of cellular proteins, causing nutrients to leak out of the cell, and killing or inhibiting the growth of bacterial cells. Additionally, turmeric's antibacterial properties are also related to the main chemical content of essential oil, which consists of five main classes, namely monoterpenoid hydrocarbons, oxygenated monoterpenoids, sesquiterpenoid hydrocarbons, oxygenated sesquiterpenoids, and esters. Turmeric essential oil contains sesquiterpenoids with strong antibacterial effects (Ramadhani et al., 2018).

Until now, turmeric extract (Curcuma longa Linn.) is easily found and commonly used, but there has been no research examining the cytotoxicity of turmeric extract on fibroblast cells at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78% to determine a safe concentration for use. This research aims to provide additional information regarding the cytotoxicity of turmeric extract on fibroblast cells. These cells are chosen because they are easily obtained, easy to grow, easily cultured in media, and can serve as a model in various conditions and diseases. Fibroblast cells are also the largest component of the gingiva, periodontal ligament, and pulp (Takii et al., 2002; Mizoguchi et al., 2018; Zhang and Zhang, 2020; Winkler et al., 2020).

Based on this information, the researcher initiated this study with the aim of proving that the extraction of turmeric extract (Curcuma longa Linn.) is not toxic to BHK21 fibroblast cells. Additionally, this study is conducted to demonstrate the toxicity of turmeric extract (Curcuma longa Linn.) at various concentrations of 0.78%, 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100% on BHK21 fibroblast cells.

B. LITERATURE REVIEW

1. Turmeric

Turmeric, known as Curcuma longa Linn., is a plant belonging to the Zingiberaceae family (Prabowo, et al., 2019). Turmeric is a medicinal plant originating
from Asia. In Indonesia, turmeric is commonly used in daily life, such as in traditional herbal drinks and as a culinary spice. The classification of turmeric is as follows: Kingdom (Plantae); Division (Magnoliophyta); Class (Liliopsida); Subclass (Zingiberidae); Order (Zingiberales); Family (Zingiberaceae); Genus (Curcuma); Species (Curcuma longa Linn.) (Shan & Iskandar, 2018).

Turmeric is a perennial plant that forms clusters and rhizomes. The plant reaches a height of about 40-100 cm. Turmeric has a pseudostem consisting of overlapping leaf sheaths. Its ability to retain water well makes the turmeric stem wet. The stem is round and has a purplish-green color. Turmeric leaves are elongated oval, roughly 31-84 cm long and 10-18 cm wide. In one turmeric plant, there are 6-10 leaves. Turmeric also produces pointed flowers that are either white or light yellow. Each turmeric flower has three sepals, three petals, and four stamens. The rhizomes of turmeric spread and branch, forming clusters. The rhizomes are elongated and have branches underground. The skin of the rhizomes is orange-brown or slightly yellow, while the flesh is yellowish-orange with a distinctive odor (Shan & Iskandar, 2018).

Curcumin, found in turmeric, plays a crucial role in maintaining health and treating various diseases. In vitro studies on the anti-inflammatory effects of curcumin indicate that it can inhibit the activity of lipoxygenase and cyclooxygenase enzymes. The expression of these enzymes tends to increase in inflammatory and cancerous conditions. Curcumin also enhances antioxidant activity in the body. Besides blocking free radicals, curcumin stimulates the body’s antioxidant mechanisms. Additionally, curcumin can reduce the production of reactive oxygen species (ROS) in the body by maintaining the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Verma, et al., 2018).

Key components found in turmeric rhizomes include curcumin, essential oil, resin, demethoxycurcumin, oleoresin, bisdemethoxycurcumin, gum, fat, protein, calcium, phosphorus, and iron. Turmeric essential oil contains compounds such as ar-turmerone, α and β-turmerone, turmerol, α-atlantone, β-caryophyllene, linalool, and 1,8 cineole (Shan & Iskandar, 2018). Turmeric (Curcuma longa Linn.) contains chemical compounds such as curcumin, essential oil, flavonoids, alkaloids, and
saponins. Curcumin and essential oil are the main constituents found in turmeric (Curcuma longa Linn.) (Zulmardi et al., 2022).

Curcumin, present in turmeric, plays a crucial role in maintaining body health and is used as a treatment for various diseases. In vitro research on the anti-inflammatory effects of curcumin indicates that it can inhibit the activity of lipoxygenase and cyclooxygenase enzymes. The expression of these enzymes tends to increase in inflammatory and cancer conditions. For example, celecoxib is a selective anti-inflammatory drug that inhibits COX-2 and is known to have a synergistic effect with curcumin. Celecoxib can inhibit the catalytic activity of this isoenzyme, while curcumin itself can inhibit the transcription of COX-2 protein, glutathione peroxidase, and superoxide dismutase (Mutiah, 2015). Curcumin also has the effect of enhancing antioxidant activity in the body. Besides blocking free radicals, curcumin stimulates the body’s antioxidant mechanisms. Additionally, curcumin can reduce the production of reactive oxygen species (ROS) in the body. This is achieved by maintaining the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Verma et al., 2018). Some previous studies also show that curcumin has antibacterial effects. For instance, curcumin has been proven to inhibit the growth of Helicobacter pylori CagA+ strains in vitro. Curcumin also has the ability to suppress the growth of certain bacteria, such as Streptococcus, Staphylococcus, and Lactobacillus. Moreover, curcumin has demonstrated antiviral activity, inhibiting the Epstein-Barr virus (EBV). Importantly, curcumin has also shown anti-HIV (human immunodeficiency virus) activity by inhibiting HIV-1 integrase required in the virus’s replication (Verma et al., 2018).

