Bioactivity and toxicity evaluation of nutraceuticals using *in vitro* cell-based models: A review

Tran Hung Son[1,2,3*]

[1]*National Institute for Food Control, Hanoi, Vietnam*

[2]*Institute of Natural Products, Korea Institute of Science and Technology (KIST), Gangneung, Republic of Korea*

[3]*Division of Bio-Medical Science & Technology, University of Science & Technology (UST), Daejeon, Republic of Korea*

(Received: 18/08/2022; Accepted: 20/9/2022)

Abstract

Nutraceutical is one of the new concepts appearing in recent years, referring to food products derived from natural sources that benefit human health in preventing and treating diseases, besides providing nutritional value. During the development of these products, it is important to evaluate the toxicity and bioactivity of potential compounds and to study the mechanism of action of these nutraceutical compounds at the cellular and molecular levels. Among the many different experimental models, the *in vitro* cell-based model has emerged as a model with many advantages for preliminary screening of nutraceuticals activity and toxicity before conducting further studies on *in vivo* models. This review summarizes some basic techniques commonly used to screen nutraceuticals' toxicity. Recent studies of the bioactivity of nutraceuticals in various areas, such as antioxidant, anti-inflammation, anti-obesity, neuroprotection, and gut health improvement, are also reviewed to introduce the application of the cell-based model in nutraceutical bioactivity research. New modern techniques using *in vitro* cell-based models have been applied in this field, such as high-throughput screening, 3D-cell culture, and organ-on-a-chip are also discussed in this paper.

Keywords: *Bioactivity, toxicity, nutraceuticals, in vitro, cell-based models.*

1. INTRODUCTION

"Nutraceutical" is a concept that combines "nutrient" and "pharmaceutical". In 1995, the term "Nutraceuticals" was first introduced by Stephen DeFelice, and the term was defined as "foods (or a part of a food) that provide medical or health benefits, including the prevention and/or treatment of disease" [1]. Nowadays, besides paying attention to the nutritional value of foods, the beneficial effects of food on human health are increasingly interested and widely studied. Furthermore, with the diversity and abundance of plant

[*]*Corresponding author: Tel: +82 4287 2808 Email: tranhungson113@gmail.com*

species in Vietnam, more and more functional foods, including bioactive compounds derived from natural sources, are researched, produced, and available in the market. Along with that, the number of food products formulated with a combination of synthetic bioactive ingredients and natural bioactive ingredients also increased dramatically in number. These products, whether functional foods, dietary supplements, or formula foods, are all fundamental constituents of the concept of nutraceuticals, with the ultimate goal being to bring positive values for human health, both in the prevention and treatment of disease.

For a nutraceutical product, the two most important factors to consider are the toxicity and bioactivity of the constituents in this product. Nutraceutical compounds can be proteins, peptides, amino acids, carbohydrates, lipids, vitamins, or small molecules isolated from natural sources. Regardless of which class the compound belongs to, toxicological and bioactivity tests on animals are necessary to ensure the safety of the compound as well as to ensure the preventive/therapeutic effect that the compound can bring to humans. However, with a large number of potential compounds that can be used as a nutraceutical, it is challenging to test all of these compounds in animal models. Therefore, along with the current trend of limiting the use of animals in experiments involving drugs, food, and cosmetics development, the utility of other experimental models is necessary. Thus, the *in vitro* cell-based model with many advantages has emerged as an effective way to study the toxicity and biological effects of nutraceutical compounds.

In this review, we describe basic knowledge related to *in vitro* cell-based model as well as some standard techniques to evaluate the cytotoxicity of the nutraceuticals. In addition, some biological effects of nutraceuticals discovered using cell-based models are reviewed, and trends in nutraceuticals bioactivity research with the support of emerging technologies are also summarized.

2. AN OVERVIEW OF IN VITRO CELL CULTURE MODEL

Cell culture is one of the popular techniques used to study the normal physiological activity, biochemical reactions, and metabolism occurring in cells. The basic principle of the technique involves separating cells from tissues and culturing them in a suitable artificial medium. Cell culture is a well-suited model for studying physiological and pathological states at the cellular level and allows the discovery of cellular mechanisms of action of drugs, bioactive compounds, and toxicants.

Based on the shape and growth properties of the cell lines, they can be divided into three main types, fibroblastic cells (long shape, adherent growth), epithelial-like cells (polygonal shape, adherent growth), and lymphoblast-like cells (spherical shape, suspension growth) [2]. Culture conditions vary between different cell types, but the basic conditions of the culture medium need to be ensured and maintained appropriately, such as supplying the essential nutrients (such as amino acids, carbohydrates, vitamins), hormones, growth factors, gases (O_2, CO_2), and other conditions such as pH, temperature [2].

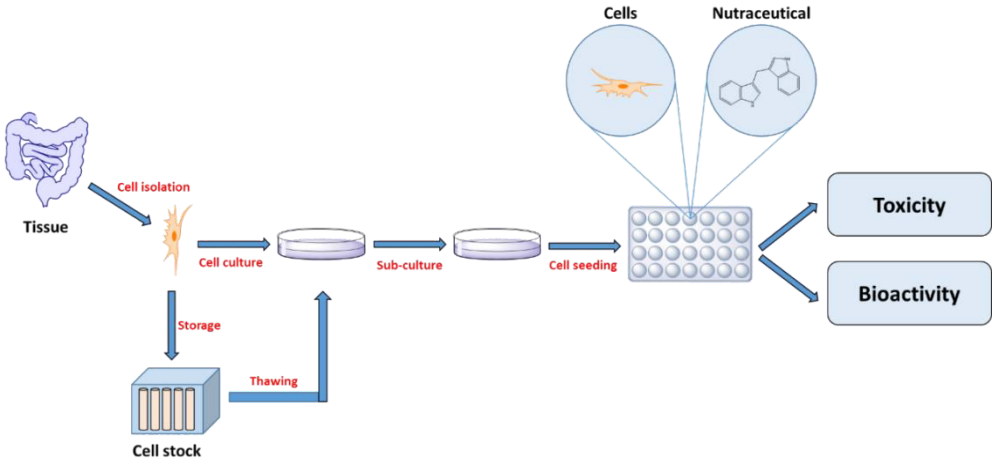

***Figure 1**. The basic process in cell culture*

Using in vitro cell models offers many advantages, such as rapid, low cost, and the ability to screen simultaneously on different compounds. In addition, it is a stable model and offers high repeatability as the experimental conditions can be easily controlled. Therefore, with the support of biochemistry and molecular biology techniques, the cellular and molecular mechanisms involved in the toxicity and the bioactivity of nutraceutical compounds can be discovered. In addition, ethical issues are not a problem in this experimental model.

However, cell culture models also have some disadvantages in studying the toxicity and activity of nutraceuticals. It is necessary to understand that human diseases are not simple because many different pathogenesis and pathways lead to the disease. So, using only simple *in vitro* cell models to simulate perfectly human disease conditions is impossible. Moreover, the human body is a complex system, which includes the interaction with cells and cells, tissues, and organs with each other through the nervous, vascular, and immune systems. Therefore, *in vitro* cell models cannot be used to study these complex interactions. In addition, cells can be differentiated during the cell culture process, leading to changes in the original cells' physiological and biochemical characteristics, affecting the research results. Finally, cell cultures should be maintained in a strictly sterile environment to limit bacterial or mold contamination.

3. TOXICITY EVALUATION OF NUTRACEUTICALS USING THE CELL-BASED MODEL

3.1. Cytotoxicity and cell viability

Cytotoxicity is defined as the negative effect of a compound on living cells that causes cell damage or cell death. Besides the definition of cytotoxicity, cell viability is a concept that contrasts with cytotoxicity. Cell viability is defined as the number of living cells in a cell sample, and the viability of cells is a basic indicator for evaluating the toxicity of a compound [3].

