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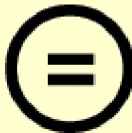

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A THESIS

FOR THE DEGREE OF MASTER'S

Effect of Methanolic Extracts of *Acer Cappadocicum* on HepG2 Cancer Cell Line in a Liver Microphysiological system

Muhammad Awais Farooqi

DEPARTMENT OF MECHATRONICS ENGINEERING

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

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Effect of Methanolic Extracts of *Acer cappadocicum* on HepG2 Cancer Cell Line in a Liver Micro-physiological system

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A thesis submitted in partial fulfillment of the requirements for the degree of Masters

February 2022

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Dedication

I want to dedicate my thesis to all my beloved ones who left this world and my family back in Pakistan. As I look back to the last 25 years, the journey is like a roller coaster ride while it takes just a few moments over there. We never knew about how heavenly powers work for us. One day, we all get the dreams we wish for but trust me, the real happiness is to share it with our loved ones. The journey is a moment of joy and pain. I want to thank all people who came into my life and made me vulnerable and sensitive.

I don't know how I have driven myself till now, yet inside there is a fire that brings all of us somewhere in the center of the ocean. A hundred saints sail my boat. I dedicate all my puny efforts and tiny contributions to the poised souls of this universe. It's hard to pin down all my tenderness. I also like to acknowledge the Republic of Korea; it gave me a new perspective of life. It made me think more about togetherness and made me more appreciative of family and social values.
I desire to mention some names that meant everything to me.

Mama Jaan: I admire all your struggles and prayers you did for me.
Baba Jaan: Your practical teachings saved me till now.
Umar: I shall never be able to pay back the strains you endorse for me.
Tuba: You inspired me and taught me.
Ali: The trust and confidence you gave will keep with me forever.
U. Sardar: I am sorry to get to know about you too late.
Ahmed: I adore your innocent personality.
Habib: I miss the baby version of Habib.
Baji Fatima: I want to thank you for listening to all my lame problems and tolerating me like my mother in Korea.
Dawood & Yousuf: They gave me daily motivation to pass this synthetic exam of life.
Sumaira: Welcome to Farooqi's house.
My future wife: Where are you now?

I mean it, it all matters, all's well that ends well.

Muhammad Awais Farooqi

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Abstract

The liver is a vital organ for many crucial functions within the human body, and should it become diseased or injured, the loss of those integral functions can cause real damage to the human cells. Therefore, hepatotoxicity measurement of any plant extract is essential because liver toxicity such as hepatitis, cirrhosis, and hepatocellular carcinoma affect human life and health because they alter the metabolism and detoxification of the human body. Thus, this study depicted the estimation of the hepatotoxicity potentials of an accessed *Acer cappadocicum* (AC) population plucked from the Kohistan Valley mountains in northern Pakistan. The leaf extracts of the plant were made in organic solvents of different polarities. The other proportion ratios of extracts were prepared through the process of maceration. Hepatotoxicity is griping about evaluating for any anticancer drug toxicity; this study was directed by applying a liver chip to estimate the hepatotoxicity assessment. We first assembled a bilayer liver MPS by executing the four hepatic cell line types (HepG2 cancer cell line, HUVEC, stellate cells, and Kupffer cells) in a bilayer microchannel with the help of laminal flow in the microchannel using micropumps. This chip was fabricated and operated by MPS protocol. It also described the better hepatic activity (ROS synthesis, and liver enzyme activities. Then, we added Methanolic extracts of A. cappadocicum into the chip. It successfully accessed the hepatotoxic effect of this *A. cappadocicum* in the organ-on-a-chip. Methanolic extracts of Acer cappadocicum were administrated to anticancer and antioxidant activities. Still, they accessed its hepatotoxicity using the HepG2 cancer cell line in the Liver Micro-physiological system, impacting ROS, and SOD, ALT, and AST assays. A. cappadocicum extract vigorously inhibited ALT and AST levels. Thus, this extract decreased the growth of enzymes in hepatocytes. The inhibitory evidence of extracts showed the ability and low level of enzymes viability of the HepG2 cancer cell line.

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Introduction

There are about two million casualties worldwide due to chronic liver diseases each year. The fact is liver cancer is ranked 4th for its most invasive cancer nature around the globe.[1] The prevalence of hepatic hepatitis is increased due to alcohol abuse, hepatocellular carcinoma, metabolic disorders, daily lifestyle, liver cirrhosis, and elements that flatulate the pressure towards hepatocytes are the main causes of viral hepatitis.[2] Due to insufficient in-vitro models, the discovery of new therapies for hepatic cancers is difficult to achieve. While early screening of liver disease along with antiviral treatments and vaccinations have dramatically decreased liver disruption. Although in vitro method for screening is very expensive, requires a lot of labor, and proved as a misleading tool because of huge cross species variation in the gene of species along with ethical permission and concerns. In-vitro models for cancer culture are rapid and executed in high throughput techniques. They cannot relate to complicated physiological and pathological functions of tissues and cells because of less shear stress.[3] There are also other factors like Extracellular constituents and crosstalk between cellular structures and histology etc. A new cutting-edge technology named the micro physiological system is revealed with microfluidic technology. It has the features to translate the vital functions of organs and tissue-specific interactions and implications of in vivo pathways. Compared to conventional cell culture and animal models, MPS is far better for understanding efficacy studies and paves the path for medicinal compounds toxicity studies. MPS provides a Microphysiological environment to evaluate the agent's effects same as in vivo environment. It recapitulates the tissue for the analysis of and tumor microenvironment.[4] Therefore, MPS is emerging as the weapon to reduce the traditional rodents' models for pre-clinical testing. There are certain tight junction proteins in the

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microfluidic chip that portray the microfluidic environment's pathophysiological behavior and monitoring of extracellular constituents. Moreover, emerging biochemical assays such as Transepithelial electrical resistance (TEER) and the other electrical cell-substrate (ECIS) for the measurement of the molecular assay. These biochemical assays are non-invasive and rapid as well. The application of TEER varies from barrier integrity and cell-mono; layer measurement along with cell-to-cell tight junction, toxicity testing with ECM quantification.[5] TEER doesn't sense to screen and monitor the plant extract effects in tumor micro-physiological. There are numerous species of unidentified plants with various natural metabolites that carry a high potential for anticancer diseases; they can be categorized by their biological activities and different spectroscopic methods. The extracts from natural sources have been given more attention due to their procession of numerous biochemical agents to inhibit or destroy cancerous cells.[6] For therapeutic purposes, around 80% of the under-developed counties rely on natural plants. Some of these plants are of indigenous origin. Acer species are reported in the Himalayan region of north Kohistan valley of Pakistan, which processes the medicinal features. This plant is very effective against certain diseases such as hepatic diseases, autoimmune diseases like rheumatoid arthritis, conjunctivitis, and inflammation. Several analyses show that Ace species have great bioactivity and confirmed huge ethnomedical significance towards liver hepatoprotective function.[7] Some studies evaluate the phytochemical analysis, antimicrobial activities, safety testing, cytotoxicity of Acer cappadocicum extracts against many primary human cells and cancer cell lines that predicted Acer Cappadocia potential against cancer cells. This study aims to utilize the Microphysiological system for the plant extracts to evaluate their anticancer activities and compare the conventional cell culture system. Acer cappadocicum Gled is tested against the HepG2 tumor cell line of MPS composed of certain hepatocellular carcinoma cells and to rule out the anticancer activity of

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methanolic extracts in a microfluidic environment. [1,4,5] Different concentrations of the extracts are perfused in the cell culture media. The chip is embedded with a TEER sensor and is applied to analyze the impact of the extracts of plant and their effect on tight junction protein cell-cell interaction. This study was carried out on a 96-well cell culture plate. This is the traditional model (in vitro cell model). The biomarkers are measured among the considerable difference among the activities. [2,6] The wide range of solid tumors are metastasized in the liver. The liver microenvironment carries a cellular and biological complex liver system responsible for the modulation and distinctions of the primary cells. The tumor cell biology metastasis study is complicated to achieve the desired results; thus, Microphysiological systems provide a pathway with clear insights for tumor studies. Both liver and tumor cells are isolated in a microenvironment within microfluidic systemic events like liver functioning in human physiology. For that study, we checked the influence of organs on the other tissues. Therefore, we linked the Microphysiological system. This study discloses the metastases of complex integrating models of primary tumor cells. First, it is provided by the liver tumor microenvironment. [8] Then certain biomarkers with inflammatory organs are incorporated with the immune system to investigate the effects on HepG2 cells and cancer treatment prognosis. The leading cause of mortality in patients is metastatic disease. Recurrence is the major drawback of treatment. Only 25% of solid tumor patients carry a 5-year survival rate. The eradication of disease does not advance in removing primary disease, which is translated against metastases.[9] The tumor cells, along with the foreign metastatic processes of a microenvironment, have disseminated and exceedingly multifaced and intricate interactions. This metastasis process starts within the tumor's primary cells, and it undergoes a cancer-associated epithelial-to-mesenchymal transition (EMT). This opens the detachment from the surroundings of the cell and transmits the motility into intravasate circulation. The

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dissemination of tumor cells then transits through the vasculature and eventually causes the colonization of extravasate distant organs. A slight reversion back for the more epithelial phenotype is the cancer-associated mesenchymal-to-epithelial reverting transition (cMErT), which is initially required for colonization. E-cadherin is one of the common elements of cEMT AND cMErT (down-regulation or upregulation, respectively). [10] They are present on the tumor surface for homotypic, cellular elements of phenotype, and heterotypic binding. The dormant state of metastatic cancer is the main hurdle because it outgrows immediately after a certain clinical evident event. It processes the dormant state as single or in the form of clusters. The tumor cells dissemination occurs in the earliest stages because primary tumor development originates from the primary cell. The dormant state of the tumor can remain for months to years or maybe a decade because it can escape from the initial therapies before emerging to a lesion which is clinical evidence. Therefore, it results in poor patient outcomes and less survival rate because disseminated tumor cells are often associated with immense therapeutic resistance. [6,9,11]

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Chapter 1

1. MPS platforms of the liver HepG2 Cancer Cell Line

Drug discovery has great importance in the medical field; therefore, new platforms like Liver MPS are advanced technology for that pathway. MPS systems have certain desirable features, which include, 1) primary human hepatic cells residents complete complement inclusions (both non-parenchymal cells and parenchymal cells) for the proper functioning of the liver and the specific cell-cell interactions along with accurate recapitulation of species signaling. 2) Relevant Physiological shear stress along with the controllable fluid flow. 3)Multicellular tissue, 3D matrices, scaffolding, and self-organization. 4) The examination of array readouts and their ability to access different values. (e.g., proteomic, therapeutic efficacy, indices, genomic, phenotypic, metabolic indices, etc.) [6,9,11]