Essential oil is reported to have biological properties such as antibacterial, anti-inflammatory, antioxidant, antitumor, and detoxifying. Essential oils containing terpene compounds have similar properties to alcohols. These compounds have the ability to damage the cytoplasmic membrane and cause protein denaturation. This disruption can lead to the leakage of macromolecules and ions from cells, causing the loss of cell shape, ultimately resulting in bacterial cell lysis (Ramadhani et al., 2017).

Flavonoids are phenolic compounds that also contribute to the color of plants. Flavonoids are derivatives of phenolic compounds that have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities (Antonio, 2019). Flavonoids capture ROS and prevent the regeneration of ROS, leading to increased activity of cellular antioxidant enzymes. Prevention of ROS formation is one of the benefits of flavonoids by inhibiting the action of xanthine oxidase and Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase enzymes, as well as chelating Fe2+ and Cu2+ ions, thereby inhibiting redox reactions that can produce free radicals (Dyah et al., 2014).

Alkaloids and saponins are identified in both fermented and non-fermented products. Both compounds have specific physical, chemical, and biological properties that make them potential drugs. The pharmacological uses of alkaloids include stimulating the nervous system, combating microbial infections, and acting as antioxidants because alkaloids can counteract free radicals by inhibiting redox
reactions. The presence of phytochemical compounds such as saponins and alkaloids in plants makes them act as natural antioxidants as they can bind free radicals (Sulasiyah et al., 2018).

2. Properties of Turmeric

Previous studies have explained that variations in concentration in turmeric ethanol extract result in different effects on the ability to inhibit the growth of Staphylococcus aureus. Curcuma longa Linn. rhizome extract contains active compounds believed to have therapeutic effects, such as curcumin containing phenolate hydroxyl groups, and essential oil containing terpene compounds similar to alcohols. These compounds have the ability to damage the cytoplasmic membrane and cause protein denaturation (Ramadhani et al., 2017). Phenol and terpene compounds can cause instability in cell walls and the cytoplasmic membrane, disrupting the functions of selective permeability, active transport, and protein regulation. This disruption can lead to the leakage of macromolecules and ions from cells, causing the loss of cell shape and ultimately resulting in cell lysis. Phenolate compounds in turmeric extract have the ability to inhibit the growth of bacterial cells, and their effect depends on the concentration used (Ramadhani et al., 2017). Therefore, turmeric can be used as an antioxidant.

In addition to having antioxidant properties, turmeric is also toxic. Toxicity can occur depending on the amount of toxic substances entering the body and the duration of exposure to these toxic substances. Toxic substances entering the body can cause disturbances in organ function (Lawrence et al., 2022). Compounds like curcumin and its derivatives most often cause liver injury depending on the dosage used. Therefore, it can be concluded that turmeric has hepatotoxic properties if consumed in high doses and for an extended period. Individuals with liver disease, heavy drinkers, and those taking medications antagonistic to turmeric should avoid consuming turmeric products. Long-term use of turmeric also affects skin conditions and may lead to gastric ulcers. Curcumin also stimulates the gallbladder, so patients with gallbladder disease should reduce curcumin use or do so only under a doctor's advice. Based on in vitro research, high doses of turmeric have been shown to have hepatotoxic effects on rats, such as coagulation necrosis, focal necrotic changes in the spleen and kidneys (Balaji et al., 2010). Toxicity can be observed during histopathological examination, revealing necrosis, edema, and cell inflammation (Lawrence et al., 2022). Necrosis refers to the death of tissue or cells that undergo stages such as karyorrhexis, karyolysis, or pyknosis in the cell nucleus. Necrosis can be caused by various factors, such as drug use, exposure to toxic substances, mechanical trauma, lack of blood supply, loss of nerve innervation, and exposure to radioactive rays (Lawrence et al., 2022). In a state of necrosis, Reactive Oxygen Species (ROS) and intracellular calcium levels increase. Stimuli causing these changes will alter the permeability of the cell membrane, causing an imbalance of intracellular potassium, sodium, and calcium ions. The increased calcium ion levels will affect mitochondrial function, influencing ROS activity. Continuously high calcium ion
levels will disrupt the integrity of the membranes inside the mitochondria, affecting their ability to produce ATP. This ion imbalance will activate the intracellular cysteine protease calpain. Calpain is an inactive intracellular cysteine protease that becomes active in the presence of increased calcium ions. Active calpain will affect lysosome membranes by releasing cathepsins B and L. This process will cause destabilization of the final membrane system, leading to cell membrane loss, and the cell’s contents will be released into the extracellular space. Characteristics of cells undergoing necrosis include reduced ATP production, leading to cell death (Escobar et al., 2015). ATP loss will cause cellular edema. Cellular edema, also known as cytotoxic edema, is a process where cells experience onkotic or oncosis swelling caused by the entry of extracellular Na+ and other cations that accumulate inside the intracellular space (D. Liang et al., 2009). The edema process is associated with disturbances in ion pumping, especially in the function of Na+/K+ ATPase pumping. This disturbance can result in an increased concentration of intracellular Na+ ions because Na+ ions cannot efficiently exit the cell, causing edema (Lawrence et al., 2022).