There are many methods to assess the cytotoxicity of a compound. The simplest way is to observe cells under a microscope, which includes observing the shape and size of cells. However, this method highly depends on the researcher's experience and has poor accuracy. Therefore, other methods have been developed for determining the cytotoxicity of chemicals, such as cyto-staining or spectrometric methods (colorimetric, fluorometric, luminometric methods) based on the change in the concentration of biomolecules produced by biochemical reactions in the cell. Table 1 summarizes the principles of the most commonly used cytotoxicity and cell viability determination methods used in nutraceutical studies.

Table 1. *Common assays to evaluate the cytotoxicity of nutraceutical compound*

Assays	***Classification***	***Principle***
Trypan blue	***Dye exclusion assay***	- Live cells possess intact cell membranes that exclude this dye, whereas dead cells do not. Viable cells will have a clear cytoplasm, whereas dead cells will have a blue cytoplasm. - In this method, cell viability is determined using a hematocytometer or automated cell counter under light microscopy [4].
3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay	***Colorimetric assay***	- This assay determines cell viability by determining the mitochondrial function of cells via mitochondrial enzymes such as succinate dehydrogenase. - In this assay, MTT is reduced to purple formazan by NADH. The light absorbance of the formazan product correlates with the activity of mitochondrial enzymes and then evaluates the percentage of living cells [5].
Water-soluble tetrazolium salt (WST-1) assay	***Colorimetric assay***	- This assay is based on converting the tetrazolium salt WST-1 into a water-soluble formazan by mitochondrial dehydrogenase enzymes in the presence of an intermediate electron acceptor. - The water-soluble salt is released into the cell culture medium. The absorbance of the reaction produces a color change which correlates to the amount of mitochondrial dehydrogenase in cell culture and thus, measuring the metabolic activity of living cells [6].

Assays	*Classification*	*Principle*
Lactate dehydrogenase (LDH) assay	***Colorimetric assay***	- LDH assay is a colorimetric method of assaying cellular cytotoxicity. LDH is an enzyme normally found in the cell cytoplasm. LDH is released into the cell culture medium when the cells are damaged. - LDH level is determined by an enzymatic reaction with the diaphorase catalyst that converts a tetrazolium salt (iodonitrotetrazolium) into a red color formazan [7].
Calcein-AM assay	***Fluorometric assay***	- Calcein AM is a membrane-permeable cell marker that can permeate into intact cells. It has been used for studies of cell membrane integrity and cell viability. - Inside the cells, calcein AM is hydrolyzed by intracellular esterases into the green, fluorescent dye calcein, which is retained in the cell cytoplasm. The fluorescent signal generated linearly correlates to the number of living cells [8].
Adenosine triphosphate (ATP) assay	***Luminometric assay***	- Cell viability can be measured based on the detection of ATP, which represents the most important chemical energy in cells. When cells are damaged, the ATP level of cells decreases significantly. - The ATP assay is based on the reaction of luciferin to oxyluciferin, with the catalyst of luciferase in the presence of oxygen and Mg^{2+}. - Oxyluciferin forms a luminescent signal, and the ATP level is calculated based on the linear correlation with the intensity of the luminescent signal [9].

In general, methods for the evaluation of cytotoxicity can be divided into four main groups: cell-staining, colorimetric, fluorometric, and luminometric methods. The method of staining cells with trypan blue is the most popular in the group of cell-staining methods. The advantage of this method is that the procedure is simple, very fast (within seconds), and cheap, however, the error of the method is quite large, so now it is rarely used to determine cell viability. The methods of colorimetric and fluorescence measurement are most commonly used nowadays with many commercial kits available. These methods are easy to

perform, repeatable, high accurate, and reagents such as MTT, WST-1, and Calcein-AM are safe for cells. The disadvantages of the above methods are time-consuming, such as MTT (maybe up to 4 hours) or WST-1 (2 hours). Luminometric methods such as ATP assay overcome the disadvantages by forming luminescent signal rapidly. However, in these methods, background, fluorescent, or luminescent interference from test compounds is possible. Currently, there are different methods to determine the cytotoxicity of a nutraceutical compound. There is no perfect method for determining the cytotoxicity of all compounds, the choice of method depends on the cell line used, the properties of the nutraceutical compounds, and the conditions of the laboratory as well.

3.2. Genotoxicity

Besides assessing nutraceutical toxicity based on cytotoxicity, genotoxicity is another concept also used to evaluate toxicity, which is often confused with cytotoxicity. Genotoxicity is defined as the ability of a compound that causes damage or changes to genetic material (DNA, RNA, or chromosomes) [10]. Depending on the mechanism of action, genotoxicity could be classified into three types: mutagenesis, carcinogenesis, and teratogenesis. Because genotoxicity could lead to many serious chronic diseases in humans, such as neurodegenerative disorders, chronic inflammation, aging, or cancer, it is necessary to check genotoxicity of compounds before using them as food products [11].

It is essential to distinguish between cytotoxicity and genotoxicity. Cytotoxicity is the ability of a compound to cause damage to living cells, while genotoxicity is the ability to enhance the changes in the structure or number of genetic material (DNA or chromosomes). Cytotoxic compounds could lead to the necrosis or apoptosis of cells, while genotoxic compounds change the structure, sequences, and the number of genes within a cell. Therefore, it can be remarked that genotoxicity may lead to cytotoxicity.

There are several methods to evaluate genotoxicity with different models, such as bacteria (Ames assay), yeast/fungus (*S. cerevisiae* assay, *A. nidulans* assay), and cell-based assay [11]. The Comet assay is one of the most popular methods to evaluate the genotoxicity of a compound. The comet assay or single-cell gel electrophoresis is a common method for measuring DNA strand breaks in cells. This method was first introduced in 1984 and then modified by Singh et al. in 1988, and it was called the Alkaline Comet assay [12]. Under alkaline conditions, DNA is allowed to unwind and undergoes electrophoresis. After electrophoresis and staining with DNA-fluorescent dye, the image of DNA can be observed under fluorescence microscopy. The head represents the intact DNA, while the tail includes various broken pieces of DNA, and the fluorescent intensity and length of the tail correlate with the percentage of DNA damage [12]. The Comet assay is a valuable tool to evaluate the genotoxicity of nutraceutical compounds and the protective effect of compounds against DNA-damage factors.

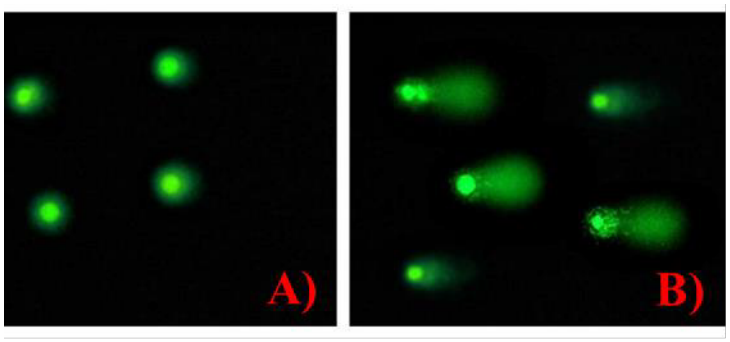

Figure 2. *Image of A) Intact DNA and B) Broken DNA with tail in Comet assay (Source: https://www.cellbiolabs.com/comet-assay-kits-96-well)*

4. BIOACTIVITY EVALUATION OF NUTRACEUTICALS USING THE CELL-BASED MODEL

The cell-based model is a prevalent model in studying the biological effects of nutraceutical compounds, mainly involving compounds from natural sources (bacteria, fungi, microalgae, plant) or probiotic strains. *In vitro* cell model is also a good model for understanding the cellular, biochemical, and molecular mechanism underlying the bioactivity of nutraceuticals. This paper reviewed some well-known bioactivities, such as anti-oxidant, anti-inflammation, anti-obesity, neuroprotection, and gut health improvement.

4.1. Anti-oxidant effect

Oxygen is one of the essential elements, playing an important role in maintaining the existence and biochemical reactions in cells. However, oxygen can be converted during cellular metabolism to reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can cause oxidative stress and damage cell structure and function. ROS or RNS can exist as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), and singlet oxygen ($^1O_2$) [13]. Although small amounts of ROS or RNS can have beneficial effects on the body, in large quantities, they can damage the structure and biosynthesis of essential components in the body, such as nucleic acids and proteins, thereby leading to cell death, which is the cause of many chronic and acute human diseases, such as cancer, hypertension, neurodegenerative disease, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, etc [14].