It's challenging to execute the tricky concepts and the system illustration, which typically falls in favor of high throughput or content carrying high information. Multiple liver MPS models have been constructed, but few subsets of them have been validated to demonstrate metastatic disease. This liver MPS system is commonly used to study the progression of metastasis and the investigation of liver niche, which is distributed by the liver MPS platform. Our liver organ consists of a complete primary human donor-matched hepatic cells entire complement (non-parenchymal cells and hepatocytes). It is determined by the different factors like cells viability, phenotype retention, and active metabolism by the tissue functions out of 4 weeks. [2,6,10] The liver chip regenerates the physiologically reflective situation of human organs along with metastatic progression. This system is a handful of gadgets to mimic the metastatic progression processes dormant emergence. Some introductions about the breast cancer cells into the system (cells behave similarly as prostate cancer carrying attainment of dormancy with random effect is

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observed in the subpopulation of tumor cells. These are both the highly aggressive cancer cell lines (MDA- MB 231) and (MCF-7) indolent cell lines. [12] This subpopulation portrays the properties of dormancy of cellular structures, chemotherapeutic resistance, reversible growth injury, and foreign microenvironment survival. Inflammatory stimuli stimulate the physiological relevant tumor cells after the latency period and emergence into the cell cycle induced by their re-entry.[13]

Some studies also depict the insights about the potential emergence drivers which different groups provide. Activated HSCs, specifically non-parenchymal cells, are shown to create a role in the production of IL-8 through dormant tumor cells by promoting emergence. Also, some factors originating those lipophilic stains carrying high cholesterol prescribed drugs can play a vital role in stopping emergence. Other studies show that colonization is promoted by tumor-derived exosomes where it is constructed by the bidirectional role of metastatic exosomes processes metastatic progression. All these characteristics carry by the hepatic niche and its promoted dormancy. [7,12]

However, Liver on-chip in vivo technology alters the physiological properties of the scaffolds; it creates the dormant phenotype which closely relevant to the liver organ. Some works are also produced the micro-metastases, which show the unsuspected behavior of dormant phenotype. The doses used have a sub-therapeutic dosage of chemotherapy and promote the emergence of the inactive cell with nodules. [4,6] The implication of human physiology on liver on-chip is significantly essential because the regimens that have to b administrated to patients must be inadequate, causing metastatic-related mortality carrying specific doses. Technically, the system that exhibits the biomarkers from the outgrowing tumor cells can identify actively detect signals from the host tissue and tumor-derived cells. The fact is those breast cancer cells that survived are

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still a threat because of their metastatic recurrence nature. Sometimes it is incurable. The main reason is its metastatic nodule in the stage of detection recurrence.[14]

1.1. Opportunities for integrating MPS platforms

Cell biology and the tumor's metastatic sites are executed in the liver Microphysiological system, which provides an unexpected and rapid insight into the study and quantitative analysis of metastasis. The liver tissue structures present in the MPS system show the therapeutic effect and tumors cell interaction inside the cellular and biological structure of the liver. The liver is not an isolated organ; in vivo tissue correlation is vital; in this scenario, the model must process the cascade to complete the metastatic cycle. Thus, we can assess the effect and influence of cancer cell lines on other organs, which can be carried out within the multi-organ MPS platforms. [15] The multi-organs Microphysiological systems vary in their complexity; it differs from specific organ modules 2-up to 10-way. Many systems replicated the progressive metastatic module, which has the primary tumor to the liver module and carries the remote organ systems. [4,9]

1.2. Perspectives on Integration

Cross-talking interaction organs, which are interconnected in the Microphysiological system, open different opportunities to explore the effects of new drugs and their toxicity and efficacy in a better way. The conventional animal models have some drawbacks, which is the main hurdle in the drug discovery pathway. Although this new technology is just 20 years old and still in its infancy, therefore still ow some problems, we must face while preparing the metastatic models.[14,15] The main question that arose to develop the metastatic biological model is the metastatic phases, e.g., how to access the tumor cell tropism, what are the investigation of efficacy, the therapeutic effects against the dormant sub and total population, what are the elucidation of emergence, cell tropism and distant drivers which are responsible for ending up

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the metastatic cycle.[13,14] The mentioned points can be, and after the readouts after the assays, all those points determine the platform and its properties. There is a slight shift and variation in the future forum. This required experimental readouts and real-time monitoring, cell tracking, biological sensors, physiological sensors, endpoint testing, immunofluorescent imaging, effluent sampling. Meanwhile, the above variations include a level of connectivity, the integration type, number of modules. First, we must set up the integration platforms; the configurable platforms, e.g., (inclusive, noncontiguous, configurable, or singular) we operated 3- and 4-way systems will most likely gather to form the bulk of the metastatic models, instead of to more 10-way complex systems. [9,16]

MPS platforms offer different perspectives to integrate a system. It provides opportunities to explore crosstalk, system-level effects on organs and depicts the conventional animal models confined in the past decades. From the past two decades, there has been a revolutionary set-in drug discovery because of MPS integrated platforms. It opens a new face for toxicity and efficacy testing in a rapid and accurate time motioning scenario. The assessment of cell tropism and therapeutic effects are only possible because of MPS platforms. It has different characteristics like inclusive and configurable integration. [17]

Drug discovery has a greater failure rate because of the highly complex process; the process is highly inefficient; therefore, many compounds wouldn't make it to the pipeline due to lack of efficacy and more significant toxicity. It takes more than ten years for any new drug to come into the pharmaceutical world. The ten years average time takes due to different phases of drug discovery, and it costs about US$ 2.62 billion. [3] The drug failures in the preclinical and clinical trials occur due to the low capability of models which can access the drug toxicity and efficacy. First, it comes to identifying the toxic effect of certain drugs. The conventional animal models

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cannot match with the human physiological cell-cell interaction. [18] Most medicines that can reach the finishing line are first identified in the in vitro stages. The drug metabolism, the drug discovery investigation carries information regarding the toxic metabolites which are not specified in the early stages. The animal models have one main benefit: they have the actual cell-cell interaction and the physiological effect of any specific drug. The process is compromised on two phases; in vitro studies, animal testing (animal models), but it commonly fails in the last stages of clinical trials. There must be a need to continue to enhance the understanding of anabolism and catabolism of toxic drugs. The phenomena are defined as absorption, distribution, metabolism, and excretion. [19]

The liver is a vital organ of the human body. Some studies show that the liver performs 800 functions at a single time. The liver carries out the drug metabolism in drug development; the metabolism is the main area of focus by the liver. There is still a need to modify the drug discovery pathway to eliminate the toxic drugs early in the pipeline of drug development. [9,13,15]

Drug metabolizing enzymes (DMEs) are the leading central group of liver proteins. These enzymes accelerate the metabolism in the liver. DMEs are divided into two groups of phases; comprised of phase I and phase II metabolizing enzymes. Emerging and conventional in vitro techniques in metabolism studies are testified on the basics of the above drug-metabolizing enzymes and their action to mimicking the drug action Phase I enzymes are mainly Cytochrome P450 and flavin-containing monooxygenases (FMOs).[20] Most of the drugs are lipophilic. These enzymes make the suitable lipophilic environment for the drug to metabolize and distribute into the blood. These two enzymes are categorized as the main factors to metabolize the vast range of pharmaceuticals (75% of all marked drugs). When a drug-driven through phase I, the conversion occurs by the process of catalyzing its metabolites to enter the phase II drug cycle, this cycle of metabolism goes

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through by UDP-dependent glucuronosyltransferases (UGT), N-acetyltransferases (NATs) sulfotransferases (SULTs), and glutathione S-transferases (GSTs). The phase II enzymes increase the solubility of drugs, especially in water, and it's also facilities the subsequent excretion of waste metabolic products. [21] In the compression, phase I has some contradictory effects. In contrast, phase II enzymes are studied less in drug development and testing because they catalyze a minimal number of substrates. All the drugs in the discovery pipeline are all the prediction outcomes driven by the enzymes produced by the liver. These enzymes depict the metabolism of drugs and the toxicity of each chemical compound, pharmaceutical by using improved cell-based techniques, which are the now center of attention to all the think tanks. [22]

Presently, drug prediction and in vivo bioactivity are made using a physiologically based animal model. It carries pharmacokinetic patterns. However, the lack of relevance between animal data and human physiology has been identified as the main hurdle because the pharmacokinetic-based model shows a difference in the physiological relevance of animal and in vitro models. Additionally, ethical concerns are also there for animal models. Animal research ethics processes all the rights for animal testing. [23] Proper animal testing training and certification are required to do animal testing; therefore, in vitro culture wells are widely used to cope with this problem. It is also called the alternative to conventional animal testing. [21,22] The screening is also quite useful before the in vivo models in drug development. The major drawback and limitations of these in vitro cell cultures are the sample preparations. It isn't easy to separate the constituents and extracts in the matrix compounds such as proteins, salts, lipids, and endogenous compounds) and second is the low range of accurate results. Other factors are the limited physiology and cell

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function accuracy. All these factors lead to preliminary and limited clinical trials and preclinical testing. [22,23]

Microphysiological is the technology of new generation such as organ-on-chip platforms which proposed the rapid screening in vitro models for the drug testing for the drug development in the preclinical phase. These platforms are 3D-based cell microchannels, and specific engineering techniques are used to fabricate the microfluidic chip. These microfluidic channels are then seeded with human cells, e.g., hepatocytes. MPS system assists the dynamic flow of cells through the cell media; this microenvironment aims to mimic the anatomy and physiology closely of the human body.[24] The Organ-on-chip allows the functional integrity of human physiology, which comes out as the better source for drug prediction. The in vivo responses are predicted by the MPS system to certain pharmaceutical compounds. The chief benefit of those MPS systems is the early detection of toxic compounds; this platform could cut down the expense of drug testing. This MPS system can also reduce the cruelty of animal testing. The liver-on-chip devices are categorized into different types of complexities; the application includes disease modeling, drug screening, and liver function studies. These applications get a more significant opportunity to predict compounds growing every year. [21,22] In the past few years, several comprehensive reviews have outlined liver-on-chip fabrication, which carries advanced study in the research market hereafter this LOC distinguished by the human-on-chip (organs-on-a-chip). A wide range of MPS applications have been reported, reviewed, and focused on liver-on-chip platforms: the drug-induced responses and their metabolism allowed by the LOC platforms in the past few years. In recent decades, understanding the MPS system has opened new doors to the acceleration of drug discovery [23,24]. Here we mainly focus on drug hepatotoxicity, the assessment, and employed platforms for predicting liver injury, which is induced by drug-drug interactions. Along with LOC, multi-organ

