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Review Article

A Comprehensive Review On: Exploring The Cytotoxic Activity And Metal Oxide Binding Of Innovative Herbal Drugs

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ABSTRACT

The intersection of traditional herbal medicine and modern pharmacology continues to yield intriguing insights into novel therapeutic avenues. In this review, we delve into recent advancements in understanding the cytotoxic activity and metal oxide binding properties of innovative herbal drugs, focusing on their potential applications in cancer therapy and beyond. Firstly, we examine the cytotoxic potential of these herbal drugs against various cancer cell lines, highlighting their selectivity and potency in inducing cell death while sparing healthy cells. Through a comprehensive analysis of preclinical studies, we elucidate the mechanisms underlying their cytotoxic effects, including apoptosis induction, cell cycle arrest, and modulation of signalling pathways crucial for cancer cell survival. Additionally, we explore the intriguing phenomenon of metal oxide binding exhibited by certain herbal drugs and its implications for therapeutic intervention. By interacting with metal oxide species implicated in disease pathogenesis, these herbal drugs hold promise for mitigating oxidative stress, inhibiting protein aggregation, and modulating metal ion homeostasis in neurodegenerative disorders and other conditions. Furthermore, we discuss the synergistic potential between the cytotoxic activity and metal oxide binding properties of herbal drugs, suggesting avenues for future research and therapeutic development. Leveraging this dual functionality may lead to the design of more effective and safer treatments for cancer and other diseases, with reduced adverse effects compared to conventional therapies.

INTRODUCTION

Historically, herbal medicine has played a significant role in primary healthcare. Due to their

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supposed therapeutic benefits, 80% of people on the planet are thought to utilize herbal medical items 1-3. Over the past few decades, the phyto-preparations sector has grown at a rapid pace, resulting in a wide range of products being utilized in complementary and alternative medicine. There is more urgency to determine a product's safety as well as its effectiveness when there is strong customer demand. Standardization and quality control procedures are particularly important since the increasing demand was also followed by an increase in the frequency of fraudulent practices (such as the inclusion of synthetic chemicals and the substitution of herbal material).1,4 A thorough toxicological evaluation is necessary to address this issue and rule out any safety issues. Finally, just as importantly, exogenous pollutants such as chemicals (such as pesticide residues, heavy metals) or microbes may also have negative impacts5. The primary goal of this study is to provide the methods and approaches that are currently being employed and suggested by regulatory bodies to look into the legitimacy and toxicity of therapeutic herbal products. Global databases including Scopus, PubMed, Web of Science, Google Scholar, and Science Direct were used for the data gathering. The literary sources were evaluated comprised books, reviews, and original papers. In recent times, there has been increasing concern regarding the adverse effects associated with synthetic drugs and medications, prompting the quest for alternative classes of pharmaceuticals that offer both safety and efficacy.6-8 Natural compounds have emerged as promising candidates, given their perceived reliability and reduced risk of adverse reactions, though potential toxicity and safety issues must be thoroughly investigated in human subjects9-12. Throughout history, humans have sought remedies to alleviate pain and manage various ailments, with evidence of medicinal plant usage dating back thousands of years.13-15 Today, numerous pharmaceuticals are derived from medicinal herbs, which play a significant role in both medical practices and everyday food preparation in modern society.16-17 The genetic variety of cancer patients and the varied nature of tumor cells have forced a reevaluation of the "magic bullet" strategy for treating cancer. Multi-targeted treatments are becoming more popular as a means of limiting chemo-resistance and improving the pharmacological activity of anticancer medications. This refers to the synergistic acts on a single site or the simultaneous actions of several drugs on numerous molecular targets. Cause medicinal plants produce a variety of phytochemicals that frequently work together to confer certain biological traits, they are being investigated as potential sources of new drugs. The US Food and Drug Administration (US FDA) has authorized over 80% of anticancer medications in the previous few decades that are either natural compounds or their derivatives.18 But as the pharmaceutical industry becomes more dependent on chemically produced molecules for cancer therapy discovery, enthusiasm in their research is dwindling.19 The development of novel plant-derived medicines is sometimes hampered by poorly understood or unidentified pharmacological and biochemical mechanisms of action. The progress in the discovery of drugs derived from plants has been slowed by the difficulty in determining the exact mechanism of action, in addition to other difficulties like plant material authentication, toxicity concerns, and difficulties in producing lead phytochemicals on a large scale due to insufficient plant material.20 Clarifying the connections between intricate combinations of phytoconstituents and potential targets (cellular and molecular) that cause the pharmacological response is necessary to comprehend the mechanism of action of therapeutic plants. Target-based screens provide many benefits for drug development, but they also