Some popular *in vitro* antioxidant activity determination methods are 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS), or Oxygen Radical Absorbance Capacity (ORAC) assay. These methods are based on an oxidation-reduction reaction between a potential compound and a reagent to determine their antioxidant properties. Although these are simple, easy-to-implement methods and can pre-screen the antioxidant activity of compounds, the mechanism of the antioxidant effect is not shown through these reactions. It must be understood that an antioxidant molecule must not only exhibit antioxidant properties (reducing). It must exhibit antioxidant effects in a living

system, for example, through regulating gene expression related to the cell's resistance to ROS or RNS. Hence, the cell-based model becomes a reasonable model to study these mechanisms. Human hepatocyte HepG2 is one of the most popular cell lines to study antioxidant effects because it highly expresses antioxidant-related enzyme lines in this cell, such as superoxide dismutase, catalase, and glutathione peroxidase. In addition, the human neuronal cell line SH-SY5Y is a popular research model to find protective agents for neuron degradation induced by H_2O_2.

One well-known antioxidant mechanism is the KEAP1/Nrf2/ARE pathway. This pathway represents the ability of cells to resist oxidative stress induced by ROS and RNS. Under oxidative stress, the Keap1 protein is activated and released from binding to Nrf2. The Nrf2 transcription factor is translocated from the cytoplasm into the nucleus and binds to the promoter region of the ARE gene, then activating cellular antioxidant mechanisms such as neutralizing free radicals or enhancing the synthesis of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase [15].

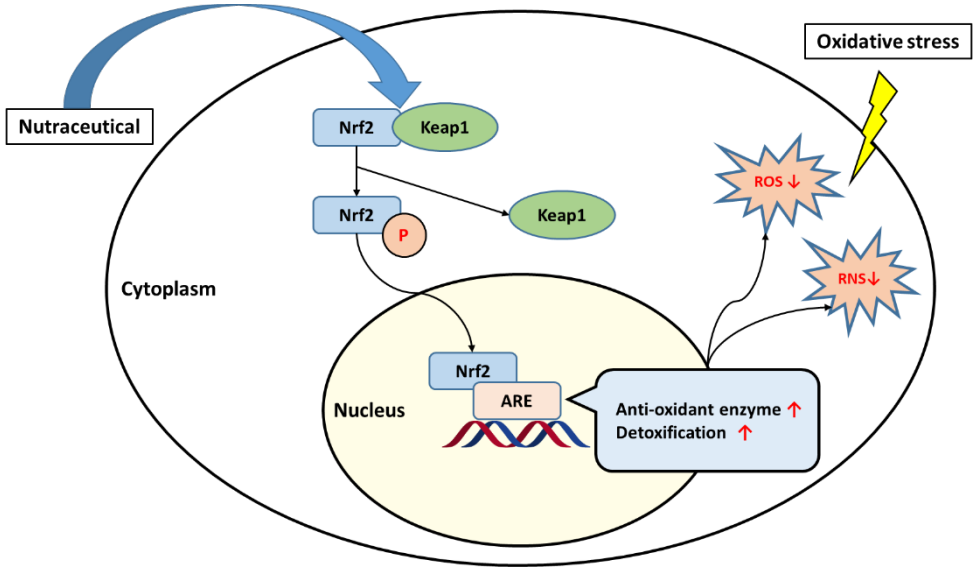

Figure 3. *KEAP1/Nrf2/ARE pathway*

Besides common antioxidants such as vitamin C, vitamin E, lycopene, glutathione, or melatonin, many naturally occurring compounds are reported with antioxidant effects. Sulforaphane, a class of isothiocyanate extracted and first isolated from broccoli, was reported to protect HepG2 and HHL5 hepatocytes against H_2O_2-induced damage through effects on the Nrf2 pathway [16]. Corn peptides – products of enzymatic hydrolyzation of corn glutens, are reported to protect HepG2 hepatocytes from ethanol-induced injury through inhibition of hepatocyte apoptosis and increased levels of ALT, AST, LDH, and MDA [17]. Lee et al. (2022) have demonstrated the antioxidant activity of three lactic acid bacteria, *Lactococcus lactis MG5125*, *Bifidobacterium bifidum MG731*, and *Bifidobacterium animalis subsp. lactis MG741* in a model of HepG2 cells with H_2O_2-induced oxidative stress

by mediating lipid peroxidation and glutathione levels and upregulating antioxidant enzymes, including SOD, catalase, and glutathione peroxidase [18].

4.2. Anti-inflammatory effect

Inflammation is a protective mechanism of the body against various infections and injuries. However, chronic inflammatory processes can cause various diseases, such as inflammatory bowel disease, rheumatoid arthritis, chronic hepatitis, pulmonary fibrosis, and cancer [19]. To determine the anti-inflammatory effect of a compound, the mice RAW 264.7 cells with lipopolysaccharide (LPS) as an inducer is the most common model. RAW 264.7 cells are macrophage-like cells, which initiate an inflammatory response via the overwhelming production of pro-inflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE2), and inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) [20]. Therefore, levels of pro-inflammatory mediators and cytokines reflect the severity of inflammation and can be used to evaluate the protective effect of nutraceutical compounds on inflammatory processes.

LPS is a component of Gram-negative bacteria's membrane, which has normally been used to mimic inflammatory conditions of macrophages *in vitro*. LPS induces the expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, and other mediators (NO and PGE2), which are the final products of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), respectively. The LPS-mediated expression of inflammatory cytokines depends on the expression of specific genes regulated by the Mitogen-activated protein kinase (MAPK) and Nuclear transcription factor Kappa-B (NF-κB) signaling pathways [21].

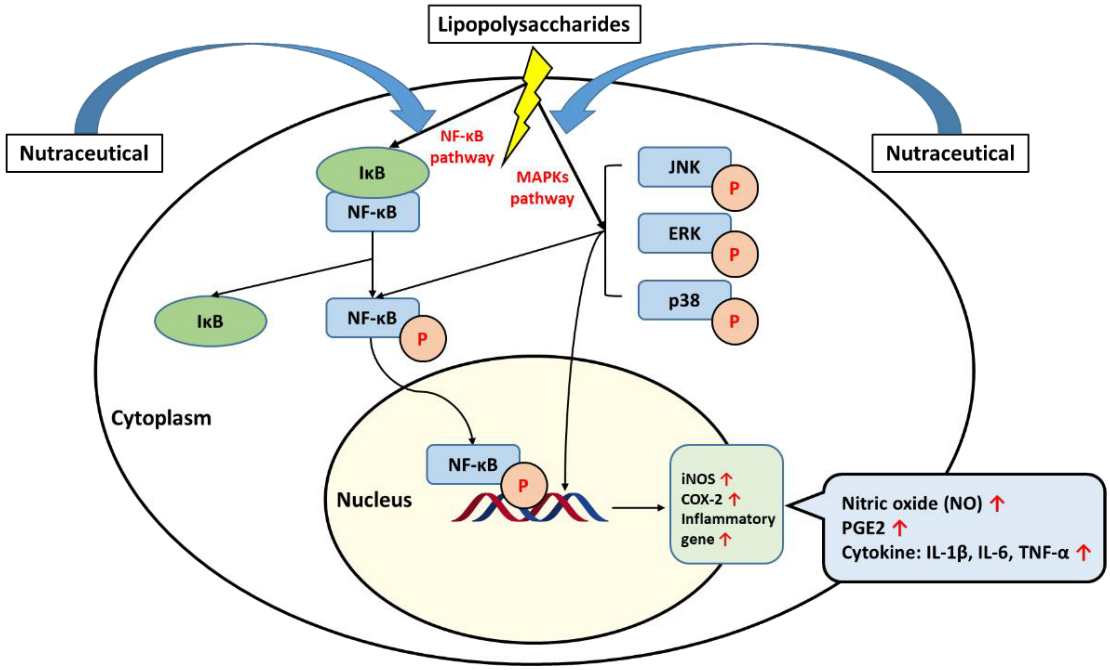

Figure 4. *NF-κB and MAPK signaling pathway in inflammation*

The NF-κB is the most common pro-inflammatory signaling pathway. Under normal conditions, NF-κB is located in the cytoplasm in non-stimulated cells. After stimulation, IκBα, combined with NF-κB, induces phosphorylation and ubiquitylation, which enhance the translocation of NF-κB p65 from the cytoplasm into the nucleus. Then, NF-κB stimulates the expression of inflammatory cytokines and mediators (COX-2, iNOS) [22]. Another pathway is the MAPK pathway, consisting of three subfamilies: the extracellular signal-regulated kinase (ERK), c-Jun N terminal kinase (JNK), and p38, which is also a well-known pathway to control the expression of pro-inflammatory mediators and cytokines by sequential phosphorylation [23].