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platforms also study the other organs' crosstalk. They also learn the specific effects caused by any compound on liver metabolism, toxicity, drug efficacy for organs systems.[25]

Chapter 2

2. Liver-On-Chip (LOC) Platforms

The liver is the vital organ for metabolic drug activities. It's the drug binding site and the target for drug-induced and chemical-induced toxicity. Most drugs are metabolized in the liver. The phenomena are called in-vitro ADME (adsorption, distribution, metabolism, excretion). This toxicity testing is based on the liver cells. The liver is the essential organ for metabolic processes like lipid, protein, carbohydrate metabolism. It is also the site of the production of complement proteins and clotting factors. The liver also carries out the detoxification of harmful compounds and the synthesis of enzymes. [23,24] Traditionally, in-vitro 2D and conventional animal models are replaced by perfused Microphysiological platforms, e.g., liver-on-chip (LOC). These LOC platforms have attracted wide attention over the old techniques. Many LOC microfluidic chips have been developed to simulate human metabolism using diverse microfabrication techniques based on liver cells, human liver microsomes, or rats. These microfluidic chips are embedded in human tissue samples. Because of using the engineering techniques, we achieved the medium flow by the science of applied microfluidics. [25] Scientific microfluidics techniques control us. That's how we can mimic blood flow in the microchannel, the same as in the human vessels. Furthermore, the composition of the medium can effectively be changed amid the course of a try, and more physiological medium-to-cell proportions can be accomplished due to little framework volumes. In addition, metabolites can reach higher concentrations due to the low-volume microchannel structure, making online discovery less demanding. [26] This is often different from metabolites

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produced in well plate assays, where metabolites can be significantly impaired due to larger well volumes. In microbiology, media contains metabolites that can coordinate with other cells found elsewhere on the same chip. To accurately demonstrate liver function in vitro, bio-converting proteins must be conveyed to allow testing of maternal sedation and its metabolite generation. The LOC steps aim to closely mimic the vital, physiological, and functional liver systems using different bio-transformed protein sources. Thus, combining microfluidic with tissue design can better simulate the complexity of organ engineering and the calm digestive system in vivo. Liver-on-chip frameworks can be coordinated within research settings for preclinical trials to better predict in vitro human experiments. spheres, or organoids shaped with mono or co-culture and liver intaglio sections. [27,28] Moreover, they permit computerized analytics such as checking of pH, temperature, squander expulsion, supplement supply, liquid weights, and shear push in culture compartments. [28]

2.1. Liver-On-Chip Platforms Based on Immortalized cell lines

Cell source is an important aspect in developing liver-on-a-chip (LOC) platforms used for drug metabolism applications. In general, primary hepatocytes (PH) remain the gold standard for predicting drug toxicity responses. However, while primary hepatocytes can provide accurate results due to their physiological relevance, there are problems with their extraction, donor-to-donor variability, and dedifferentiation, which negatively affect the reproducibility of the results. [29] In the development of Microphysiological platforms, researchers often prefer immortalized cell lines due to their stability and ease of handling. In comparison with primary cells, cell lines have lower functionality and weaker reflection of the susceptibility of human liver cells to injury caused by drug toxicity and interactions; however, through careful construction of a 3D liver

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sinusoid-on-a-chip (LSOC) incorporating four immortalized cell lines (HepG2, LX2, EAhy926, U937), we're able to propose an alternative to primary hepatocyte-based models with comparable results in testing hepatotoxicity caused by drug-drug interactions. [27,28] LOC systems employing immortalized cell lines (e.g., HepG2, Huh7, HepaRG), notwithstanding their less powerful functionality, are very valuable for early-stage drug development due to their high proliferation and extensive characterization. LOC platforms seeded with immortalized cell lines are being used in research settings to conduct toxicity and metabolism studies. For example, the 'Homunculus' LOC device based on HepaRG spheroids was employed to test HIF prolyl hydroxylase inhibitor adaptations that show promising in vivo hemorrhagic stroke models. The enzyme isoforms of cytochrome P50 (CYP50) responsible for the metabolism of the compound have been identified, and studies confirm hepatotoxicity of the liver. [21,22]

Liver Microphysiological frameworks (MPSs) are promising models for foreseeing hepatic medicate impacts. However, after a decade since their presentation, MPSs are not routinely utilized in medicate improvement due to the need for criteria to guarantee reproducibility. We characterized the possibility of a liver MPS surrender reproducible results of tests assaying medicate poisonous quality, digestion system, and intracellular amassing. The capacity of the liver MPS to duplicate hepatotoxic impacts was evaluated utilizing trovafloxacin, which expanded lactate dehydrogenase (LDH) discharge and diminished cytochrome P450 3A4 (CYP3A4) movement. These perceptions were made in two test locales and with distinctive clumps of Kupffer cells. [19,21] Upon refined comparable hepatocytes within the MPS, spheroids, and sandwich societies, CYP3A4 action and egg whites' generation identified contrasts between culture designs. Cells in all culture groups showed distinctive sensitivities to hepatotoxicant introduction. [17,18]

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Hepatocytes within the MPS were more practically steady than those of other culture stages, as CYP3A4 movement and egg whites discharge remained noticeable for more prominent than 18 days in culture. However, practical decay happened in spheroids (12 days) and sandwich societies (7 days). The MPS was too illustrated to be reasonable for digestion system considers, where CYP3A4 movement, troglitazone metabolites, diclofenac clearance, and intracellular collection of chloroquine were evaluated. To guarantee reproducibility between considers with the MPS, the combined utilize of LDH, and CYP3A4 tests were actualized as quality control measurements. By and large, comes about demonstrated that the liver MPS can be utilized reproducibly in common medicate assessment applications. Ponder results-driven to common contemplations and proposals for utilizing liver MPS. [23,25]

2.2. Microphysiological systems (MPSs) are micro-engineered cell culture platforms

Microphysiological systems (MPS) are microengineering cell culture steps used to mirror the physiological activity of cells by reconstructing key features of the extracellular microenvironment in a particular tissue type. body. When examining the toxic qualities of drugs, digestive systems, Microphysiological systems (MPS) are microengineering cell culture steps used to mirror the physiological activity of cells by reconstructing critical features of the extracellular microenvironment in a particular tissue body type. When examining the toxic qualities of drugs, digestive systems, and transport, the liver is the central organ of thought and one of the prominent MPS modalities common for calm applications to develop. Liver MPS aims to preserve essential human hepatocytes or hepatocytes (PHH) in three-dimensional (3D) culture, in continuous liquid trade and co-culture with other cell types, such as human Kupffer basal cells (PHKC); conditions are known to promote the joint work and physiological relevance of these cells. As has been studied elsewhere about sedation evaluation criteria, hepatic MPS can make

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advances in estimating drug pharmacokinetics, visualizing drugs, Human harm prediction, and harm identification depending on a calm digestive system. Despite further advances in this area, information from MPS is not routinely used by the pharmaceutical industry during the administrative or silent evaluation phases. [29,30] Usually, these are limitations due primarily to the need for accessible performance and quality control criteria for frequent use of instruments and reproducibility of results between test destinations. Critical in deciphering trials with MPS in test sites for parameters used in drug advancement. Validation of this concept comes from experimental studies performed on prototyping MPS that maintain their potential usefulness in drug discovery and convince development partners to begin looking at them calmly. Performance criteria for randomization or prediction of the storage, dispersion, digestion, and excretion (ADME) system. This study addresses the need to measure the MPS performance of the liver for ordinary uses in drug pharmacology and drug toxicology, as applications in these areas depend on the metabolic work of the cell., strength and endurance of work, and their response to hepatotoxic compounds. [31]

A way to replicate performance must be sought before establishing specific usage parameters for MPS, where innovation can clearly illustrate points of concern compared with current methods and equipment. Of the system to ensure consistent quality satisfactory for the controlled assessment, especially when considering the application. in the later stages of drug improvement, MPS is recognized as having impressive potential to replace, reduce, or refine biological or clinical trials. [29]

Here, liver MPS was evaluated for reproducible results, considering quality control criteria to maintain consistency across multiple test sites and MPS clusters. Previous examinations using MPS of the liver have included a series of conclusive score measurements of cell migration and

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liver activity, several of which are also used here. When expanding the criteria needed to reduce batch or inter-batch inconsistencies in MPSs, the complexity of working MPSs can present further challenges in creating products that can be reproduced. When utilizing MPS, guaranteeing cell steadiness based on framework get together, groundworks, and more complex cell culture strategies can require a level of exploratory arranging and the ability to distant past what is necessary to preserve a two-dimensional (2D) culture. [21,22,23] Based on the detailed comes about and discourse, standard contemplations and suggestions are made for liver MPS to be utilized in a reproducible way and built up to create a sedate chance expectation framework with liver MPS. In expansion to later commitments, different master viewpoints encourage portray openings within the field of calm improvement where hepatic MPS might serve as a paradigm-shifter. As a result, generator. The natural product can recover and stabilize cell work. [28]

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Chapter 3

3. Liver MPS used in the current study

The liver MPS is based on microfluidic processes and has been used in several laboratories and many experimental projects for applications with modifiability in drug testing, recommending its functional strength and replicability. As an extension of the description made so far with this framework, liver MPS can also extend the capabilities of current cell culture methods, mainly used in pharmaceuticals. study and toxicology/safety applications. To investigate this plausibility, we tried the utility of the liver MPS for common sedate advancement applications related to identifying sedate harmfulness, digestion system, and intracellular collection, which are key pharmacological determinants for medicating improvement thinks about. Reproducibility of MPS comes about was tried considering already distributed work and the test results from two test destinations, from utilizing diverse cell bunches and from other cell culture stages (spheroids and sandwich cultures). [30,31]