require a trial-and-error process that only provides a limited knowledge of the mechanisms of action. Target inhibition may not always result in the intended pharmacological impact or effectiveness, potentially because the target is different from what was first thought.21 Because of this, very few medications survive phase 1 trials; those that do usually fail for toxicity, off-target action, or lack of effectiveness. The majority of clinically authorized anticancer drugs originating from plants were approved decades after their therapeutic efficacy was first discovered.22

METHOD

Criteria for Evaluating Cellular Response in Cytotoxicity Assays of Herbal Medicine

The goal of in vitro toxicity testing is to evaluate the possible toxicity of chemicals, herbal extracts, essential oils, and other materials using non-human experimental animals23-26 As platforms for assessing cell viability, proliferation, and cytotoxicity, cell-based assays are widely utilized.27 Viability and toxicity have been measured using a variety of evaluation techniques, and choosing the best test method for cell health can be difficult.28-29 Determining the effectiveness of a natural substance so much depends on selecting an appropriate test technique for cytotoxicity assessment in an effort to enhance the study's quality and efficacy.30-31 Many quantifiable measures that may be used as indicators of cytotoxicity include those based on the measurement of the total protein content, cell number or growth, vital dye decrease, particular enzyme release, and vital dye release.32-33 It is possible to ascertain the cellular reaction to natural substances or herbal extracts using several measures. The reliability and reproducibility of results in cytotoxicity and cell viability assays are greatly influenced by a number of factors, such as drug solvents, concentrations of natural compounds or herbal extracts, length of drug exposure, cell-seeding density optimization, and assay timing. Solvent compatibility is crucial for performing cytotoxicity tests in cell culture systems because the cells are subjected to varying amounts of the test substance.34-35 In cell-based assays, standardization is essential, and selecting the right cell lines for a cytotoxicity assay might affect test performance and lead to an accurate evaluation. The cell lines are chosen based on their functional characteristics, the apparatus that is accessible, the preferred mode of administration, the results of the cytotoxicity test, and the relevant exposure circumstances.36 It is advisable to take these factors into account before starting a cytotoxicity test.37 In vitro experiments for biological evaluations frequently employ three types of cells: primary cells, self-renewing cells, and transformed cells (continuously produced cells or cell lines). Primary cells are those that have been extracted straight from the tissues of humans or animals and have been applied to translational and biological research38

Methods for Assessing Viability and Cytotoxicity of Herbal Medicine

Dye Exclusion Assays

One of the most used in vitro techniques for counting and estimating the number of living and dead cells is dye exclusion experiments. These approaches can be used to assess the fraction of living cells because in these experiments, the plasma membranes of alive cells reject the specified dye (unstained with the dye), whereas dead cells do not exclude them (stained with the dye).39 For these tests, a variety of staining methods, such as erythrosine B, congo red, eosin-nigrosin, and trypan blue, are employed, each having its own benefits and drawbacks.40,41 Although dye exclusion tests are straightforward and reasonably priced, their experimental protocols include a number of challenging and time-consuming stages in order to cover a significant number of samples. To get precise and reliable findings, a number of elements that are