Because of the side effects associated with NSAIDs or glucocorticoids, nowadays, more and more natural compounds have been reported to have anti-inflammatory effects and are used as dietary supplements to reduce inflammation, such as curcumin, quercetin, chlorogenic acid, cordycepin, and sulforaphane [24-28]. Not only natural compounds have been reported with anti-inflammatory effects, but also recently, probiotic bacteria strains have been reported to exert strong anti-inflammatory effects through studies on RAW 264.7 cells, such as *Lactobacillus plantarum CAU1055*, *Lactobacillus reuteri CRL1098*, *Weissella cibaria JW15*, *Bifidobacterium bifidum BGN4*, *Lactobacillus fermentum* (*MCC 2759* and *MCC 2760*) [29-33].

4.3. Anti-obesity effect

Obesity is defined as excessive fat accumulation in the body, leading to uncontrolled weight gain. Obesity is usually associated with other diseases, such as type 2 diabetes, hypertension, hypercholesterolemia, and hyperlipidemia. Obesity is associated with increased levels of fat within adipocytes and an increase in the number of adipocytes [34]. Therefore, studies on adipocytes are required to discover potential compounds with anti-obesity effects and establish the molecular mechanism underlying the activity.

In assays for the anti-obesity activity of nutraceuticals *in vitro* cell models, 3T3-L1 preadipocytes were the most commonly used cell lines. 3T3-L1 preadipocytes are the source of mouse embryos, having the morphology of fibroblast cells and the ability to differentiate into mature adipocytes under the stimulation of classic hormonal cocktails, including insulin dexamethasone and 3-isobutyl-1-methyl xanthine [35]. Therefore, they are commonly used in obesity studies and the *in vitro* differentiation of adipocytes. 3T3-L1 differentiation is an economical and convenient way to generate adipocyte-like cells for experiments. The most common test on the 3T3-L1 cell line is evaluating the lipid accumulation in these cells using the Oil-Red O staining method. Oil Red O is a fat-soluble dye that stains neutral triglycerides, lipids, and lipoproteins. This is a basic experiment based on observation and color comparison to assess the anti-obesity activity of nutraceuticals before conducting biochemical analyses such as determining the content of triglycerides formed in 3T3-L1 cells.

Grape skin extract was reported to inhibit the differentiation of 3T3-L1 adipocytes and decrease triglyceride content. The mechanism of action for this adipogenesis inhibitory effect was evaluated with the inhibition of adipocyte hormone secretion, such as leptin and adiponectin, and inhibited glycerol-3-phosphate dehydrogenase activity. The gene and protein expression levels were also examined to understand the antiobesity effect of grape skin extract. 3T3-L1 cells treated with grape skin extract could decrease the adipogenic transcription factor (PPARγ, C/EBPα, and SREBP1) gene and protein expression levels, and preadipocyte secreted factor-1 mRNA was up-regulated [36]. Interestingly, peptides isolated from tuna extract were reported to have anti-obesity effects by inhibiting 3T3-L1 cell differentiation and decreasing triglyceride levels. In addition, the molecular mechanism was also investigated with the inhibited adipocyte formation by downregulating the expression of C/EBPs and PPAR-γ. PPARγ, a factor mainly expressed in adipose tissue, promotes the differentiation of adipocytes, while C/EBPα expression is induced during late adipocyte differentiation [34]. Several isolated probiotic bacteria, such as *Lactiplantibacillus plantarum KU15117*, *Lactobacillus gasseri MG2855*, *Leuconostoc mesenteroides* and *Lactobacillus plantarum* isolated from fermented kimchi also showed good anti-obesity activity using a 3T3-L1 cell model [37-39].

4.4. Neuroprotective effect

Parkinson's and Alzheimer's are the two most common neurological diseases with complex and unexplored pathogenesis. In addition, current treatments and the effectiveness of drugs for these diseases are also suboptimal. Therefore, studying and discovering potential nutraceutical compounds with preventive effects is very important. Furthermore, the pathogenic mechanisms are also gradually found, thereby providing the basis for studying compounds with preventive and therapeutic effects.

Oxidative stress is involved in neuronal cell death, which is one of the leading causes of neurodegenerative diseases, especially Alzheimer's and Parkinson's diseases [40]. Studying the neuroprotective effects of nutraceuticals has mainly been performed on the human cell lines SH-SY5Y, SK-N-SH, or mouse neuronal cell lines such as PC12, HT-22, and the protective mechanisms of nutraceuticals were also gradually discovered by using these cell lines.

Research by A. Gonzalez-Sarrias et al. has shown that dietary polyphenols, after undergoing SH-SY5Y neuronal metabolism, are effective in preventing neuronal apoptosis induced by H_2O_2 via attenuation of ROS levels, increased REDOX activity, and decreased oxidative stress-induced apoptosis by preventing the caspase-3 activation [41]. S. Thummayot et al. have demonstrated that purple rice and its main active ingredient, cyaniding, protected SK-N-SH neurons damaged by Aβ25-35 by reducing ROS and RNS formation, enhancing SOD antioxidant enzyme activity, and suppressing neuronal apoptosis induced by Aβ25-35. Furthermore, purple rice and cyanidin were found to inhibit the expression of Bax, caspase-3, and caspase-9 – proteins that activate the apoptosis and

increase the activation of the Bcl-Xl protein anti-apoptosis factor [42]. Recently, the effect of probiotic bacteria strains on nerve cells has also been studied. Some microbial strains isolated from Korean fermented foods, which are *Lactobacillus fermentum KU200060*, *Lactobacillus delbrueckii KU200171*, *and Lactobacillus buchneri KU200793*, could prevent SH-SY5Y neuronal cell death induced by MPP^+ with mechanism are reduce Bax/Bcl-2 ratio and increase the expression level of Brain-Derived Neurotropic Factor (BDNF), which enhance the survival of dopaminergic neurons and can protect them against the neurotoxic effects and also promote synaptic transmission, synaptic plasticity, and synaptic growth [43].

4.5. Gut health improvement

Currently, the studies related to acute and chronic intestinal diseases are increasingly attractive. Hippocrates had a famous quote to describe the importance of gut health to human health: "All disease begins in the gut" [44]. Many probiotics have been discovered and isolated, with beneficial effects on gut health being reported. On the other hand, foods that enter the body can change the human intestinal microbiota, thereby bringing about positive or negative effects on the body. Furthermore, the gut is known to be connected to other body parts through pathways such as the gut-brain axis, gut-lung axis, gut-liver axis, gut-kidney axis, etc. Therefore, developing drugs and functional foods that affect these organs based on changes in gut health is a new research trend in recent years.

Studying the improvement of gut health based on intestinal cell lines is an effective model and has been widely applied, and molecular mechanisms are gradually being elucidated. HT-29 and Caco-2 cells are the most commonly used intestinal cancer cell lines in research on improving intestinal conditions and symptoms of inflammatory bowel disease as well as intestinal cancer. In particular, Caco-2 cells differentiated from 12 - 14 days can simulate the permeability of intestinal cells because differentiated Caco-2 cells have morphological and biochemical properties similar to those of the actual human intestines [45]. Altering the permeability of intestinal cells is an important research target because when intestinal cells are injured, it increases the mucosal barrier's permeability, thereby exposing enterocytes to toxicants, food allergens, and pathogenic bacteria. Therefore, these conditions the intestinal inflammation and ulceration [46].