Turmeric interacts with drugs through several mechanisms. The main mechanism of turmeric interaction with drugs through the CYP450 enzyme involves the inhibition and induction of CYP450 enzymes. Based on existing research, curcumin compounds found in turmeric can inhibit the CYP2C9 enzyme, which is part of the CYP450 enzyme group that can inhibit the liver’s ability to metabolize drugs dependent on that enzyme. Curcumin activity inhibiting CYP2C9 can be observed from enzyme kinetic analysis. The analysis shows that NADPH is a non-competitive cofactor inhibitor for CYP2C9, making curcumin considered non-competitive with the substrate. This process increases the bioavailability of drugs, raising the risk of toxicity when given simultaneously with CYP2C9 substrate drugs such as warfarin, tolbutamide, and phenytoin. The presence of curcumin will increase the AUC and CMax of these drugs. In another study, curcumin has been proven to trigger an increase in the activity of the CYP1A2 enzyme, also part of the CYP450 enzyme group, thus accelerating the metabolism of certain drugs in the body (Wang et al., 2015).

Consuming turmeric excessively can have negative effects on digestive system health and overall body health. One of the impacts is irritation of the digestive system, which can cause symptoms such as abdominal pain, bloating, and nausea. Hydrolytic curcumin has been proven to cause instability in intestinal pH, quickly metabolizing and conjugating in the liver, and being excreted in feces (Dulbecco et al., 2013). The use of turmeric in high doses has been associated with other health conditions, including gastroesophageal reflux disease (GERD), endometriosis, and potential issues in pregnant and breastfeeding women. It is important to note that if you are taking medications regularly, consult with a doctor before consuming turmeric. This is because turmeric can interact with medications and affect their effectiveness (Veronika et al., 2022).

The use of turmeric as a natural remedy generally does not cause serious allergic effects. Allergic reactions to turmeric rarely occur, and most people tolerate
turmeric well. However, turmeric contains proteins that can trigger allergic reactions in sensitive individuals. Turmeric proteins identified as allergens can interact with previously produced IgE antibodies in the body. This process triggers the release of inflammatory mediators, such as histamine, responsible for the onset of allergic symptoms. Allergic symptoms that may arise due to turmeric allergy include skin rashes, itching, swelling, nausea, vomiting, or, in severe cases, anaphylaxis (Asnia et al., 2019).

Previous studies have demonstrated that curcumin compounds found in turmeric can disrupt the activity of certain hormones in the body. Traditionally, turmeric has been used as a remedy to regulate the smoothness of the menstrual process. Curcumin has the ability to inhibit the aromatase enzyme, influencing the hormonal balance between estrogen and androgen in the body (Liang et al., 2009). Additionally, based on studies, curcumin has been found to lower elevated androgen levels in patients with PCOS (Kamal et al., 2021). Furthermore, curcumin also affects the hormone receptors present in cells. Previous research has shown that curcumin can interact with estrogen hormone receptors, leading to changes in estrogen hormone responses in the body (Nejati-Koshki et al., 2014). Curcumin has anti-inflammatory properties that create hormonal imbalance, affecting hormone production and utilization. Curcumin provides anti-inflammatory effects by regulating inflammatory signaling pathways and inhibiting the production of inflammatory mediators, reducing inflammation, and potentially restoring hormone balance. Curcumin binds to toll-like receptors (TLRs) and regulates nuclear factor kappa-B (NF-KB), mitogen-activated protein kinase (MAPK), activator protein-1 (AP-1), and other signaling pathways that control inflammatory mediators, reducing inflammation (Peng et al., 2021).

3. Cytotoxicity Test

Cytotoxicity testing is a toxicity test conducted in vitro using cell cultures to detect the antineoplastic activity of a compound in order to obtain cytotoxic drugs. It is a quantitative test by determining cell death (Indrayudha et al., 2013). There are two common methods used in cytotoxicity testing, namely the direct calculation method using trypan blue and the MTT assay method. The MTT assay method is a colorimetric method used in cytotoxicity testing. In this method, MTT reagent is used, which is a water-soluble tetrazolium salt that can be converted into formazan crystals by the succinate tetrazolium reductase enzyme active in living cells through the mitochondrial respiratory pathway. The formazan crystals have a purple color, which can then be measured for absorbance using an ELISA reader (Nurani et al., 2015).

The principle of the MTT method is a colorimetric-based measurement based on the reduction reaction of water-soluble tetrazolium, producing a yellow solution. This reaction produces insoluble purple formazan salt that can be measured spectrophotometrically. The MTT reagent only reacts with living cells and is cleaved through the reduction reaction by the succinate tetrazolium reductase system to form formazan (Nurani et al., 2015).
4. **Fibroblasts Cell**

Fibroblasts are cells commonly found in connective tissue and play a role in synthesizing various components of the extracellular matrix, such as collagen, elastin, and glycosaminoglycans. These cells also secrete growth factors and cytokines that can influence the proliferation and differentiation of cells. In in vitro culture, fibroblasts can produce various types of proteins, including factors that inhibit cell differentiation. Fibroblasts have two stages of activity, namely active and quiescent, characterized by differences in morphology and protein synthesis activity (Wirata, 2019).

Fibroblasts are the main cells responsible for the production of fibers in soft tissue and the extracellular matrix. In addition to fibroblasts, chondroblasts also produce similar substances. Connective tissue originates from the mesenchyme, which is embryonic tissue consisting of elongated undifferentiated cells. Mesenchymal cells have distinctive features such as oval nuclei with prominent nucleoli. These cells develop from the mesoderm and serve as the origin for various types of cells in connective tissue, as well as other structures such as blood cells, endothelial cells, and muscle cells (Wirata, 2019).