The overall objective of this study was to examine the performance of hepatic MPS by predicting regenerative sedation-induced hepatotoxicity, analysis of the gastrointestinal drug system, and intracellular sedation combination. We first illustrate the regenerative ability of liver MPS to recognize hepatotoxins using trovafloxacin, recently comparing it with other liver culture designs for their response to other toxins and their uses. Therefore, hepatic MPS was evaluated for potential use for gastrointestinal laxatives, clearance, and transport schemes for the use of troglitazone, diclofenac, and chloroquine. Finally, the use of quality control criteria was investigated to ensure that reproducible information was generated from MPS reflections. The regenerative capacity of hepatic MPS has been characterized by its ability to predict hepatotoxicity, evaluate the gastrointestinal system, and drug combinations, and reflect the effects of drugs requiring action—

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elongation of cells. Cellular strategies for drug enhancement must be robust, reproducible, have well-defined quality control criteria, and depend on materials that can be easily obtained or manufactured. [22,25,28] We have followed this rule by using commercially available cells, devices, instruments, and supplies according to current thinking. One of the challenges facing large-scale use of MPS is the difficulty of procuring MPS for quiet cutting-edge research facilities from school groups, often with unique skills advantages in microfabrication and tool strategy, or semi-exclusive access cell types. As an extension of the industry's efforts, test centers have been established to overcome these translational obstacles. The beginning demonstrates the need for separate development partners to create information describing the main characteristics of MPS use. As reflected in the present, the tests performed in the PHH used the same set of cells to ensure an essential point about the unshakable quality of the MPS operation and to correct for group effects. Previous studies on the use of MPS in the liver have shown that cluster effects can affect performance, and future evaluations will help explore criteria for cellular viability. In our trials of trovafloxacin, calm responses were compared from two specific PHKC groups and different test sites to confirm the reproducibility of the information gathered. [17,19,23]

Based on previous work delivered using liver MPS and the prior performance of other cell culture stages used to reflect on liver work (v. On (ii) usefulness, and (iii) ability to generate parameters for future use in a controlled assessment Due to the complexity of MPS operation, anomalies between discovery replicas may arise due to several variables, several cells, utilities, and device handling. [14,17,19] Overall, our data allowed us to examine variability between MPS wells, MPS plates, distinct batches of qualified PHKCs, and between test sites. The standard error of the mean in LDH production or CYP3A4 activity obtained with experimental replicates was low enough to

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allow detection of differences between drug concentration groups and groups treated or untreated with LPS. Concentration-dependent effects induced by tamoxifen, digoxin, and troglitazone on CYP3A4 activity and LDH and albumin production were also clearly detected with qualified replicates from different MPS batches per condition. By performing quality control measures (LDH and CYP3A4) earlier, exception tests were effectively avoided from tests due to impeded cellular action. In expansion to this strategy, the quality of liver MPS get together was moreover surveyed. Duplicating already distributed comes about advance affirmed the plausibility to utilize the liver MPS with reproducibility. [29] The measured diclofenac clearance, stage I and stage II digestion system of troglitazone, the capacity to preserve the liver MPS for weeks, and the watched microtissue morphology inside platforms were all observed in a way steady with that of past reports. Despite potential contrasts between destinations or cell clusters, 100 μM trovafloxacin with LPS initiated a critical increment in LDH, and trovafloxacin and levofloxacin have already been utilized to test liver frameworks. Despite the merging incomes between test conditions, the size of medicating impact was variable between tests. Inconsistencies in LDH and CYP3A4 movement levels between test sites outline the ought to standardize operations and methods concerning MPSs. [31,32] In rundown, we come about demonstrated that experimentation with the liver MPS is likely to produce reproducible comes about when upheld by quality control measurements based on LDH and CYP3A4 action.

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Chapter 4

4. Materials and Methods

Microfluidic chip fabrication

The microfluidic chip was built with two top and bottom glass chips (soda-lime glass, 56 mm long, 41 mm wide, and 1.1 mm thick). A multi-head 3D printer was utilized to print the microfluidic channel on the best glass with PDMS (Sylgard 184, Dow Corning, USA). The glass chips were degassed thoroughly sometime recently stacking into the printer arrange, the creation tallness and width of the channels were set to be 300 µm and 800 µm, individually. A customized, attractive chip holder was utilized to amass the beat and foot glasses of the microfluidic chip. Moreover, the silicon gaskets were set within the attractive chip holder to maintain a strategic distance from leakage.

4.1. Cell seeding and liver fibrosis on microfluidic chip

A human derived immortal HepG2 hepatoma cell line (Korea Cell Line Bank, South Korea) and human foreskin fibroblasts cell line Hs68 (Korea Cell Line Bank, South Korea) were utilized to create a co-culture model of hepatocytes and fibroblast. Both cell lines were grown in Roswell Park Memorial Institute (RPMI) 1640 cell culture media (cat# 11875093, ThermoFisher, USA) supplemented with 10% fetal bovine serum (FBS) (cat# 16000044, ThermoFisher, USA) and 1% v/v penicillin/ streptomycin (P/S) antibiotic solution for cell culture (cat# 15070063, ThermoFisher, USA). The HepG2 and Hs68 cells were kept in a humidified incubator (with 5% CO2 at 37 °C).

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All glass chips were sterilized with 90% isopropyl liquor and UV illuminated for 1 hour in a biosafety cabinet sometime recently utilized. An attractive ECM & cell seeding unit was used to apply the ECM and seeding cells on microfluidic glass chips' cell culture range. ECM arrangements of collagen, poly-l-lysine and fibronectin were utilized sometime recently, cell seeding for cell connection to the microfluidic chip surface. The cells were extended and passaged thrice sometime recently, seeding on the microfluidic chip at the physiological proportion of 1:8. Then, cells were permitted to join the microfluidic chip for 4 hours in a standard cell culture incubator at 37 °C with 5% CO2. After that, the glass chip best and foot parts were collected in an attractive chip holder.

Sensors manufacture and characterization TEER sensor were manufactured on both glass chips by printing a 500 nm ITO design utilizing the CVD method. The sensor was characterized as portrayed already [34]. While the ROS sensor design was printed on the best glass chip downstream using an in-house multi-head 3D printer. A schematic of the method appears in Fig. 1a. The manufacture handle included, begin with cleaning the substrate utilizing ethanol, acetone, and DI water. After drying the chip surface, oxygen plasma was treated for 20 s to clean the substrate. The print speed was set at 1 ms−1 to manufacture gold and silver terminals. Gold (Au) ink [cat# Au-LT-20 (20 wt.%) Fraunhofer, Germany] was printed, to begin with, dried at 40 °C for 10 min and sintered at 190 °C for 12 h, taken after by printing the silver (Ag) ink [cat# bit (TEC-PA-060) Dissolvable (DA-030) INKtec, Republic of KOREA] and sintering at 130 °C for 20 min.

The sensors were characterized employing a commercial PalmSens4 convenient framework (PalmSens, Netherlands) for cyclic voltammetry (CV) with standard arrangements of 10 mM potassium ferricyanide (K4 [Fe (CN)6]) and 0.1 M KCl. Be that as it may, a custom-developed

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framework was utilized for the sensor's chronoamperometric reaction to plot its calibration bend. The sensor was at that point washed with Phosphate buffered saline (PBS) and twofold refined water to utilize within the tests.

4.2. ECM evaluation in the liver-on-chip device

Three diverse ECM have been utilized for examining the impact of ECM on the cell connection to the microfluidic glass chip surface. Rodent Tail Collagen (the sort I) (cat#C3867-1 VL, Sigma-Aldrich, South Korea) was utilized at concentrations of 200 µg/mL in PBS (cat# 10010023, ThermoFisher Logical, USA). Poly-l-lysine 1 mg/mL (cat# 0403, ScienCell, USA) was weakened in sterile twofold refined water to induce 5 µg/mL concentrations. Agreeing to the manufacturer's instruction, fibronectin 1 mg/mL (cat# 33010018, ThermoFisher, USA) was resuspended in 1 mL of sterile twofold refined water. Fibronectin was further mixed with the PBS to urge the concentrations of 25 µg/ mL. The 400 µL of each ECM arrangement was connected to the microfluidic chips' cell culture zone utilizing the ECM & cell seeding pack. The chips were at that point brooded overnight at 4 °C in a sterile environment.

4.3. ROS, SOD, AST, and ALT enzyme measurements

Albumin, urea, and CYP450 chemical measures were performed as valuable biomarkers of the hepatocytes. AST ELISA Pack (cat# ab108787, Abcam, USA), Urea Measure Unit (cat# KA1652, Abnova, USA), and P450-Glo CYP3A4 Test Pack (cat# V9001, Promega, USA) were utilized for AST, ALT, ROS and SOD measurement, individually. In brief, cell culture media tests were collected at an indicated time focuses and put away at −80 °C. Media tests were defrosted at 37 °C in a water shower some time recently tests. A microplate reader (SpectraMax i3 Multimode

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Microplate reader, Atomic Gadgets, USA) was utilized for taking readings by taking after the manufacturer's information.

4.4. Fabrication of microfluidic device

The glass-based microfluidic chip has comprised of two glass slides (soda-lime-silica glass, 1.1 mm thick, 56 mm long, and 41mm wide), which were stacked on each other. A biocompatible microfluidic channel isolated the two glass slides to make a single chip. The biocompatible microfluidic channel was 3D printed by employing a multi-head 3D printer, and polydimethylsiloxane (PDMS) (Dow SYLGARD®, Dow Corning, USA) was utilized as a substrate. Legitimate degassing was performed sometime recently stacking glass chips into the 3D printer arrange (channel tallness 300 µm, width 800 µm). A custom-built attractive chip holder bolstered the microfluidic glass chip gathering. Silicon gaskets were utilized within the magnetic chip holder to dodge liquid spillage.

4.5. Cell culture and seeding on the microfluidic chip

An undying human origin HepG2 hepatocellular carcinoma cell line was acquired from Korea Cell Line Bank (Seoul, Republic of Korea). HepG2 cells were refined in elevated glucose Dulbecco's adjusted hawk cell culture medium (DMEM) (catalog # 11995040. ThermoFisher Logical, USA) supplemented with 5% fetal bovine serum (FBS) v/v (catalog # 16000044, ThermoFisher Logical, USA) and 1% v/v penicillin & streptomycin (P&S) anti-microbial arrangement for cell culture (catalog # 15070063, ThermoFisher Logical, USA. The cellular culture was kept up in a cell culture incubator (humidified at 37 °C, 5% CO2). The extension of HepG2 cells was carried out by passing three times some time recently seeding on the microfluidic chip. Dulbecco's phosphate-

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buffered saline (DPBS) (catalog # 14190144, ThermoFisher Logical, USA) was warmed in a water shower at 37 °C for washing the cells washing out the metabolic toxins, debris, and waste.