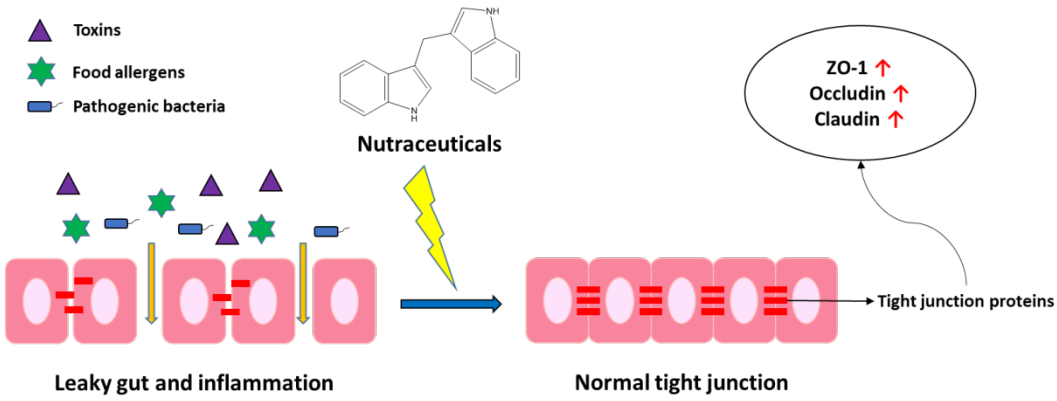

Figure 5. *Leaky gut conditions*

Related to the molecular mechanism, tight junction proteins (TJ proteins) in intestinal epithelial cells play a crucial role in maintaining intestinal permeability. Some TJ proteins are essential in maintaining intestinal membrane permeability for intestinal cells: ZO-1, occludin, claudin-1, and claudin-4. Expression levels of these proteins assess the extent of damage to intestinal cell membranes and are mainly determined through Western blot techniques. For example, Kim et al. demonstrated that 3,3′-Diindolylmethane, a digestive metabolite originating from cruciferous vegetables, such as broccoli, cabbage, and kale, could improve the leaky gut conditions induced by IL-1β by increasing the expression level of two TJ proteins, which are ZO-1 and occludin [47]. Another study has shown that yogurt can improve membrane permeability stimulated by a mixture of IL-1β, TNF-α, and lipopolysaccharides from *E. coli* through increasing ZO-1 and occludin expression levels [48]. Furthermore, several strains of probiotic microorganisms are also known to alter intestinal cell permeability, such as *Bacillus subtilis 29784*, with the ability to increase the expression levels of ZO-1, occludin, and claudin-1 [49].

5. EMERGING TECHNOLOGIES IN CELL-BASED STUDIES

5.1. Cell-based high-throughput screening (HTS)

With the diversity of potential nutraceutical compounds, increasing the performance of the screening stage is an important issue that needs to be evaluated. Nowadays, with the development of many HTS methods, thousands to millions of potential compounds can be screened simultaneously to discover bioactive compounds. Among many experimental, *in vitro* cell model is the most suitable for HTS because of its advantages such as ease of maintenance, cost-effectiveness, and rapid. Potential nutraceutical compounds were evaluated for bioactivity by evaluating various biochemical and phenotypic characteristics depending on the targeted bioactivity. Currently, more and more cell-based HTS system has been developed to screen nutraceutical compounds' toxicity and bioactivity. Along with that is the appearance of the compound libraries to serve the screening process to find potential candidates to develop nutraceutical products.

Gong et al. have developed a high-throughput screening protocol for screening and identifying the natural compounds that can enhance the NK-cell-mediated killing of cancer cells. In this study, the cell lines used in this HTS assay are NK cells co-cultured with non-small cell lung cancer (NSCLC) cells, and a library of 502 natural compounds was screened. The results showed that 28 compounds showed potential activity, and andrographolide was demonstrated to could increase the IFN-γ significantly by NK cells and induce NK cell-mediated killing of NSCLC cells [50]. In another study, Tan et al. have screened 1597 small molecules and 21 575 natural product extracts originating from plants, bacteria, and fungi to check the ability to reduce the cell viability of SALL4hi live cancer cells. The result showed that one small molecule (PI-103) and four natural compounds (oligomycin, efrapeptin, antimycin, and leucinostatin) could reduce the viability of SALL4hi cells, and the molecular

mechanism of oligomycin was also discovered by inhibiting the oxidative phosphorylation; therefore, inhibit the ATP synthesis in cancer cells [51]. José Pérezdel Palacio et at. have developed an HTS assay for screening the natural compounds with anti-inflammatory/immunomodulatory effects. The cell-based HTS model is based on quantitative analysis of NO levels in RAW 264.7 macrophage and interleukin-8 (IL-8) in Caco-2 cells. In a total of 5976 microbial extracts screened, two extracts were reported that contain new compounds with potent anti-inflammatory effects. The bioactive compounds were then isolated and characterized using Nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HR-MS) [52].

5.2. 3D-cell culture

The traditional *in vitro* 2D-cell culture model has some disadvantages: cells are grown on a flat surface using a dish or flask, while under *in vivo* conditions; the cell-surrounded extracellular environment interacts in all three dimensions. Therefore, 3D-cell culture models such as organoids and spheroids have been studied in recent years. The 3D-cell culture model provides a system in which cells are cultured and grown in their natural shape, and there are three-dimensional interactions between cell-to-cell and cell-to-extracellular environments. The 3D-cell culture model is a kind of connection between the *in vitro* and *in vivo* models, helping researchers to overcome the limitations of the traditional *in vitro* model while at the same time maintaining the benefits of an *in vitro* model: easy-to-control experimental conditions, low cost, and can be used for high throughput screening. Therefore, 3D-cell culture modeling stands out as a modern technique to study the toxicity as well as the biological activity of compounds. Table 2 compares some of the advantages and disadvantages of the 2D-cell culture, 3D-cell culture, and animal models [53-54].

Table 2. *Comparison of 2D cell culture, 3D cell culture, and animal models*

	2D-cell culture model	***3D-cell culture model***	***Animal models***
Model complexity	Simple	Complex	Very complex
Physicological similarity	Poor	Good	Very good
Disease modeling	Poor	Good	Very good
High-throughput screening	Yes	Yes	No
Manageability	Very good	Good	Poor
Cost	Low	Low	High
Ethical issues	No	No	Yes

Currently, the intestinal organoid is the most common model used in research. Kim et al. have demonstrated that Schisandrin C, a lignin compound isolated from Schisandra chinensis, could improve the intestinal permeability dysfunction in the mouse intestinal organoids model by increasing the expression level of TJ molecules such as ZO-1 and occludin. This result was similar to the research results on the 2D-cell culture with the human intestinal Caco-2 cells model, which is also shown in this study. It demonstrates the

compatibility between 2D-cell culture and 3D-cell culture models in studying the molecular mechanism of biologically active compounds [55]. Another study by Cai et al. was also performed using intestinal organoids to determine the effect of some common dietary nutrients, such as caffeic acid, chlorogenic acid, L-glutamic acid, curcumin, vitamin C, and m-hydroxyphenylpropionic acid on the growth of this organoid. The result showed that of the above compounds, caffeic acid could inhibit intestinal organoids' development, which is a dose-dependent effect. At the same time, curcumin could increase the growth rate of the organoid at a moderate dose (300 µg/mL) [56]. Besides studies on intestinal organoids, research on cerebral organoids has also gradually been interested and developed. Wang et al. have evaluated the neuroprotective mechanism of resveratrol using cerebral organoids 3D-cell culture. The result showed that resveratrol could protect the neuronal cell from damage caused by D-galactose by inducing neuronal cell proliferation, inhibiting apoptosis, and enhancing the differentiation of germ layers [57]. These results show the initial applications and potential of using 3D-cell culture models in the study of toxicity and bioactivity of nutraceutical compounds.