BHK21 cells appear as oval cells with a maximum size of about 8 microns. The cytoplasm shows rough endoplasmic reticulum distributed diffusely throughout the cell without being connected to the plasma membrane. Instead, they are extensively connected to perinuclear cisterns. Cytoplasmic islands are immersed in lacunar reticulum space. In some electron micrographs, rough endoplasmic reticulum appears as isolated circles surrounded by cytoplasmic matrix. Ribosomes attached to the membrane of rough endoplasmic reticulum have an average size of 120 Å. Many free ribosomes of the same size are scattered throughout the cytoplasm, both isolated and grouped. Fine fibers occur in longitudinally oriented bundles in the cytoplasm. Mitochondria show structural features described elsewhere (Soto & Castejon, 1969).

BHK21 cells have less developed Golgi apparatus. Vacuolated lysosomes with sizes ranging from 0.3 to 1 micron are visible. They contain partially digested materials such as membranes and granules. Dense granular clusters without bounding

![Figure 2. Chemical Structure of Formazan (Source: Shi, et al., 2021)](image-url)
membranes are also observed. The cell surface is smooth with few short processes. At this level, many pinocytic vesicles are clearly visible. Adhesion between neighboring cells is present. They have attachment plaques resembling the central zone of epithelial cell (zonula adherens). The large cell nucleus is located centrally and has an irregular shape, with folds in the nuclear membrane. The outer nuclear membrane, carrying ribosomes on its surface, is continuous with the membrane of the rough endoplasmic reticulum. Chromatin clumps are seen aggregated toward the inner nuclear membrane, interrupted by a clear pathway with a width of about 0.1 micron at the nuclear pores. The description of the nucleolus corresponds to mammalian cells by Bernhard.1 Multiple nucleoli are found in each cell and are almost always associated with the nuclear membrane (Soto & Castejon, 1969).

BHK21 fibroblast cells are often used to test the toxicity of substances because BHK21 fibroblast cells are the best culture material originating from embryonic tissue cells, making them easy to grow and subculture (Wulandari, 2006).

5. **Hypothesis**

Turmeric extract (Curcuma longa Linn.) is not toxic to BHK21 fibroblast cells.

C. **METHOD**

This study is an in vitro laboratory experimental research. The research design used is a post-test only control group design. BHK21 fibroblast cells that have been cultured are placed in each well of a 96-well microplate. Wells are divided into two groups: the treatment group and the control group.

The research sample consists of fibroblast cells obtained from BHK21 cell culture. The determination of sample replication used in this study refers to the Federer formula (1963). From the 8 test groups used, 3 sample replications are obtained per group. This research is divided into 10 groups:

1. Positive control group: BHK21 fibroblast cell culture sample with Minimum Essential Medium Non-Essential Amino Acids (MEM-NEAA) without the addition of extract to demonstrate 100% cell viability.
2. Negative control group: Sample group containing MEM-NEAA without the addition of extract to demonstrate 0% cell viability.
3. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 100% with BHK21 fibroblast cells and MEM-NEAA.
4. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 50% with BHK21 fibroblast cells and MEM-NEAA.
5. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 25% with BHK21 fibroblast cells and MEM-NEAA.
6. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 12.5% with BHK21 fibroblast cells and MEM-NEAA.
7. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 6.25% with BHK21 fibroblast cells and MEM-NEAA.
8. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 3.12% with BHK21 fibroblast cells and Eagle Minimum Essential Medium (EMEM).

9. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 1.56% with BHK21 fibroblast cells and MEM-NEAA.

10. Turmeric extract (Curcuma longa Linn.) treatment group at a concentration of 0.78% with BHK21 fibroblast cells and MEM-NEAA.

This research uses three types of variables: independent variables, dependent variables, and control variables. The independent variable used is turmeric extract with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%. The dependent variable studied is the percentage of viable Baby Hamster Kidney fibroblast (BHK-21) cells. The control variables in this study are the MTT test technique, incubation temperature, and cell incubation time.

This research was conducted at two locations: Balai Materia Medica, Batu, Malang, and the Research Center of the Faculty of Dentistry, Universitas Airlangga, carried out from August 2023 to September 2023. The equipment used in this research includes an oven, cutting tools, grinder, digital shaker, Erlenmeyer flask, jars, gauze, rotary evaporator, water bath, alcoholmeter, micropipettes (200 ul, 1000 ul), tube rack, 15 ml centrifuge tubes, yellow and blue tips, CO2 incubator, inverted microscope, ELISA reader spectrophotometer, 96-well microplate, and centrifuge. The materials used in this research are turmeric rhizomes (Curcuma longa Linn.), 96% ethanol, sterile distilled water, Minimum Essential Medium Non-Essential Amino Acids (MEM-NEAA), 0.25% Trypsin EDTA, Phosphate Buffer Saline, DMSO, and 5 mg/mL MTT solution.

The working procedure to obtain the results of this research involves several stages. Firstly, the process begins with the extraction of turmeric from 1.5 kg of turmeric rhizomes. The turmeric is thoroughly washed, cut, and dried in an oven at a temperature ranging between 40-50 degrees Celsius for a duration of 24 hours. Once the turmeric rhizomes are adequately dried, they are placed into a grinding machine to produce a fine powder. The turmeric powder is then subjected to extraction using the maceration method. The steps of the maceration method, as outlined by Ramadhani in 2018, are as follows:

1. Turmeric powder is placed into a container, and 7500 mL of 96% ethanol solvent is added.
2. The container is positioned on a digital shaker at a speed of 50 rpm for 24 hours to ensure homogeneity.
3. The homogenized mixture of turmeric powder and 96% ethanol becomes a liquid extract. This liquid extract is filtered using cloth and collected in an Erlenmeyer flask.
4. The residue from filtering the liquid extract undergoes maceration twice. The residue is placed in a container, and 96% ethanol solvent is added until submerged. The container is then positioned on a digital shaker for 24 hours.
5. The obtained filtrate from the entire filtering process is combined and evaporated using a rotary evaporator for 3 hours.