important in determining cell viability should be taken into account. Dye does not interact with wounded cells in dye exclusion studies unless the cell membrane is destroyed, since biological reactions to cytotoxic substances would take longer in damaged cells to lose membrane integrity. Additionally, surviving cells may continue to proliferate after the test. Lethally wounded cells do not show up in the cellular disintegration phase, therefore the culture period is important for dye exclusion studies.42-44 In vitro chemosensitivity testing benefits greatly from the use of dye exclusion experiments, which may identify the kill target cells in populations of non-dividing cells.44 Trypan blue is a cheap, easy-to-use technique that reliably measures membrane integrity. However, because it takes a long time and is harmful to cells, it is not appropriate for use on a large number of samples where cytotoxic effect over time is needed. Furthermore, since it is hard to distinguish between healthy cells and live cells that have lost their capacity to perform certain activities, this approach is not appropriate for studies where the number of cells with normal activity is critical.45-47 Eosin-nigrosin is a simple and inexpensive approach, but it takes a lot of time, and with extended exposure, its chemical components change the morphometric dimensions of the cell. Thus, the cells should be exposed to eosinnigrosin for the shortest amount of time when the morphometric dimensions of the cells are significant.48-49

Trypan Blue Assay

The trypan blue stain test was created in 1975 to evaluate the viability of cells in suspension culture. It has been extensively utilized to identify dead cells and ascertain the possible hazardous effects of various chemicals and substances.50 Only non-viable cells with broken membranes will stain with the strongly negatively charged dye trypan blue.51

Eosin-Nigrosin Assay

The 1975-developed eosin-nigrosin assay is one staining method for evaluating sperm vitality. Additionally, this fluorescent red dye can be used to stain muscle fiber, red blood cells, collagen, and cytoplasm,52 and makes it easier to see under optical microscopes to assess cell viability.53 Eosin is used to quantify living and dead cells. It can enter unviable cells with broken membranes and turn dead cells pink. Viable cells, on the other hand, do not absorb eosin and are visible as clear, white cells. The assay has been extensively employed to ascertain the viability of sperm when the percentage of sperm motility falls below 25%.54-55 The nigrosin component gives the dead sperm a dark pink stain that helps to visualize them. It may also be used to examine and assess the size and form of sperm. On the same semen sample, the eosinnigrosin test should be performed regularly following the evaluation of sperm motility. In order to conduct these assays, prepare the reagents, which include 10% nigrosin (add 5 g of nigrosin to 50 ml of deionized water) and 1% eosin Y (add 0.5 g of eosin Y to 50 ml of deionized water). Next, thoroughly combine 1 drop of the collected semen with 2 parts of eosin 1% on a Boerner slide, and stir the mixture for 15 seconds at room temperature using a wooden stirrer. Add two drops of 10% nigrosin to the following step.56

Colorimetric Assays

Cellular viability is frequently assessed using colorimetric assays. Viability and to determine whether compounds in the medication development process are harmful or effective.57 The ratio of NADH to NADPH shows the metabolic activity of cells. The reaction of biochemical markers including MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide), MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), XTT (2,3-bis (2-methoxy-4-nitro-5-sulphophenyl) 5-carboxanilide-2H- tetrazolium

monosodium salt), WST1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium monosodium salt), WST8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt), LDH (lactate dehydrogenase), SRB (sulforhodamine B), NRU (Neutral Red Uptake) and CVS (crystal violet staining) as the enzymatic reduction compounds can be used in cytotoxicity examinations (Figure 3). Spectroscopic techniques can be used to identify the color change that occurs when these chemicals are reduced in colorimetric experiments.58

Adenosine Tri-Phosphate (ATP) Assay

ATP bioluminescence was initially developed to investigate the possibility of a linear relationship between the quantity of cultured cells and luminescence measurements using the luciferin-luciferase reaction.59-61 In luminometric ATP cell viability tests, the luciferase enzyme cannot interact with internal ATP until the cells become ATP permeable. When intracellular ATPases have been inhibited, the light is measured using luminometers to estimate intracellular ATP levels. Most assays are sensitive enough to detect the luminous signal from as far out as 50 cells. Within a few hours, the luminous signal may be assessed and is quite steady. Cells are separated into 96-well plates and placed in 100 µl of growth media.62-64

SELECTIVITY, POTENCY STUDIES, AND CLINICAL IMPLICATIONS

Selectivity in Cytotoxic Activity:

Evaluation of selectivity is crucial to assess the potential therapeutic relevance of herbal drugs. Selectivity refers to the ability of a drug to target cancer cells while sparing normal cells, thereby minimizing adverse effects. Potency studies involve determining the concentration of the herbal drug required to induce cytotoxic effects specifically in cancer cells. This is typically assessed using in vitro assays on various cancer cell lines and normal cell lines to compare selectivity ratios.65

Mechanisms of Selectivity:

Understanding the mechanisms underlying selectivity is essential for elucidating the therapeutic potential of herbal drugs. Mechanisms may include differential expression of molecular targets or pathways in cancer cells compared to normal cells, leading to preferential cytotoxicity.66

Potency Studies:

Potency studies involve dose-response experiments to determine the concentration-dependent cytotoxic effects of herbal drugs on cancer cells. These studies aim to establish the optimal therapeutic dose that achieves maximal efficacy with minimal toxicity. Various cytotoxicity assays, such as MTT assays or flow cytometry-based assays, are utilized to assess the potency of herbal drugs. Data from these studies are analyzed to generate dose-response curves and calculate parameters such as half-maximal inhibitory concentration (IC50) values.67

Clinical Implications:

The selectivity and potency of herbal drugs have significant implications for clinical translation. Herbal drugs with high selectivity and potency may offer promising alternatives or adjuncts to conventional chemotherapy, potentially enhancing treatment outcomes while reducing side effects. Clinical trials are essential for evaluating the safety and efficacy of herbal drugs in human subjects. Phase I trials assess safety and dose escalation, while phase II trials evaluate efficacy in specific cancer populations. Phase III trials compare herbal drugs to standard treatments in larger patient cohorts to establish their clinical utility.67-68

IMPORTANCE OF METAL OXIDE BINDING

Pathological Implications:

Metal ions, such as copper, zinc, and iron, play crucial roles in various physiological processes. However, dysregulation of metal homeostasis can lead to pathological conditions, including neurodegenerative disorders like Alzheimer's and Parkinson's diseases. Metal ions can aggregate and form toxic complexes, contributing to disease progression.68

Oxidative Stress and Protein Aggregation:

Metal ions, particularly transition metals like iron and copper, can catalyze the production of reactive oxygen species (ROS), leading to oxidative stress and damage to cellular components. Moreover, metal ions can interact with proteins, promoting their misfolding and aggregation, which is a hallmark of neurodegenerative diseases.69-70

Role of Metal Oxides:

Metal oxide nanoparticles, such as iron oxide (FeO), zinc oxide (ZnO), and copper oxide (CuO), are implicated in neurodegenerative processes due to their ability to generate ROS and induce protein aggregation. These metal oxides can interact with biomolecules, including proteins and nucleic acids, altering their structure and function.71

Therapeutic Potential:

Herbal drugs that possess the ability to bind to metal oxides offer potential therapeutic benefits in mitigating metal-induced toxicity and oxidative stress. By sequestering metal ions and preventing their interaction with biomolecules, these herbal drugs may inhibit protein aggregation and alleviate neurodegenerative pathology.72

Chelation Therapy:

Chelation therapy, which involves the administration of chelating agents to bind and remove metal ions from the body, is a recognized approach for treating metal toxicity. Herbal drugs with metal oxide binding properties may act as natural chelators, facilitating the removal of excess metal ions and restoring metal homeostasis. 73

Neuro-protective Effects:

Herbal drugs that target metal oxide binding may exert neuroprotective effects by reducing oxidative stress, inhibiting protein aggregation, and preserving neuronal function. These drugs have the potential to slow down disease progression and alleviate symptoms associated with neurodegenerative disorders.74

Synergistic Effects with Cytotoxic Activity:

The ability of herbal drugs to bind to metal oxides may synergize with their cytotoxic activity against cancer cells. Metal oxide binding may enhance the selective targeting of cancer cells while sparing normal cells, thereby improving the therapeutic index of herbal drugs in cancer therapy.75

EMERGING TRENDS IN HERBAL MEDICINE RESEARCH

Integration of Traditional Knowledge and Modern Science:

There is a growing trend towards integrating traditional knowledge of medicinal plants with modern scientific approaches. Ethnopharmacological studies are being conducted to validate the therapeutic properties of traditional herbal remedies and identify bioactive compounds responsible for their efficacy.76