The cell culture was trypsinized when it accomplished 90% confluency, with 0.50% trypsin EDTA arrangement (catalog # 25300054, ThermoFisher Logical, USA). The microfluidic glass chips were cleaned with 90% isopropyl liquor and washed thrice with twofold refined water in a biosafety cabinet. After that, chips were air-dried and UV lighted for 60 minutes for accomplishing sterilization. A customized, attractive extracellular framework (ECM) & cell seeding unit was utilized to apply ECM and for cell seeding on the cell culture zone of the microfluidic glass chip. The fibronectin (ThermoFisher Logical, USA) arrangement was arranged in double-distilled water at the concentration of 25 µg/ml for the hepatocellular carcinoma cell connection to the surface of the microfluidic glass chip. Cells were seeded at the concentration of 400000 cells/ml in DMEM through the attractive cell seeding pack and were permitted to join the ECM surface for 6 hours in a cell culture incubator (humidified at 37 °C, 5% CO2). After that, the cell culture media was expelled, and the microfluidic chip's beat and foot glass slides were amassed within the custom-built magnetic chip holder. The amassed microfluidic chip was situated in a microfluidic stage to create a cancer tissue monolayer in an energetic cell culture microenvironment. The microfluidic chip was associated with the cell culture media supply (5 ml) through the microfluidic tubing.

A microfluidic peristaltic pump was set up at 60 µl per diminutive speed to make the shear stress of 0.5 dyn/cm2 within the microfluidic cell culture channel. The shear push was calculated by the condition given underneath $\tau = 6\mu Q/wh^2$ Though "µ" speaks to the thickness of the cell culture media, "Q" means the stream rate of the cell culture media, "w" shows the width of the channel whereas "h" stands for the stature of the microfluidic channel. 2.3 Collection and planning of plant

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extricates Acer cappadocicum test (branches and green clear out) was collected from the north Himalaya (Pallas Valley, Kohistan, Pakistan. After the plant confirmation, extricates were performed through mechanical maceration. The dry clear out and the plant branches were ground in a process (work number 60) and splashed within the dissolvable (20g/200ml). The arrangement was shaken for 72 h in a robotized shaker. Whatman Number 1 channel was utilized to channel the account. The collection was put beneath a shade at room temperature to vanish the dissolvable and extract the rough extricates. The rough disengage at that point was suspended in H2O and apportioned with methanol.

The working concentration (100ng/ml, 10ng/ml, 1ng/ml) of the unrefined extricates were arranged within the dimethyl sulfoxide (DMSO) and stored at 4 °C within the dim. 2.4 Live/Dead measure and ROS estimation measure the cells were washed thrice with DPBS arrangement. A live/dead measure unit (catalog # 15070063, ThermoFisher Logical, USA) was utilized to recolor the cells after the manufacturer's enlightening. Cellular Receptive Oxygen (ROS) Test Unit (catalog # ab113851, Abcam, USA) recolored the cells for 45 minutes. After the recoloring strategies, the cell culture range of the microfluidic chip was washed with DPBS, and a mounting media (Fluoromount Fluid Mounting Media. Sigma-Aldrich, USA) was utilized to put the coverslip on the tissue. At the same time, CellTiter 96® Watery One Arrangement Cell Multiplication Test (MTS) Unit (catalog # G3581, Promega, USA) was utilized for the MTS measure. The confocal imaging reader (Cytation C10, BioTek, Vermont, USA) was being used at the excitation wavelength (530-560) and outflow wavelength (530-645) for getting confocal micrographs. The fluorescent pictures were prepared to utilize the ImageJ computer program (Form 1.52 p, NIH, USA). 2.5 Biomarker Investigation ALT, AST, Urea, albumin, and Turf measures were performed

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to appraise the effect of plant extricates on the liver tumor MPS. Alanine Transaminase Movement Test Unit (catalog # ab105134, Abcam, USA), Aspartate Aminotransferase Action Measure Pack (catalog # ab105135, Abcam, USA), Urea Test Pack (catalog # ab83362, Abcam, USA), Human Egg whites ELISA Unit (catalog # ab179887, Abcam, USA) and Superoxide Dismutase Movement Measure Unit (catalog # ab65354, Abcam, USA) units were utilized for ALT, AST, Urea, Egg whites, and Grass measurement, separately. Briefly, cell culture media tests were taken at time interims and quickly put away at -80 °C. In differentiate, a Turf test was performed on the cell lysate arranged by the strategy already depicted. Cell culture media tests were defrosted in a water shower at 37 °C sometime recently the biomarker estimation method.

A semi-automated microplate reader (SpectraMax iD3, Atomic Gadgets, USA) was utilized to require the readings per the manufacturer's enlightening. 3. Comes about and talk 3.1 Real-time observing liver tumor MPS The liver tumor MPS was established, as appeared within the figure. The real-time observing of the MPS was performed for six days employing a chip inserted TEER sensor. In vivo, physiochemical conditions help cells to engender and keep up their atomic prompts. The characterizes cell morphology, and separation happens due to the fluidic shear push shown within the living bodies.[20] Whereas ordinary cell culture models don't offer unconventional share push or controlled microenvironment required by cells for solid engendering. Physio-mechanical components of MPS control the microenvironment by applying shear stress.[21] It has already appeared that the shear push of 0.5 dyn/cm2 yields a prevalent monolayer in an MPS compared to the stationary cell culture systems.[9] The tumor MPS observed TEER values with six-hour interims, as displayed in figure 4 (a). A noteworthy increment within the TEER values was watched within the control tests, which portrays the cells proliferation, separation, cell to cell tight intersection arrangement, and inevitably a tumor monolayer

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arrangement. The display thinks about uncovers the TEER extend of 343-392 Ω/mm2 for a compact tumor monolayer structure.

The comes about to take after a past think about where the TEER run of 345-395 Ω/mm2 was the esteem for monolayer formation.[9] It has been demonstrated that a continuous supply of FBS and cellular separation comes about in a reliable increment of TEER, and the same marvel was watched within the current study.[7] DMSO is broadly utilized in biomedical sciences and is considered one of the most excellent widespread solvents. The prepared disintegration of plant-based extricates made it a perfect transaction medium between the cells and the chemical molecules.[22, 23] Jang et al. claimed that DMSO confers no impact on the cell-to-cell tight intersection protein expression and physiology.[24] Be that as it may, it was found that DMSO contrarily influenced the TEER values with a liver tumor MPS, and the TEER esteem dropped to 397 Ω/mm2 on the six days, whereas the control tumor MPS displayed the TEER esteem of 404 Ω/mm2 for the same period. The biomarker discharge and cell practicality information of tumor MPS encourage the contention that indeed the minor amounts of DMSO essentially affect the liver tumor MPS. Three concentrations (100 ng/ml, 10 ng/ml, and 1 ng/ml) of the methanolic extricates of the A. cappadocicum were connected to the tumor MPS on the 3rd day of the trial, and their impact on the liver tumor MPS was watched for three days. The most elevated concentration of the extricates diminished the TEER esteem up to 327 Ω/mm2, implying the cell-to-cell tight intersection disturbance. Essentially, other engagements of the plant extricate too brought about within the drop of TEER; detailed the presence of quinones within the A. cappadocicum, which are known to disturb the obstruction keenness in cancer epithelial cells, an interferometer with the cellular transcriptional control utilizing the histone deacetylase (HDAC).

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Besides, quinones are moreover known to boost responsive oxygen species (ROS), a demonstrated source of tight intersection protein debasement. ROS discharge was straightforwardly corresponding to cell closed intersection harm and lower than traditional TEER values in a past study. Impact of A. cappadocicum of liver work tests such as albumin, urea, ALT, and AST are the set of biomarkers speaking to the general pathophysiological state of the liver-specific cells. Hepatocellular carcinoma cells ceaselessly deliver liver-specific proteins and other biomarkers, which can be utilized to screen the impact of an anticancer agent. MPS is known to impact the abdicate of by HepG2 cells incredibly. Comparing the ordinary cell culture demonstrated (96-well cell culture plate), liver tumor MPS appeared an impressive contrast in albumin discharge between the two cell culture models.

The albumin generation by liver tumor MPS was onefold expanded than the ordinary cell culture demonstrates. A similar reaction was observed in urea discharge by the liver tumor MPS appeared in the cells. On the other hand, the application of A. cappadocicum extricates essentially decreased the egg whites and urea discharge from the hepatocellular carcinoma cells. A triple diminish was famous in albumin generation with the 100 ng/ml plant extricate, whereas the same extricate concentration decreased the urea discharge by twofold. A related phenomenon was watched within the urea and albumin discharge by the ordinary cell culture demonstrate. Interests, there were impressive contrasts between the tissue tumor MPS' biomarker surrender and the conventional cell culture reveal. The most reduced concentration of plant extricates (1 ng/ml) appeared a less remarkable impact on the area and albumin generation than the ordinary cell culture models, which estimate the compromised anticancer movement comes about by the conventional cell culture models. ALT and AST are intracellular hepatic proteins, and their extracellular nearness bodes the

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fundamental harm to the hepatic cells. Anticancer extricates harm the hepatic intracellular cells, and the intracellular hepatic proteins cells.

Subsequently, ALT & AST estimations were performed for liver tumor hepatic intracellular that appeared to be less reliable ALT & AST discharge than DMSO. The release of higher concentrations of ALT and AST are related to consequent harm to the hepatocellular carcinoma cells.[32] The plant extricates of A. cappadocicum expanded ALT & AST within the liver tumor MPS, speaking to the plant extracts' viability against the hepatocellular carcinoma cells. Comparative investigation of cell reasonability: Cellular well-being comprises omnipresent instruments taking part in a few pathophysiological operations, counting cancers. In this manner, assessing cell practicality is crucial to evaluate cells' physical astuteness and evaluate the effect of different ambushes such as poisons, mechanical variables, and chemical compounds. A few biochemical and optical tests have been outlined and utilized to gauge cell practicality. Be that as it may, a significant contrast rests in them comes about owing to a few uncontrolled variables such as color infiltration, non-standardized conventions, chances of individual blunder, etc. Consequently, two specific cell practicality appraisal measures have been performed in this ponder. In expansion, impedimetric relative cell record was measured by utilizing the TEER values as portrayed already. The contrast in cell practicality displayed by three distinctive cell practicality appraisal strategies highlights the significance of a standardized cell practicality strategy. In any case, TEER-based impedimetric relative cell record speaks to predominance over MTS and live/dead test as both appear as it were supreme cell reasonability.