6. The evaporated filtrate is placed in a water bath for 2 hours to ensure the removal of any remaining 96% ethanol. The filtrate undergoes testing with an alcoholmeter to confirm the absence of 96% ethanol.

Figure 3. Turmeric Extract (Materia Medica Institute)

Second stage: Preparation of turmeric extract concentration groups (Curcuma longa linn.) involves dilution steps using distilled water, as outlined by Prehananto et al., 2023:

1. 100% concentration is directly taken from the prepared turmeric extract.
2. 50% concentration is obtained by taking 2 ml from the 100% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
3. 25% concentration is obtained by taking 2 ml from the 50% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
4. 12.5% concentration is obtained by taking 2 ml from the 25% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
5. 6.25% concentration is obtained by taking 2 ml from the 12.5% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
6. 3.12% concentration is obtained by taking 2 ml from the 6.25% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
7. 1.56% concentration is obtained by taking 2 ml from the 3.12% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.
8. 0.78% concentration is obtained by taking 2 ml from the 1.56% concentration stock solution using a micropipette, then adding it to 2 ml of distilled water.

Third stage: Cytotoxicity testing is conducted using the MTT method (Vajrabhaya et al., 2018, and Kurniawan et al., 2016):

1. Cells are retrieved from the incubator and observed under a microscope to ensure there are enough cells for the treatment.
2. Cells are harvested using Trypsin EDTA.
3. Cells are centrifuged for 5 minutes at a speed of 2000 rpm.
4. Minimum Essential Medium Non-Essential Amino Acid (MEM-NEAA) and cells with a density of 2x10^4 are added to a 96-well microplate.
5. The 96-well microplate is incubated for 24 hours.
6. Turmeric extract samples (Curcuma longa linn.) at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78% are added to the wells, with each treatment group replicated three times.
7. The 96-well microplate is incubated for another 24 hours.
8. Medium and samples are collected, washed using Phosphate Buffer Saline (PBS).
9. MTT reagent is added in 20 µl.
10. Samples are incubated for 4 hours.
11. Cell conditions are observed under a microscope, and DMSO is added in 100 µl.
12. Absorbance values are read using an ELISA reader at a wavelength of 540 nm to determine optical density.
13. Cell viability percentage is calculated using the formula (Freshney, 2010):

\[
Cell\ \text{Viability} \ (%) = \frac{OD_{\text{treatment}} - OD_{\text{control media}}}{OD_{\text{kontrol sel}} - OD_{\text{control media}}} \times 100
\]

Where:
- Cell Viability = percentage of live cells after testing
- OD treatment = nilai optical density pada setiap sampel
- OD media = optical density value for each sample
- OD control cells = optical density value for control cells

Fourth stage: Data processing and analysis. The acquired data is subjected to normality testing using the Shapiro-Wilks test and variance homogeneity using the Levene Test. Subsequently, analysis is performed to observe differences using the Kruskall Wallis Test with a significance level of 5%. If significant differences are found, the Mann-Whitney Test is then conducted.

D. RESULTS AND DISCUSSION

The results of the study on BHK-21 fibroblast cells after the administration of turmeric extract (Curcuma longa linn.) are presented as readings obtained using an ELISA reader to observe the outcomes in each treatment and control group, indicating the optical density or absorbance levels. The percentage of cell viability can be inferred from these optical density values. The denser the color observed in the well, the higher the optical density, or the greater the absorbance value.

To assess the cytotoxicity of turmeric extract, the viability percentage of BHK-21 fibroblast cells is examined to evaluate the cells’ ability to survive after exposure. This is calculated using the percentage of live cells formula. Cells absorb the yellow-colored MTT solution, which is then broken down through the reduction reaction by mitochondrial succinate dehydrogenase enzyme. This enzyme converts MTT into blue formazan crystals, marking the viability of the cells.
Based on the observations and readings of the absorbance values for the cytotoxicity test of turmeric extract on BHK-21 fibroblast cells, divided into treatment groups with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, with three replications for each treatment and control group. After obtaining the data from the ELISA readings, data processing is conducted to determine cell viability. The absorbance or optical density values in each well are used to measure viability or the number of live cells, following the formula for cell viability according to Freshney. The results are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>n (Number of Experiments)</th>
<th>Average optical density</th>
<th>% Cell Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cells</td>
<td>3</td>
<td>0.381</td>
<td>100%</td>
</tr>
<tr>
<td>Control Media</td>
<td>3</td>
<td>0.057</td>
<td>0%</td>
</tr>
<tr>
<td>Turmeric Extract 100%</td>
<td>3</td>
<td>0.078</td>
<td>6.48%</td>
</tr>
<tr>
<td>Turmeric Extract 50%</td>
<td>3</td>
<td>0.116</td>
<td>18.1%</td>
</tr>
<tr>
<td>Turmeric Extract 25%</td>
<td>3</td>
<td>0.136</td>
<td>24.28%</td>
</tr>
<tr>
<td>Turmeric Extract 12.5%</td>
<td>3</td>
<td>0.139</td>
<td>25.1%</td>
</tr>
<tr>
<td>Turmeric Extract 6.25%</td>
<td>3</td>
<td>0.21</td>
<td>47.01%</td>
</tr>
<tr>
<td>Turmeric Extract 3.12%</td>
<td>3</td>
<td>0.339</td>
<td>86.93%</td>
</tr>
<tr>
<td>Turmeric Extract 1.56%</td>
<td>3</td>
<td>0.353</td>
<td>91.25%</td>
</tr>
<tr>
<td>Turmeric Extract 0.78%</td>
<td>3</td>
<td>0.370</td>
<td>96.50%</td>
</tr>
</tbody>
</table>