5.3. Organ-on-a-chip system

Along with the appearance and development of microfluidic technology, its applications in human health research are increasingly interested and developed. Organ-on-a-chip is a new technology that has the potential to help researchers better understand the toxicity and bioactivity of nutraceutical compounds in the human body. It is a specialization of the microfluidic chip that mimics the normal and pathological conditions of human organs *in vitro*. It includes interactions between cell-to-cell, cell-to-extracellular environment, and organ-to-organ. The organ-on-a-chip model can easily adjust and maintain suitable environmental conditions for different cultured cell lines. By using microfluidic systems, the supply of nutrients and removing the metabolites from the culture medium could be easily performed. Currently, the combination of the 3D-cell culture with microfluidic chip technology can provide a powerful tool to mimic the *in vitro* model for understanding the molecular mechanism of nutraceutical toxicity and bioactivity.

Up to now, many organ-on-a-chip systems have been developed to screen nutraceutical compounds' toxicity and bioactivity, especially gut-on-a-chip systems to study gut health. Jeon et al. have developed a microfluidic-based intestinal model which allows the co-culture of human intestinal cells (Caco-2) and microbial cells to investigate the protective effect of some probiotics on damaged intestinal cells. The gut-on-a-chip system was simulated by LPS, which led to the gut model's inflammatory conditions. The result showed that *Lactiplantibacillus plantarum HY7715*, a probiotic strain, could suppress LPS-induced by increasing the transepithelial electrical resistance (TEER) value on co-cultured damaged intestinal models [58]. This gut-on-a-chip showed the potential to become a model to explore the interaction between intestinal cells and the gut microbiome and investigate the probiotic's molecular mechanism on gut health.

Multi-organ-on-a-chip is a helpful model for simulating the interactions between organs in the human body. One of the critical links in the human body is the connection and interaction between the gut and the brain through the gut-brain axis. Previous studies in animal models have shown evidence that alterations in the composition and numbers of the gut microbiota can affect the normal physiological as well as pathological states of the brain and vice versa. The mechanism of this interaction is explained by the effect on the gut microbiota by potential pathogens or therapeutic agents, causing the gut microbiota to release soluble neurotransmitters, hormones, immune molecules, and neuroactive metabolites. These substances circulate in the circulatory system, cross the blood-brain barrier and act on neuronal cells [59]. For traditional *in vitro* cell culture models, it would be difficult to study these interactions. Therefore, multi-organ-on-a-chip has become a powerful tool to simulate and evaluate organ interaction. Kim et al. have developed a modular microfluidic chip where gut epithelial (Caco-2) and brain endothelial cells (hBMECs) were co-cultured to form the gut epithelial barrier and blood-brain barrier (BBB) [60]. LPS and sodium butyrate were used in this study to evaluate toxicity and protective activity, respectively. To simulate an inflammatory condition, the chip was stimulated by LPS. The results showed that both the intestinal barrier and BBB were affected by LPS. Both barriers were damaged, leading to increased permeability (TEER values decreased in both intestinal and neuronal cells). Butyrate is known to enhance the barrier function of gut epithelium and is thought to be important in improving gut health. The results showed that sodium butyrate has beneficial effects on mono-cultured hBMECs cells by protecting the BBB (by increasing TEER value). At the same time, when sodium butyrate was administrated to the intestinal cells, the integrity of both the intestinal barrier and BBB was improved, based on the increase in the measured TEER values. This result demonstrated that sodium butyrate-treated in the gut enhanced a protective effect on the brain endothelium barrier, and the designed chip could accurately simulate the beneficial effect of sodium butyrate via interaction with gut epithelium and BBB.

6. CONCLUSION

This article has included some general information about the *in vitro* cell culture model and its application in researching the safety and bioactivity of nutraceutical compounds. Many techniques to determine the toxicity, including cytotoxicity and genotoxicity, are now applied to evaluate the safety of nutraceuticals. Bioactivities of nutraceuticals have also been investigated using cell-based models, such as antioxidant, anti-inflammation, anti-obesity, neuroprotection, and gut health improvement. The mechanism of action at the biochemical, cellular, and molecular levels underlying the biological activity of nutraceuticals has also been elucidated through studies on the cell-based model. With their low cost, ease of maintenance, and excellent reproducibility, the cell-based model has become a powerful tool in studying nutraceutical toxicity and bioactivity. Moreover, research using cell-based

models is also developing daily with the support of modern technologies, such as high-throughput screening, 3D-cell culture, and organ-on-a-chip systems. These emerging technologies minimize the limitations of traditional 2D-cell culture models and make *in vitro* cell models a strong tool for screening before application in mammalian animal models.

ACKNOWLEDGMENTS

The authors would like to thank Professor Phan Tuan Nghia (Hanoi University of Science, Hanoi National University, Vietnam) for critical reading and helpful comments on manuscript revision.

REFERENCES

[1]. C. M. Dominguez, N. Oturan, A. Romero, A. Santos, and M. A. Oturan, "Removal of lindane wastes by advanced electrochemical oxidation," *Chemosphere*, vol. 202, pp. 400-409, 2018.

[2]. ThermoFisher Scientific, "*Cell culture basic Handbook*," 2020.

[3]. A. Adan, Y. Kiraz, and Y. Baran, "Cell Proliferation and Cytotoxicity Assays," *Current Pharmaceutical Biotechnology*, vol. 17, no. 14, pp. 1213-1221, 2016.

[4]. W. Strober, "Trypan Blue Exclusion Test of Cell Viability," *Current Protocols in Immunology*, 111, pp. A3 B 1-A3 B 3, 2015.

[5]. F. Denizot and R. Lang, "Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability," *Journal of Immunological Methods*, vol. 89, no. 2, pp. 271-277, 1986.

[6]. P. Ngamwongsatit, P. P. Banada, W. Panbangred, and A. K. Bhunia, "WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line," *Journal of Microbiological Methods*, vol. 73, no. 3, pp. 211-215, 2008.

[7]. T. Decker and M. L. Lohmann-Matthes, "A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity," *Journal of Immunological Methods*, vol. 115, no. 1, pp. 61-69, 1988.

[8]. J. Uggeri, R. Gatti, S. Belletti, R. Scandroglio, R. Corradini, B. M. Rotoli, and G. Orlandini, "Calcein-AM is a detector of intracellular oxidative activity," *Histochemistry and Cell Biology,* vol. 122, no. 5, pp. 499-505, 2004.

[9]. T. L. Riss, R. A. Moravec, A. L. Niles, et al., "Cell Viability Assays," Editors, *Assay Guidance Manual*, Bethesda (MD), 2004.

[10]. D. H. Phillips and V. M. Arlt, "Genotoxicity: damage to DNA and its consequences," *EXS*, vol. 99, pp. 87-110, 2009.

[11]. J. Alejandra Izquierdo-Vega, J. A. Morales-González, M. Sánchez-Gutiérrez, G. Betanzos-Cabrera, S. M. Sosa-Delgado, M. T. Sumaya-Martínez, Á. Morales-González,[4] R. Paniagua-Pérez, E. Madrigal-Bujaidar, and E. Madrigal-Santillán, "Evidence of Some Natural Products with Antigenotoxic Effects. Part 1: Fruits and Polysaccharides," *Nutrients*, vol. 9, no. 2, 2017.

[12]. A. R. Collins, "The comet assay for DNA damage and repair: principles, applications, and limitations," *Molecular Biotechnology*, vol. 26, no. 3, pp. 249-261, 2004.

[13]. E. B. Kurutas, "The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state," *Nutrition Journal*, vol. 15, no. 1, pp. 71, 2016.

[14]. L. A. Pham-Huy, H. He, and C. Pham-Huy, "Free radicals, antioxidants in disease and health," *International Journal of Biomedical Science*, vol. 4, no. 2, pp. 89-96, 2008.