Standardization and Quality Control:

Quality control and standardization of herbal medicines are emerging as critical areas of research. Efforts are underway to develop standardized methods for extraction, characterization, and quantification of bioactive compounds in herbal formulations to ensure consistency and efficacy.77

Pharmacogenomics and Personalized Medicine:

Pharmacogenomic studies are exploring the genetic factors that influence individual responses to herbal medicines. Personalized approaches to herbal medicine are being developed based on an individual's genetic makeup, allowing for tailored treatments with improved efficacy and safety.78-79

Bioinformatics and Systems Biology:

Bioinformatics and systems biology approaches are being applied to herbal medicine research to elucidate the complex interactions between bioactive compounds and biological pathways. Computational modeling and network analysis are being used to predict the therapeutic potential of herbal formulations and identify synergistic drug combinations.80

Novel Drug Delivery Systems:

Innovative drug delivery systems are being developed to enhance the bioavailability and efficacy of herbal medicines. Nanotechnology-based approaches, such as nanoemulsions and nanoparticles, are being explored for targeted delivery of bioactive compounds to specific tissues or cells.81-82

Combination Therapies and Synergistic Effects:

Combination therapies involving herbal medicines and conventional drugs are gaining attention for their potential synergistic effects and improved treatment outcomes. Research is focusing on identifying complementary mechanisms of action and optimal dosage regimens for combined treatments.83

Bio prospecting and Plant Biotechnology:

Bio prospecting of plant biodiversity is a promising approach for discovering novel bioactive compounds with therapeutic potential. Advances in plant biotechnology, including tissue culture and genetic engineering, are being utilized to enhance the production of bioactive compounds in medicinal plants.84

Clinical Trials and Evidence-Based Medicine:

There is a growing emphasis on conducting well-designed clinical trials to evaluate the safety and efficacy of herbal medicines. Evidence-based approaches are being employed to validate traditional uses of medicinal plants and establish scientific evidence for their therapeutic effects.85

Regulatory Considerations and Market Access:

Regulatory frameworks for herbal medicines are evolving to ensure product safety, quality, and efficacy. Harmonization of regulations and standards is facilitating market access for herbal products, while ensuring consumer protection and public health.86

Global Collaboration and Knowledge Sharing:

Collaborative research initiatives and knowledge-sharing platforms are fostering international cooperation in herbal medicine research. Multidisciplinary approaches involving researchers, healthcare professionals, policymakers, and traditional healers are being promoted to address global health challenges.86-87

CONCLUSION

The exploration of the cytotoxic activity and metal oxide binding properties of innovative herbal drugs represents a promising frontier in modern pharmacology. Through this review, we have elucidated the multifaceted potential of herbal medicines in cancer therapy and beyond. The cytotoxic activity of herbal drugs, characterized by their ability to selectively induce cell death in cancer cells while sparing normal cells, holds immense therapeutic promise. Mechanistic insights into the cytotoxic pathways and potency studies have provided valuable groundwork for the development of novel cancer treatments. Furthermore, the synergistic effects between cytotoxicity and metal oxide binding underscore the multifunctional nature of herbal drugs, offering opportunities for enhanced therapeutic efficacy and reduced adverse effects. In parallel, the metal oxide binding properties of herbal drugs offer a novel avenue for addressing metal-induced toxicity and oxidative stress, particularly in neurodegenerative disorders. By sequestering metal ions and inhibiting protein aggregation, herbal drugs have the potential to mitigate disease progression and alleviate symptoms. As we look towards the future, challenges such as safety

considerations and regulatory frameworks must be addressed to ensure the responsible development and use of herbal medicines. Emerging trends in herbal medicine research, including pharmacogenomics, combination therapies, and bio-prospecting, offer exciting opportunities for innovation and collaboration. In conclusion, the convergence of traditional herbal medicine and modern pharmacology offers a rich source of therapeutic agents with diverse mechanisms of action and clinical applications. By harnessing the cytotoxic activity and metal oxide binding properties of innovative herbal drugs, we can pave the way for more effective and personalized treatments, ultimately improving patient outcomes and advancing global health

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