**Source:** Data Analysis (Excel)

In Table 1, it can be observed that at a concentration of 100%, the average optical density is 0.078, with a cell viability percentage of 6.48%. At a concentration of 50%, the average optical density is 0.116, and the cell viability percentage is 18.1%. For a concentration of 25%, the average optical density is 0.136, and the cell viability percentage is 24.28%. At a concentration of 12.5%, the average optical density is 0.139, and the cell viability percentage is 25.10%. For a concentration of 6.25%, the average optical density is 0.21, and the cell viability percentage is 47.01%. At a concentration of 3.12%, the average optical density is 0.339, with a cell viability percentage of 86.93%. For a concentration of 1.56%, the average optical density is 0.353, and the cell viability percentage is 91.25%. At a concentration of 0.78%, the average optical density is 0.370, with a cell viability percentage of 96.50%.
Figure 3. Percentage of cell viability after treatment

Figure 4. Observation of cells using a microscope. A. BHK-21 cells after treatment with 0.78% turmeric extract, B. BHK-21 cells after treatment with 1.56% turmeric extract, C. BHK-21 cells after treatment with 3.12% turmeric extract, D. BHK-21 cells after treatment with 6.25% turmeric extract, E. BHK-21 cells after treatment with 12.5% turmeric extract, F. BHK-21 cells after treatment with 25% turmeric extract, G. BHK-21 cells after treatment with 50% turmeric extract, H. BHK-21 cells after treatment with 100% turmeric extract, I. Control BHK-21 cells after MTT solution, J. Control media BHK-21 cells after MTT solution.
The normality test results were conducted to assess the cytotoxicity test results of turmeric extract, as seen from the percentage of viable BHK-21 fibroblast cells, which have a normal distribution or not before the one-way ANOVA test. The normality test will be statistically analyzed using SPSS 26 software. In this study, the normality test will be analyzed using the Shapiro-Wilk test at a significance level of 5%, where data is considered normally distributed if it has a p-value > 0.05. The normality test results can be shown as follows:

**Table 2. The Shapiro-Wilk Normality Test Results of Cytotoxicity Test on BHK-21 Fibroblast Cells (p > 0.05)**

<table>
<thead>
<tr>
<th>Concentration Group</th>
<th>n</th>
<th>p</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cells</td>
<td>3</td>
<td>0.305</td>
<td>Normal</td>
</tr>
<tr>
<td>Control Media</td>
<td>3</td>
<td>0.862</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 0.78%</td>
<td>3</td>
<td>0.000</td>
<td>Not Normal</td>
</tr>
<tr>
<td>Turmeric Extract 1.56%</td>
<td>3</td>
<td>0.349</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 3.12%</td>
<td>3</td>
<td>0.843</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 6.25%</td>
<td>3</td>
<td>0.201</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 12.5%</td>
<td>3</td>
<td>1.000</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 25%</td>
<td>3</td>
<td>0.817</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 50%</td>
<td>3</td>
<td>0.144</td>
<td>Normal</td>
</tr>
<tr>
<td>Turmeric Extract 100%</td>
<td>3</td>
<td>0.407</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Shapiro-Wilk, p > 0.05

Source: Data Processing Results (SPSS)

Table 2 shows that the results of the cytotoxicity test of turmeric extract, seen from the percentage of viable BHK-21 fibroblast cells in the control group and the treatment group of turmeric extract concentrations 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, have a normal distribution as indicated by a p-value > 0.05. However, the treatment group with 0.78% turmeric extract has data with a non-normal distribution, as seen from a p-value < 0.05. Since there is a treatment group that does not meet the normality assumption, the difference test for the cytotoxicity test results of BHK-21 fibroblast cells will be analyzed using the Kruskal-Walls test. Subsequently, a homogeneity test will be conducted to determine the level of variance diversity in the cytotoxicity test results of BHK-21 fibroblast cells in 2 control groups and 8 treatment groups, which will be measured with the Levene Test.

**Table 3. Results of Levene Test Homogeneity Test Results of Cytotoxicity Test of BHK-21 Fibroblast Cells (p > 0.05)**

<table>
<thead>
<tr>
<th>Concentration Group</th>
<th>n</th>
<th>P</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cells</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Media</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 0.78%</td>
<td>3</td>
<td>0.001</td>
<td>Inhomogeneous</td>
</tr>
<tr>
<td>Turmeric Extract 1.56%</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 3.12%</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 6.25%</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 shows that the results of the cytotoxicity test of turmeric extract, as observed from the percentage of viable BHK-21 fibroblast cells in 2 control groups and 8 treatment groups with turmeric extract concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78%, exhibit non-homogeneous variance, indicated by a p-value of 0.001 (p < 0.05). Due to the non-homogeneous nature of the research data, the analysis of the difference in the results of the cytotoxicity test, as observed from the percentage of viable BHK-21 fibroblast cells, will be conducted using the Kruskal-Walls test, followed by a Post Hoc Test using the Mann-Whitney Test.