[15]. C. Furger, "Live cell assays for the assessment of antioxidant activities of plant extracts," *Antioxidants (Basel)*, vol. 10, no. 6, 2021.

[16]. P. Liu, W. Wang, J. Tang, R. P. Bowater, and Y. Bao, "Antioxidant effects of sulforaphane in human HepG2 cells and immortalised hepatocytes," *Food and Chemical Toxicology*, vol. 128, pp. 129-136, 2019.

[17]. X. She, F. Wang, J. Ma, and X. Chen, "In vitro antioxidant and protective effects of corn peptides on ethanol-induced damage in HepG2 cells," *Food Agricultural Immumology,* vol. 27, no. 1, pp. 1-12, 2015.

[18]. J. Y. Lee and C. H. Kang, "Probiotics Alleviate Oxidative Stress in H2O2-Exposed Hepatocytes and t-BHP-Induced C57BL/6 Mice," *Microorganisms*, vol. 10, no. 2, 2022.

[19]. R. Scrivo, M. Vasile, I. Bartosiewicz, and G. Valesini, "Inflammation as "common soil" of the multifactorial diseases," *Autoimmunity Reviews*, vol. 10, no. 7, pp. 369-374, 2011.

[20]. N. Q. C. Thanh ,T. D. Binh, P. L. A. Tuan, N. D. H. Yen, D. T. X. Trang, N. T. Tuan, K. Kanaori, and K. Kamei, "Anti-Inflammatory Effects of Lasia spinosa Leaf Extract in Lipopolysaccharide-Induced RAW 264.7 Macrophages," *International Journal of Molecular Sciences*, vol. 21, no. 10, 2020.

[21]. L. Dong, L. Yin, Y. Zhang, X. Fu, and J. Lu, "Anti-inflammatory effects of ononin on lipopolysaccharide-stimulated RAW 264.7 cells," *Molecular Immunology*, vol. 83, pp. 46-51, 2017.

[22]. Y. Tian, S. Zhou, R. Takeda, K. Okazaki, M. Sekita, and K. Sakamoto, "Anti-inflammatory activities of amber extract in lipopolysaccharide-induced RAW 264.7 macrophages," *Biomedicine and Pharmacotherapy*, vol. 141, p. 111854, 2021.

[23]. M. Cargnello and P. P. Roux, "Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases," *Microbiology and Molecular Biology Reviews,* vol. 75, no. 1, pp. 50-83, 2011.

[24]. M. R. Guimaraes, F. R. Leite, L. C. Spolidorio, K. L. Kirkwood, and C. Rossa, Jr., "Curcumin abrogates LPS-induced pro-inflammatory cytokines in RAW 264.7 macrophages. Evidence for novel mechanisms involving SOCS-1, -3 and p38 MAPK," *Archives of Oral Biology*, vol. 58, no. 10, pp. 1309-1317, 2013.

[25]. F. Xue, X. Nie, J. Shi, Q. Liu, Z. Wang, X. Li , J. Zhou, J. Su, M. Xue, W-D. Chen, Y-D. Wang , "Quercetin Inhibits LPS-Induced Inflammation and ox-LDL-Induced Lipid Deposition," *Fronties in Pharmacology*, vol. 8, pp. 40, 2017.

[26]. S. J. Hwang, Y. W. Kim, Y. Park, H. J. Lee, and K. W. Kim, "Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells," *Inflammation Research*, vol. 63, no. 1, pp. 81-90, 2014.

[27]. Y. H. Choi, G. Y. Kim, and H. H. Lee, "Anti-inflammatory effects of cordycepin in lipopolysaccharide-stimulated RAW 264.7 macrophages through Toll-like receptor 4-mediated suppression of mitogen-activated protein kinases and NF-kappaB signaling pathways," *Drug Design, Development and Therapy*, vol. 8, pp. 1941-1953, 2014.

[28]. S. S. Ranaweera, C. Y. Dissanayake, P. Natraj, Y. J. Lee, and C. H. Han, "Anti-inflammatory effect of sulforaphane on LPS-stimulated RAW 264.7 cells and ob/ob mice," *Journal of Veterinary Science*, vol. 21, no. 6, pp. e91, 2020.

[29]. S. H. Choi, S. H. Lee, M. G. Kim, H. J. Lee, and G. B. Kim, "Lactobacillus plantarum CAU1055 ameliorates inflammation in lipopolysaccharide-induced RAW264.7 cells and a dextran sulfate sodium-induced colitis animal model," *Journal of Dairy Science*, vol. 102, no. 8, pp. 6718-6725, 2019.

[30]. M. Griet, H. Zelaya, M. V. Mateos, S. Salva, G. E. Juarez, G. F. de Valdez, J. Villena, G. A. Salvador, and A. V. Rodriguez, "Soluble factors from Lactobacillus reuteri CRL1098 have anti-inflammatory effects in acute lung injury induced by lipopolysaccharide in mice," *PLoS One*, vol. 9, no. 10, pp. e110027, 2014.

[31]. H. E. Park, K. H. Do, and W. K. Lee, "The immune-modulating effects of viable Weissella cibaria JW15 on RAW 264.7 macrophage cells," *Journal of Biomedical Research*, vol. 34, no. 1, pp. 36-43, 2019.

[32]. N. Lee, S. Lee, S. Lee, S. W. Jang, H. S. Shin, J-H. Park, M. S. Park, and B-H. Lee, "Lysed and disrupted Bifidobacterium bifidum BGN4 cells promote anti-inflammatory activities in lipopolysaccharide-stimulated RAW 264.7 cells," *Saudi Journal of Biology Science*, vol. 28, no. 9, pp. 5115-5118, 2021.

[33]. A. C. Archer, N. K. Kurrey, and P. M. Halami, "In vitro adhesion and anti-inflammatory properties of native Lactobacillus fermentum and Lactobacillus delbrueckii spp," *Journal of Applied Microbiology*, vol. 125, no. 1, pp. 243-256, 2018.

[34]. Y. M. Kim, I. H. Kim, J. W. Choi, M. K. Lee, and T. J. Nam, "The anti-obesity effects of a tuna peptide on 3T3-L1 adipocytes are mediated by the inhibition of the expression of lipogenic and adipogenic genes and by the activation of the Wnt/beta-catenin signaling pathway," *International Journal of Molecule Medicine*, vol. 36, no. 2, pp. 327-334, 2015.

[35]. F. J. Ruiz-Ojeda, A. I. Ruperez, C. Gomez-Llorente, A. Gil, and C. M. Aguilera, "Cell Models and Their Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review," *International Journal of Molecule Science*, vol. 17, no. 7, 2016.

[36]. Y. S. Jeong, H. K. Jung, K-H. Cho, K-S. Youn, and J-H. Hong "Anti-obesity effect of grape skin extract in 3T3-L1 adipocytes," *Food Science and Biotechnology*, vol. 20, no. 3, pp. 635-642, 2011.

[37]. K. J. Han, N. K. Lee, H. S. Yu, H. Park, and H. D. Paik, "Anti-adipogenic Effects of the Probiotic Lactiplantibacillus plantarum KU15117 on 3T3-L1 Adipocytes," *Probiotics Antimicrob Proteins*, vol. 14, no. 3, pp. 501-509, 2022.

[38]. M. Miyoshi, A. Ogawa, S. Higurashi, and Y. Kadooka, "Anti-obesity effect of Lactobacillus gasseri SBT2055 accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet-induced obese mice," *European Journal of Nutrition*, vol. 53, no. 2, pp. 599-606, 2014.

[39]. K-H. Lee, J-L. Song, E-S. Park, J. Ju, H-Y. Kim, and K-Y. Park, "Anti-Obesity Effects of Starter Fermented Kimchi on 3T3-L1 Adipocytes," *Preventive Nutrition and Food Science*, vol. 20, no. 4, pp. 298-302, 2015.

[40]. X. Chen, C. Guo, and J. Kong, "Oxidative stress in neurodegenerative diseases," *Neural Regeneration Research*, vol. 7, no. 5, pp. 376-385, 2012.