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Impacts of A. cappadocicum on liver tumor MPS Microfluidics offers sheer stretch for the ideal development of the cells inside a microenvironment by applying mechanical strengths against the apical cell film. Fluidic automatic push essentially makes strides in cell physiology, separation, cytokine generation, and reactions to pharmaceutical operators such as drugs and plant extricates. A comparative wonder was watched within the display think about. Liver tumor MPS biomarker abdicate found to be at slightest onefold expanded compared to the customary 96-well cell culture plate show. Hepatocellular carcinoma cells appeared way better cellular reasonability with a liver tumor MPS than the conventional cell culture demonstrated. The extricates of A. cappadocicum were connected to cell culture models, such as liver tumor MPS and routine cell culture. Be that as it may, the reactions of the hepatocellular carcinoma cells were found to be more unmistakable in liver tumor MPS. The traditional cell culture demonstrates appeared less anticancer action of the plant extricates than the liver tumor MPS. ROS are fundamental for cellular signaling, but they must be balanced to preserve the characteristic cellular work in a tumor specialty. ROS discharge and antioxidant capacity of plant extricates are associated determinants of their anticancer exercises. The picture investigation comes about of ROS estimation propose more ROS found inside the liver tumor MPS than the traditional cell culture demonstrates. Turf is the cell's guardian for directing ROS discharge, and their expression may be a significant component for cell survival by inhibiting tumor development and metastasis. Grass expression within the conventional cell culture show was found more than the liver tumor MPS, which clues for higher ROS discharge within the liver tumor MPS.

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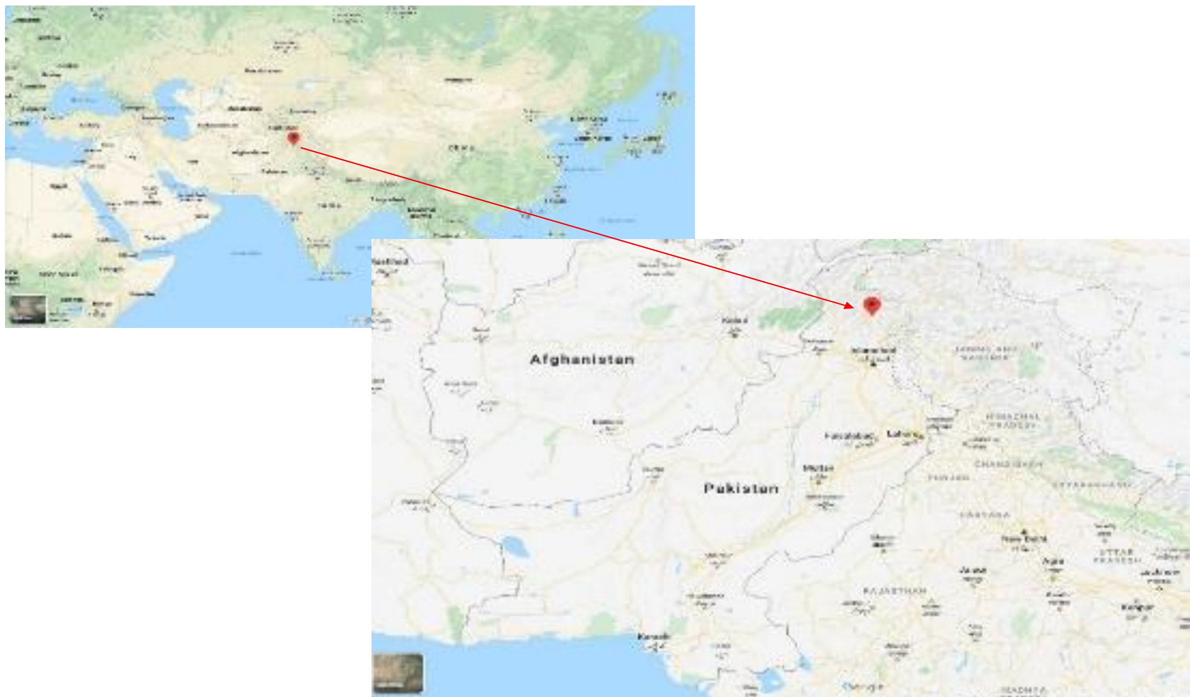
Plant description:

Plant Scientific Name	Local Name	Part Used	Description
Acer Cappadocicum	Chin	Leaves & Branches, Whole Plant	Tree up to 20m tall. Dark Brown, smooth bark

Area of Collection

Coordinates: 36.6 0N 73.00 0E Distance from Capital 315km 8hour Drive

Kohistan Valley, Chitral, Khyber Pakhtwhakhwa, Pakistan.



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Acer Cappadocicum (Chin) Plant

Tools & Field Notes:

- Scissors, Clippers, Cutter/Knives.
- Zipper bags.
- Silica gel and newspaper.
- Temperature & humidity (depends on collector choice).
- Date and time of collection.
- Altitude and latitude of the location.

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After Collection Procedure (Drying & Grinding):

- Drying: Carefully dry the plant material under shade.
- Place the material in newspaper under the weight for herbarium specimen preparation (branch with leaves with flower if the season is flowering).
- Carefully check the material so that no fungus infection occurs.
- Grinding: Grind the material from mill mesh size 1.
- Caution: during grinding, clear the mesh for subsequent grinding
- so that plant material does not mix into other plant powder.
- Carefully label the plant material during collection and after drying and milling.
- Place in airtight /zipper package.
- Permission form to transfer material (from university).

Drying of Plants:

Acer Cappadocicum (Chin) Plant

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Grinding Process (Plant Griding & Material Drying Process)

Grinding Machine Inner Blade

Drying Process Equipment

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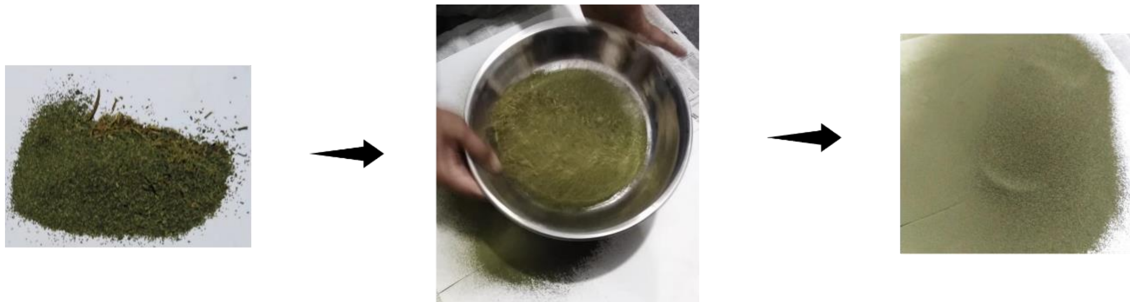
Filtration & Weighing

Refining of Plant Material:



Weighing of Plant extracts

Quantity of Plant Extracts (Chin 960 gram refined 350-gram raw powder:

Sealing & Packaging:

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Plants Coding & Solvents:

Part Used Acer Cappadocicum	Solvent	Abbreviation
Whole plant	Methanol	*ACM*
Whole plant	n-Hexane	*ACH*
Whole plant	Chloroform	*ACC*
Whole plant	Ethyl Acetate	*ACE*
Whole plant	n-Butanol	*ACB*

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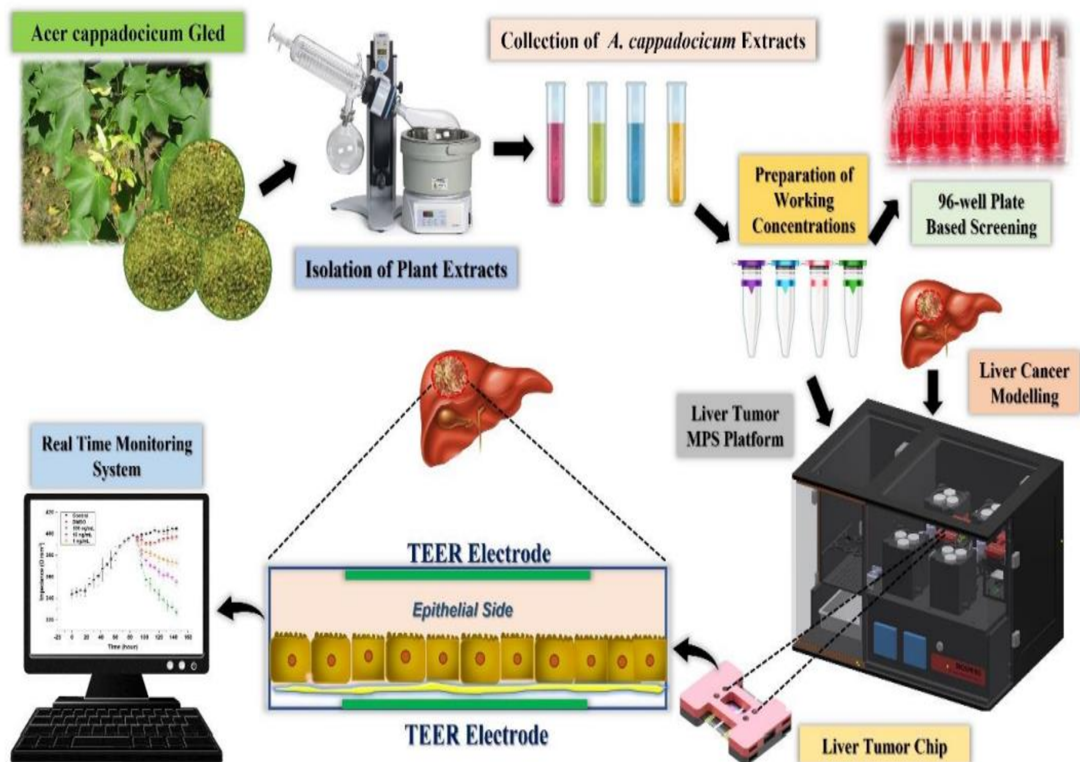
Chapter 6

Results

The plant extract preparation method and liver tumor MPS platform:



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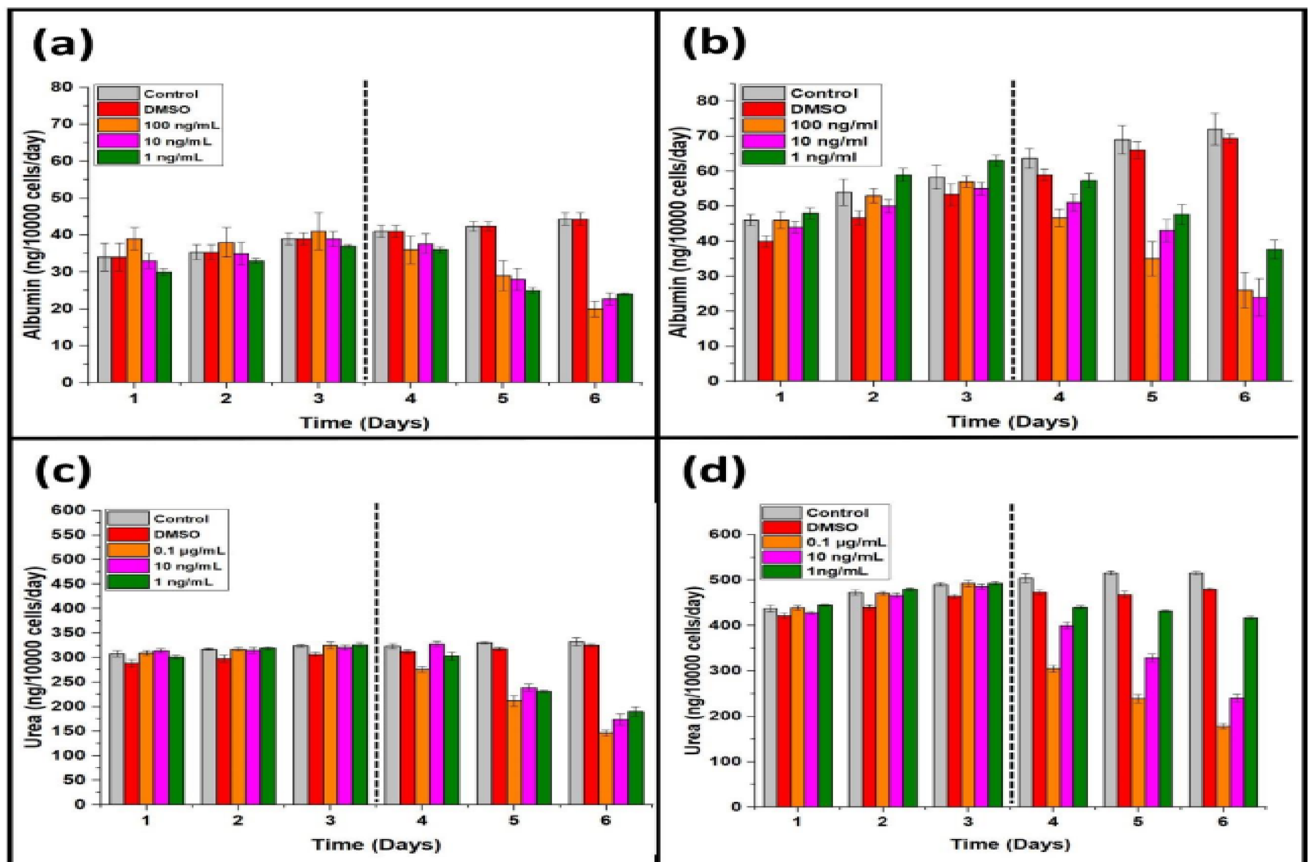
Comparison of the liver tumor MPS with the traditional 96-well plate cell culture model: The cell culture models were treated with plant extracts after 3 days of stable culture. (a) Illustration of albumin production in 96-well plate cell culture model before and after the treatment with plant extracts. (Dotted line is divining the pre-treatment and post-treatment biomarker yield) (b) Illustration of albumin production in liver tumor MPS model before and after the treatment with plant extracts. (Dotted line is divining the pre-treatment and post-treatment biomarker yield) (c) Illustration of urea release in 96-well plate cell culture model before and after the treatment with plant extracts. (Dotted line is divining the pre-treatment and post-treatment biomarker yield) (d) Illustration of urea release in liver tumor MPS model before and after the treatment with plant extracts. (Dotted line is divining the pre-treatment and post-treatment biomarker yield.



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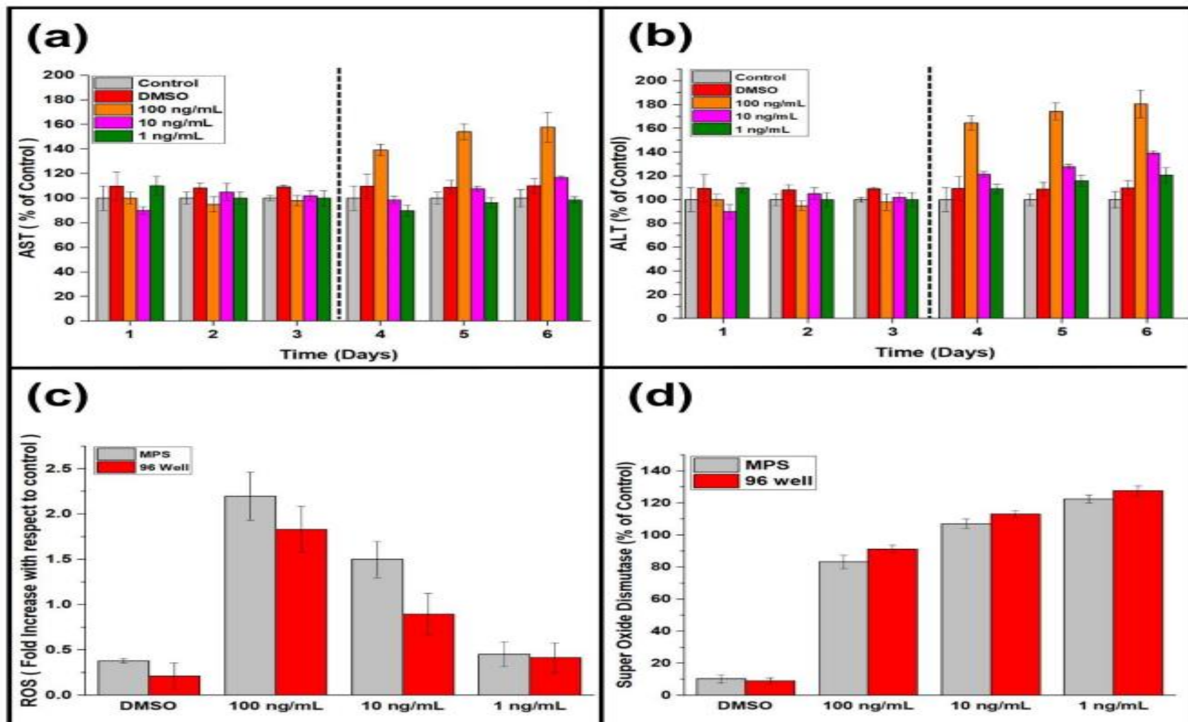
Evaluating the anticancer effect of plant extracts through cancer cell damage indicators (ALT, AST, ROS, and SOD).

(a) AST release after treatment with DMSO and plant extracts (100ng ml-1, 10ng ml-1, 1ng ml-1). (Dotted line is divining the pre-treatment and post-treatment biomarker yield) (b) AST release after treatment with DMSO and plant extracts (100ng ml-1, 10ng ml-1, 1ng ml-1). (Dotted line is divining the pre-treatment and post-treatment biomarker yield) (c) Reactive oxygen species (ROS) production in liver tumor MPS and traditional 96-well cell culture model with DMSO and different concentrations of plant extracts (100ng ml-1, 10ng ml-1, 1ng ml-1) at the end of the experiments or after 6 days. Both cell culture models were treated with the DMSO and extracts for 72 h after 3 days of stable cell culture. The columns exhibit the ratios of ROS produced by the treatment (plant extracts) and DMSO. (d) The bar graph represents the activity of superoxide dismutase (SOD) in the traditional 96-well cell culture model and liver tumor MPS after treatment with DMSO and different.



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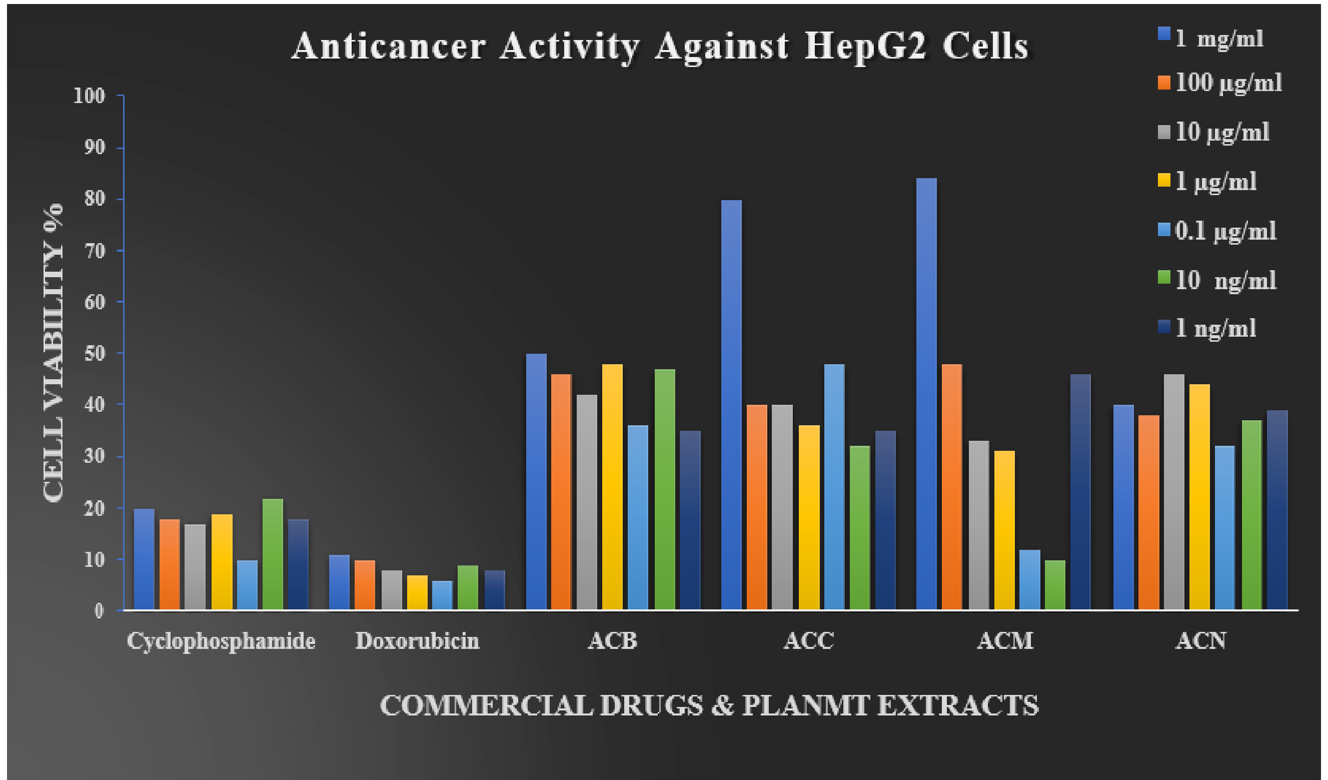
Anticancer & Cytotoxicity Activities of Plant Acer Cappadocicum