The difference in the results of the cytotoxicity test of BHK-21 fibroblast cells in the control media group, control cells, turmeric extract concentrations of 0.78%, 1.56%, 3.12%, 6.25%, 12.5%, 25%, 50%, and 100% will be analyzed using non-parametric statistical tests, namely the Kruskal-Walls test, to determine whether there is a significant difference between treatment groups. At a significance level of 5%, if the obtained p-value is < 0.05, it indicates a significant difference in cytotoxicity among treatment groups. The following are the results of the Kruskal-Walls test for 2 control groups and 8 concentration groups:

<table>
<thead>
<tr>
<th>Concentration Group</th>
<th>n</th>
<th>Median</th>
<th>Mean ± SD</th>
<th>% Viabilitas Sel</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cells</td>
<td>3</td>
<td>0,057</td>
<td>0,058 ± 0,008</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Control Media</td>
<td>3</td>
<td>0,361</td>
<td>0,382 ± 0,050</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 0,78%</td>
<td>3</td>
<td>0,395</td>
<td>0,370 ± 0,043</td>
<td>96,50</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 1,56%</td>
<td>3</td>
<td>0,365</td>
<td>0,353 ± 0,030</td>
<td>91,26</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 3,12%</td>
<td>3</td>
<td>0,339</td>
<td>0,339 ± 0,004</td>
<td>86,93</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 6,25%</td>
<td>3</td>
<td>0,239</td>
<td>0,210 ± 0,062</td>
<td>47,02</td>
<td>0,001*</td>
</tr>
<tr>
<td>Turmeric Extract 12,5%</td>
<td>3</td>
<td>0,139</td>
<td>0,139 ± 0,026</td>
<td>25,10</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 25%</td>
<td>3</td>
<td>0,135</td>
<td>0,136 ± 0,012</td>
<td>24,28</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 50%</td>
<td>3</td>
<td>0,113</td>
<td>0,116 ± 0,007</td>
<td>18,11</td>
<td></td>
</tr>
<tr>
<td>Turmeric Extract 100%</td>
<td>3</td>
<td>0,077</td>
<td>0,078 ± 0,005</td>
<td>6,48</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis in Table 4, using the Kruskal-Wallis test, indicates that the cytotoxicity test data of turmeric extract, as observed from the percentage of viable BHK-21 fibroblast cells, comprising 2 control groups and 8 treatment groups with
Turmeric extract concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78%, has a p-value of 0.001 (p < 0.05), suggesting a significant difference in the percentage of viable BHK-21 fibroblast cells between the 2 control groups and 8 treatment groups. Due to the significant difference between groups, the analysis proceeds to the Post Hoc Test using the Mann-Whitney Test to determine which groups differ significantly from each other and identify the groups with the highest and lowest percentages of viable cells. The Mann-Whitney results are presented in Table 5.

Table 5. Mann-Whitney Test Results Cytotoxicity Test BHK-21 Fibroblast Cells (p < 0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>CM</th>
<th>CC</th>
<th>0.78%</th>
<th>1.56%</th>
<th>3.12%</th>
<th>6.25%</th>
<th>12.5%</th>
<th>25%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td></td>
<td></td>
<td>0.050*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>0.050*</td>
<td></td>
<td>0.046*</td>
<td>0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.78%</td>
<td>0.050*</td>
<td>0.827</td>
<td>0.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.56%</td>
<td>0.050*</td>
<td>0.827</td>
<td>0.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.12%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.507</td>
<td>0.513</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.25%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.046*</td>
<td>0.050*</td>
<td></td>
<td></td>
<td>0.184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.046*</td>
<td>0.050*</td>
<td>0.050*</td>
<td></td>
<td>0.184</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.046*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.127</td>
<td>0.827</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.046*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.184</td>
<td>0.050*</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.046*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.050*</td>
<td>0.050*</td>
</tr>
</tbody>
</table>

Mann Whitney, p < 0.05

Source: Data processing results (SPSS)

Explanation
(*) : Significantly different between treatments
CM : Control Media
CC : Control Cell

Turmeric extract at 0.78% significantly differs from 6.25%, 12.5%, 25%, 50%, and 100% (p < 0.05), but not significantly different from 1.56% and 3.12% (p > 0.05). Turmeric extract at 1.56% significantly differs from 6.25%, 12.5%, 25%, 50%, and 100% (p < 0.05), but not significantly different from 3.12% (p > 0.05). Turmeric extract at 3.12% significantly differs from 6.25%, 12.5%, 25%, 50%, and 100% (p < 0.05), but not significantly different from 0.78% and 1.56% (p > 0.05). Turmeric extract at 6.25% significantly differs from 50% and 100% (p < 0.05), but not significantly different from 12.5% and 25% (p > 0.05). Turmeric extract at 12.5% significantly differs from 100% (p < 0.05), but not significantly different from 25% and 50% (p > 0.05). Turmeric extract at 25% significantly differs from 0.78%, 1.56%, and 3.12% (p < 0.05), but not significantly different from 6.25% and 12.5% (p > 0.05). Turmeric extract at 50% significantly differs from 0.78%, 1.56%, 3.12%, 6.25%, and 25% (p < 0.05), but not significantly different from 12.5% (p > 0.05). Turmeric extract at 100% significantly differs from all other concentrations (p < 0.05).