[41]. A. Gonzalez-Sarrias, M. A. Nunez-Sanchez, F. A. Tomas-Barberan, and J. C. Espin, "Neuroprotective Effects of Bioavailable Polyphenol-Derived Metabolites against Oxidative Stress-Induced Cytotoxicity in Human Neuroblastoma SH-SY5Y Cells," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 4, pp. 752-758, 2017.

[42]. S. Thummayot, C. Tocharus, D. Pinkaew, K. Viwatpinyo, K. Sringarm, and J. Tocharus, "Neuroprotective effect of purple rice extract and its constituent against amyloid beta-induced neuronal cell death in SK-N-SH cells," *Neurotoxicology*, vol. 45, pp. 149-158, 2014.

[43]. M. J. Cheon, S. M. Lim, N. K. Lee, and H. D. Paik, "Probiotic Properties and Neuroprotective Effects of Lactobacillus buchneri KU200793 Isolated from Korean Fermented Foods," *International Journal of Molecule Science*, vol. 21, no. 4, 2020.

[44]. J. F. Cryan, K. J. O'Riordan, C. S. M. Cowan, et al., "The microbiota-gut-brain axis," *Physiology Review*, vol. 99, no. 4, pp. 1877-2013, 2019.

[45]. I. Hubatsch, E. G. Ragnarsson, and P. Artursson, "Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers," *Nature Protocols*, vol. 2, no. 9, pp. 2111-2119, 2007.

[46]. C. Stolfi, C. Maresca, G. Monteleone, and F. Laudisi, "Implication of Intestinal Barrier Dysfunction in Gut Dysbiosis and Diseases," *Biomedicines*, vol. 10, no. 2, 2022.

[47]. J. Y. Kim, T. A. N. Le, S. Y. Lee, et al., "3,3'-Diindolylmethane improves intestinal permeability dysfunction in cultured human intestinal cells and the model animal caenorhabditis elegans," *Journal of Agricultural and Food Chemistry*, vol. 67, no. 33, pp. 9277-9285, 2019.

[48]. K. K. Putt, R. Pei, H. M. White, and B. W. Bolling, "Yogurt inhibits intestinal barrier dysfunction in Caco-2 cells by increasing tight junctions," *Food and Function*, vol. 8, no. 1, pp. 406-414, 2017.

[49]. L. Rhayat, M. Maresca, C. Nicoletti, et al., "Effect of *Bacillus subtilis* strains on intestinal barrier function and inflammatory response," *Frontier in Immunology*, vol. 10, p. 564, 2019.

[50]. C. Gong, Z. Ni, C. Yao, et al., "A high-throughput assay for screening of natural products that enhanced tumoricidal Aactivity of NK cells," *Biological Procedures Online,* vol. 17, p. 12, 2015.

[51]. J. L. Tan, F. Li, J. Z. Yeo, et al., "New high-throughput screening identifies compounds that reduce viability specifically in liver cancer cells that express high levels of SALL4 by inhibiting oxidative phosphorylation," *Gastroenterology*, vol. 157, no. 6, pp. 1615-1629 e1617, 2019.

[52]. J. Perez Del Palacio, C. Diaz, M. de la Cruz, et al., "High-throughput screening platform for the discovery of new immunomodulator molecules from natural product extract libraries," *Journal of Biomolecular Screening*, vol. 21, no. 6, pp. 567-578, 2016.

[53]. G. Alzeeb, J. P. Metges, L. Corcos, and C. Le Jossic-Corcos, "Three-Dimensional Culture Systems in Gastric Cancer Research," *Cancers (Basel)*, vol. 12, no. 10, 2020.
[54]. S Chenchula, S Kumar, and S Babu, "Comparitive efficacy of 3dimensional (3D) cell culture organoids vs 2dimensional (2D) cell cultures vs experimental animal models in disease modeling, drug development, and drug toxicity testing," *International Journal of Current Research and Review*, 2019.
[55]. M. R. Kim, S. Y. Cho, H. J. Lee, et al., "Schisandrin C improves leaky gut conditions in intestinal cell monolayer, organoid, and nematode models by increasing tight junction protein expression," *Phytomedicine*, vol. 103, p. 154209, 2022.
[56]. T. Cai, Y. Qi, A. Jergens, et al., "Effects of six common dietary nutrients on murine intestinal organoid growth," *PLoS One*, vol. 13, no. 2, pp. e0191517, 2018.
[57]. Y. Wang, T. Wei, Q. Wang, et al., "Resveratrol's neural protective effects for the injured embryoid body and cerebral organoid," *BMC Pharmacology and Toxicology*, vol. 23, no. 1, p. 47, 2022.
[58]. M. S. Jeon, Y. Y. Choi, S. J. Mo, et al., "Contributions of the microbiome to intestinal inflammation in a gut-on-a-chip," *Nano Convergence*, vol. 9, no. 1, pp. 8, 2022.
[59]. I. Raimondi, L. Izzo, M. Tunesi, et al., "Organ-On-A-Chip in vitro Models of the Brain and the Blood-Brain Barrier and Their Value to Study the Microbiota-Gut-Brain Axis in Neurodegeneration," *Frontiers in Bioengineering and Biotechnology*, vol. 7, pp. 435, 2019.
[60]. Min-Hyeok Kim, D. Kim, and J. Hwan, "A gut-brain axis-on-a-chip for studying transport across epithelial and endothelial barriers," *Journal of Industrial and Engineering Chemistry*, vol. 101, pp. 126-134, 2021.

Tổng quan đánh giá độc tính và hoạt tính sinh học của dược thực phẩm dựa trên mô hình tế bào *in vitro*

Trần Hùng Sơn[1,2,3]

[1]*Viện Kiểm nghiệm an toàn vệ sinh thực phẩm Quốc gia, Hà Nội, Việt Nam*

[2]*Viện Nghiên cứu các hợp chất thiên nhiên, Viện Khoa học và Công nghệ Hàn Quốc, Gangneung, Hàn Quốc*

[3]*Bộ môn Khoa học và công nghệ Y sinh, Đại học Khoa học và Công nghệ Hàn Quốc, Daejeon, Hàn Quốc*

Tóm tắt

Dược thực phẩm là một khái niệm mới xuất hiện trong những năm gần đây, chỉ những sản phẩm có nguồn gốc từ thiên nhiên có khả năng mang lại những tác động tích cực đối với sức khỏe con người trong việc phòng và điều trị bệnh, bên cạnh việc cung cấp giá trị dinh dưỡng như thực phẩm thông thường. Trong quá trình nghiên cứu và phát triển các sản phẩm dược thực phẩm, việc đánh giá độc tính và hoạt tính của các hoạt chất tiềm năng cũng như

nghiên cứu sâu về cơ chế tác động ở mức độ phân tử và tế bào của chúng là rất cần thiết. Trong các mô hình thí nghiệm, mô hình tế bào *in vitro* nổi lên như là một mô hình với nhiều ưu điểm cho việc sàng lọc sơ bộ độc tính cũng như hoạt tính của các hoạt chất trước khi tiến hành nghiên cứu sâu hơn trên các mô hình động vật *in vivo*. Bài tổng quan này tóm tắt những kĩ thuật cơ bản được sử dụng trong việc đánh giá độc tính của dược thực phẩm trên mô hình tế bào. Các nghiên cứu gần đây về hoạt tính của các hoạt chất dược thực phẩm như hoạt tính chống oxy hóa, chống viêm, chống béo phì, bảo vệ hệ thần kinh hay cải thiện sức khỏe đường ruột cũng được tổng hợp để cung cấp cái nhìn tổng quan trong việc ứng dụng mô hình tế bào *in vitro* trong nghiên cứu các hoạt chất dược thực phẩm. Cuối cùng, các kĩ thuật hiện đại liên quan đến mô hình tế bào *in vitro* như hệ thống sàng lọc thông lượng cao, mô hình nuôi cấy tế bào 3D và hệ thống organ-on-a-chip cũng được trình bày ở trong bài tổng quan này.

Từ khóa: *Hoạt tính sinh học, độc tính, dược thực phẩm, mô hình tế bào in vitro.*