Liver Cancer Cell Line:



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Discussion:

Real-time observing of liver tumor MPS The liver tumor MPS was set up, as appeared within the figure, and the real-time checking of the MPS was performed for six days employing a chip inserted TEER sensor. In vivo, physiochemical conditions help cells to proliferate and keep up their atomic prompts. The characterizes cell morphology, and separation occurs due to the fluidic shear stress of living bodies.[20] Whereas routine cell culture models don't offer unconventional share push or controlled microenvironment required by cells for their solid engendering. Physio-mechanical components of MPS direct the microenvironment by applying shear push. It has already appeared that the shear push of 0.5 dyn/cm2 yields a predominant monolayer in an MPS compared to the stationary cell culture systems. The tumor MPS checked TEER values with six-hour interims. A noteworthy increment within the TEER values was watched within the control tests, which delineates the cells engendering, separation, cell to cell tight intersection arrangement, and inevitably a tumor monolayer arrangement.

The display ponders the TEER extend of 343-392 Ω/mm2 for the arrangement of a compact tumor monolayer. The comes about to take after a past consider where the TEER extend of 345-395 Ω/mm2 was the esteem for monolayer formation. It has been demonstrated that a nonstop supply of FBS and cellular separation comes about in a reliable increment of TEER, and the same wonder was watched within the current study. DMSO is broadly utilized in biomedical sciences and is considered one of the leading widespread solvents. The prepared disintegration of plant-based extricates made it a perfect exchange medium between the cells and the chemical particles. However, Jang et al. claimed that DMSO confers no impact on the cell-to-cell tight intersection of protein expression and physiology. Be that as it may, it was found that DMSO contrarily influenced

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the TEER values with a liver tumor MPS, and the TEER esteem dropped to 397 Ω/mm2 on the six days, whereas the control tumor MPS displayed the TEER esteem of 404 Ω/mm2 for the same period. The biomarker discharge and cell practicality information of tumor MPS reinforce the contention that indeed the minor amounts of DMSO altogether affect the liver tumor MPS. Three concentrations (100 ng/ml, 10 ng/ml, and 1 ng/ml) of the methanolic extricates of the A. cappadocicum were connected to the tumor MPS on the 3rd day of the test, and their impact on the liver tumor MPS was watched for three days.

The most elevated concentration of the extricates essentially diminished the TEER esteem up to 327 Ω/mm2, which means the cell-to-cell tight intersection disturbance. Essentially, other concentrations of the plant extricate too brought about within the drop of TEER. Farzana et el. detailed the presence of quinones within the A. cappadocicum, which are known to disturb the obstruction judgment in cancer epithelial cells, an interferometer with the cellular transcriptional direction utilizing the histone deacetylase (HDAC). Besides, quinones are moreover known to boost receptive oxygen species (ROS), which are a demonstrated source of tight intersection protein corruption. ROS discharge corresponded to cell closed intersection harm and lower than average TEER values in the past think. [32,33]. Effect of A. cappadocicum of liver work tests. Liver work tests such as egg whites, urea, ALT, and AST are the set of biomarkers speaking to the by and sizeable pathophysiological state of the liver-specific cells. Hepatocellular carcinoma cells ceaselessly create liver-specific proteins and other biomarkers, which can be utilized to screen the impact of an anticancer operator. MPS is known to impact the abdicate of albumin by HepG2 cells significantly. Comparing the routine cell culture show (96-well cell culture plate), liver tumor MPS appeared an impressive distinction in egg whites discharge between the two cell culture models.[34] The albumin generation by liver tumor MPS was onefold expanded than the ordinary

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cell culture demonstration. A comparative reaction was watched within the case of urea discharge by the liver tumor MPS. On the other hand, the application of A. cappadocicum extricates decreased the albumins and urea discharge from the hepatocellular carcinoma cells. A triple diminish was famous in albumins generation with the 100 ng/ml plant extricate, whereas the same extricate concentration diminished the urea discharge by twofold. [35]

A related wonder was watched within the urea and albumin discharge by the ordinary cell culture demonstrate. Interests, impressive contrasts were found between the biomarker abdicate by the liver tumor MPS and the traditional cell culture demonstrated. [36] The most reduced concentration of plant extricates (1 ng/ml) appeared a less remarkable impact on the urea and albumin generation than the routine cell culture models, which figure the compromised anticancer movement comes about by the conventional cell culture models. ALT and AST are hepatic intracellular proteins, and their extracellular nearness bodes the basic harm to the hepatic cells. Anticancer extricates harm the hepatocellular carcinoma cells, and the hepatic intracellular proteins spill from the cancer cells' harmed cell membrane. Therefore, ALT & AST estimations were performed for liver tumor MPS. [36,37] The control test appeared less and reliable ALT & AST discharge in comparison with DMSO. The discharge of higher concentrations of ALT and AST are related to ensuing harm to the hepatocellular carcinoma cells.[32] The plant extricates of A. cappadocicum expanded ALT & AST within the liver tumor MPS, speaking to the plant extracts' viability against the hepatocellular carcinoma cells. [33,32]

Cellular well-being comprises omnipresent components taking part in a few pathophysiological operations, counting cancers. Hence, evaluating cell practicality is imperative to survey cells' physical judgment and assess the effect of different ambushes such as poisons, mechanical components, and chemical compounds. A few biochemical and optical tests have been outlined

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and utilized to gauge cell practicality. In any case, an impressive contrast rests in them comes about owing to a few uncontrolled variables such as color infiltration, non-standardized conventions, chances of individual blunder, etc. Subsequently, two diverse cell practicality evaluation tests have been performed in this ponder. In expansion, the impedimetric relative cell file was measured by utilizing the TEER values as portrayed already. [35] The distinction in cell reasonability displayed by three diverse cell practicality evaluation strategies highlights the significance of a standardized cell reasonability strategy. Be that as it may, TEER-based impedimetric relative cell file speaks to predominance over MTS and live/dead test as both appear as it were supreme cell viability. Effects of A. cappadocicum on liver tumor MPS Microfluidics offers shear stress for the ideal development of the cells inside a microenvironment by applying mechanical strengths against the apical cell film. Fluidic mechanical push essentially moves cell physiology, separation, cytokine generation, and reactions to pharmaceutical specialists such as drugs and plant extricates. [36]

A comparable marvel was watched within the display think about. Liver tumor MPS biomarker abdicate found to be at slightest onefold expanded compared to the routine 96-well cell culture plate show. Hepatocellular carcinoma cells appeared superior cellular reasonability with a liver tumor MPS than the conventional cell culture demonstrated.[37] The extricates of A. cappadocicum were connected to both cell culture models, such as liver tumor MPS and ordinary cell culture show. Be that as it may, the reactions of the hepatocellular carcinoma cells were found to be more noticeable in liver tumor MPS. [38] The conventional cell culture show appeared less anticancer action of the plant extricates than the liver tumor MPS. ROS are fundamental for cellular flagging, but they must be in harmony to preserve the characteristic cellular work in a tumor specialty.[39] ROS discharge and antioxidant capacity of plant extricates are associated

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determinants of their anticancer exercises. The picture investigation comes about of ROS estimation recommends more ROS found inside the liver tumor MPS than the ordinary cell culture show. [40] Turf is the cell's watchman for directing ROS discharge, and their expression could be a significant component for cell survival by inhibiting tumor development and metastasis. Turf expression within the conventional cell culture show was found more than the liver tumor MPS, which clues for higher ROS discharge within the liver tumor MPS. [41]

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Conclusion:

In conclusion, this thinks about displayed a modern strategy of anticancer movement appraisal of A. cappadocicum plant extricates or subordinates. The plant extricates appeared superior anticancer movement in liver tumor MPS and, thus can be explored assist for clean compound separation and anticancer medicate advancement. Microfluidic-based liver tumor MPS are predominant in the conventional cell culture models for evaluating the reaction of hepatocellular carcinoma cells against the plant extracts. The cell reasonability and biomarkers discharge essentially modifies with the ordinary cell culture models and can lead to false-negative come about or the lower anticancer viability of the candidate compound. Real-time observing could be a non-invasive and cheap strategy to gauge plant extricates viability compared to the conventional bioassays.

Consequently, our liver tumor MPS and real-time checking framework can be utilized for considering anticancer action evaluation of plant-based extricates and compounds. In this proof-of-concept ponder, the organ-on-a-chip method gives a tall degree of congruity with conventional verbal organization thinks in people almost the most excreted metabolites of stanozolol and DHCMT. In any case, to show the complex digestion system of anabolic-androgenic operators and other disallowed compounds more totally from distinctive classes (e.g., specific androgen receptor modulators [SARMS], hypoxia-inducible calculate [HIF] actuating operators and metabolic modulators), the complexity of the demonstrate needs to be advance expanded.

Subsequently, the current confinement of the utilized organ-chip approach is the failure to reproduce the complexity of living life forms. In any case, multi organ chips, shaped by a combination of distinctive organoids on one chip, mirror higher arrange structural physiology

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more exact than any past in vitro show. For case, these chips would be able to recreate verbal or topical applications of drugs by combining liver cells with intestinal cells or skin tissues individually. Indeed, the investigation of a counterfeit urinary network appears conceivable employing a combined liver and kidney demonstration. Another confinement concerning conventional in vivo organization ponders the complex and challenging planning of the cell cultures and the spheroid formation in a sterile environment. In common, with its fundamental thought to imitate the Microphysiological unit of an organ-on-a-chip, the stage has extraordinary potential to end up an effective elective to drug testing in clinical trials and a promising system for metabolic profiling of denied compounds or recently rising to perform improving substances in drug testing.

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