The results of the Post Hoc Test using the Mann-Whitney Test indicate that the administration of turmeric extract at concentrations of 0.78% and 1.56% has the
highest percentage of viable BHK-21 fibroblast cells, equivalent to the control cells. The administration of turmeric extract at 100% has the lowest percentage of viable BHK-21 fibroblast cells compared to all treatment groups.

Curcuma longa linn., commonly known as turmeric, is not only used as a flavor enhancer in food but also offers various health benefits, serving as a part of traditional medicine. Turmeric is known for its anti-inflammatory, wound healing, antioxidant, anti-protozoan, antibacterial, antiviral, antifungal, and anticancer properties (Singletary, 2020). Given its widespread availability in Indonesia, turmeric is a natural resource worthy of exploration. With its extensive use in the community, further research on its safety is necessary.

In this study, turmeric extract preparation was conducted at the UPT Laboratory of Herbal Materia Medica Batu. The dilution of turmeric extract (Curcuma longa linn.) to obtain the desired concentrations was carried out at the Research Center of the Faculty of Dentistry, Airlangga University. The dilution aimed to determine the concentration that would maintain the viability of BHK-21 fibroblast cells, as indicated by the percentage of viable fibroblast cells.

The research results on the different concentrations of turmeric extract (Curcuma longa linn.) on BHK-21 fibroblast cells using the MTT assay are presented in Table 5.1, showing the percentage of viable fibroblast cells. The viability test was performed to assess the cytotoxicity of a substance on the cells, determined by 3-(4,5-Dimethylthianizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). This test is based on the reduction of the yellow tetrazolium salt to purple formazan crystals, indicating the number of living cells. MTT reagent, a tetrazolium salt, is converted into formazan crystals with a bluish-purple color, measured for absorbance using an ELISA reader spectrophotometer. The living cells enhance the total formazan produced. As observed in Figure 5.1, a significant decrease in the viability of BHK-21 fibroblast cells indicates that higher concentrations of turmeric extract (Curcuma longa linn.) can induce cell death.

Toxicity of a substance can be influenced by various factors, with concentration being one of them (Gupta, et al., 2022). In this study, toxicity was assessed using BHK-21 fibroblast cell cultures with the MTT assay. Toxicity can be observed through microscopic examination, revealing necrosis, edema, and cell inflammation (Lawrence et al., 2022). The parameter used to assess the cytotoxicity of a substance is the Cytotoxic Dilution 50% (CD50). A substance is considered toxic if the percentage of living cells is less than 50% after exposure (Munadziroh et al., 2018).

In Figure 3, a significant decrease in the viability of BHK-21 fibroblast cells demonstrates that the concentration of turmeric extract (Curcuma longa linn.) affects the viability of BHK-21 fibroblast cells. High concentrations of turmeric extract prove to be toxic, leading to cell death. The results from each concentration group indicate a consistent effect of decreasing the viability of BHK-21 fibroblast cells. Turmeric extract at concentrations of 0.78%, 1.56%, and 3.12% showed values above the parameter. However, concentrations of 6.25%, 12.5%, 25%, 50%, and 100% resulted in viability percentages below 50%. Previous studies have shown that turmeric extract (Curcuma
longa linn.) with concentrations above these levels inhibits the growth of bacteria such as Streptococcus mutans, Staphylococcus aureus, and Pseudomonas sp. Comparing the results from the spectrophotometer, seen using an ELISA reader, and the Cytotoxic Dilution 50% (CD50) parameter, turmeric extract (Curcuma longa linn.) already exhibited toxic properties at a concentration of 6.25%, with a viability percentage of 47.01%. The discrepancy between these findings could be attributed to the ELISA reader capturing the bluish-purple color as impurities from the turmeric extract. Cell death can occur at high concentrations of turmeric extract (Curcuma longa linn.), leading to cell rupture or lysis and subsequent cell death (Vitria et al., 2015).

Turmeric extract (Curcuma longa linn.) contains curcumin, flavonoids, alkaloids, essential oils, and saponins, each possessing therapeutic properties. Curcumin, in particular, has therapeutic properties. Under certain conditions, such as high concentrations, curcumin and its derivatives from turmeric are likely to exhibit toxic effects, as they can show carcinogenic and toxic effects. Curcumin can suppress cell proliferation, induce apoptosis, inhibit angiogenesis, and suppress antiapoptotic proteins (Cianfruglia et al., 2019). In this study, turmeric extract not only contains curcumin but also flavonoids, alkaloids, and saponins. These compounds have antioxidant properties that can enhance the activity of cellular antioxidant enzymes, preventing cell damage (Dyah et al., 2014). Although turmeric extract (Curcuma longa linn.) contains beneficial antioxidant compounds, at high concentrations, the percentage of these active compounds is also high, potentially leading to toxic effects.

E. CONCLUSION

Based on the conducted research, turmeric extract (Curcuma longa linn.) is not cytotoxic to BHK-21 fibroblast cells at concentrations of 0.78%, 1.56%, and 3.12%. Further research is needed on the toxicity testing of the active compound curcumin contained in turmeric extract (Curcuma longa linn.) in vitro

REFERENCES


19. Indrayudha, P. *et al.* (2013) Aktivitas Sitotoksik Ekstrak Etanol (Haryoto dkk) Aktivitas Sitotoksik Ekstrak Etanol Tumbuhan Sala (Cynometra ramiflora Linn) Terhadap Sel HeLa, T47D dan WiDR.


34. Mustapa, M., Tuloli, T. & Mooduto, A., 2018. Uji Toksisitas Akut yang Diukur dengan Penentuan LD50 Ekstrak Etanol Bunga Cengkeh (Syzygium aromaticum


