SCREENING OF THE ANTI-PROLIFERATIVE EFFECTS OF BAICALEIN AND CARBON NANODOT COMBINATION ON HeLa CANCER CELLS.

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by

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DECLARATION

I hereby declare this dissertation is the result of my own investigations, except otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use this dissertation for teaching, research, and promotional purposes.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

List of symbols

(%)	Percentage
Cm	Centimeter
µg	Microgram
µg/ml	Microgram per milliliter
µl	Microliter
µM	Micro Molar
m	Meter
mg	Milligram
ml	Milliliter
NH	Amide
Nm	Nanometer
OH	Hydroxyl

List of abbreviations

AKT	Protein kinase B
CDKs	Cyclin-dependent kinase
C-dots	Carbon dots
CDs	Carbon Nanodot
CO	Carbonyl
CQD	Carbon quantum dot

DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DOSM	Department of Statistics Malaysia
DOX	Doxorubicin
GLOBOCAN	Global Cancer Observatory
GQD	Graphene quantum dot
h	Hours
HPV	Human papillomavirus
HSIL	High-grade squamous intraepithelial lesion
LLETZ	Large loop excision of the transformation zone
LSIL	Low-grade squamous intraepithelial lesion
MAPK	Mitogen-activated protein kinase
MDR	Multi-drug resistance
min	Minutes
MMP-2/-9	Matrix metalloproteinase-2 and -9
mTOR	Mammalian target of rapamycin
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay
NM	Nanomaterials
NMR	Nuclear magnetic resonance
NP	Nanoparticles
O. indicum	Oroxylum indicum (L.) Benth. ex Kurz
QOL	Quality of life

ROS	Reactive oxygen species
SK-HEP-1	SK-Hepatic Adenocarcinoma
WHO	World Health Organization

List of acronyms

B1	Baicalein treatment 1
B2	Baicalein treatment 2
B3	Baicalein treatment 3
Bai-CDs	Baicalein–Carbon Nanodot combination
Bai-CDs (1)	Baicalein–Carbon Nanodot combination treatment 1
Bai-CDs (2)	Baicalein–Carbon Nanodot combination treatment 2
Bai-CDs (3)	Baicalein–Carbon Nanodot combination treatment 3
C1	Cisplatin treatment 1
C2	Cisplatin treatment 2
C3	Cisplatin treatment 3
CD1	Carbon Nanodot treatment 1
CD2	Carbon Nanodot treatment 2
CD3	Carbon Nanodot treatment 3
T1	Treatment 1
T2	Treatment 2

PENYARINGAN KESAN ANTI-PROLIFERASI KOMBINASI BAICALEIN DAN KARBON NANODOT TERHADAP SEL KANSER HeLa

ABSTRAK

Kajian ini mengenalpasti kesan antiproliferasi daripada terapi gabungan baharu yang menggunakan baicalein, sejenis flavonoid, dan karbon nanodot (CDs) terhadap sel kanser serviks HeLa. Baicalein, yang terkenal dengan sifat antikansernya yang berkesan, mempunyai beberapa limitasi dalam aplikasi klinikal disebabkan oleh bioavailabiliti yang rendah. Karbon nanodot, dengan biokeserasian, keterlarutan, dan potensi penghantaran ubat yang cemerlang, digunakan sebagai pembawa untuk meningkatkan keberkesanan terapeutik baicalein. Kajian ini menilai kesan gabungan baicalein-karbon nanodot (Bai-CDs) berbanding rawatan tunggal baicalein, karbon nanodot, dan cisplatin terhadap sel HeLa menggunakan ujian MTT. Hasil kajian menunjukkan bahawa terapi gabungan ini secara signifikan menghalang proliferasi sel yang bersifat bergantungan kepada dos. Penemuan ini membuktikan potensi gabungan Bai-CDs sebagai strategi terapeutik baharu untuk kanser serviks, yang menawarkan peningkatan bioketersediaan dan kesan antikanser yang disasarkan, sambil mengurangkan kesan sampingan yang sering dikaitkan dengan kemoterapi konvensional.

SCREENING OF THE ANTI-PROLIFERATIVE EFFECTS OF BAICALEIN AND CARBON NANODOT COMBINATION ON HeLa CANCER CELLS

ABSTRACT

This study identifies the anti-proliferative effects of a novel combination therapy using baicalein, a flavonoid, and carbon nanodots (CDs) on HeLa cervical cancer cells. Baicalein, known for its potent anti-cancer properties, has shown limitations in clinical applications due to low bioavailability. Carbon nanodots, with their excellent biocompatibility, solubility, and drug delivery potential, were used as a carrier to enhance the therapeutic efficacy of baicalein. The study evaluated the anti-proliferative effects of the baicalein-carbon nanodot (Bai-CDs) combination in comparison to standalone treatments of baicalein, CDs, and cisplatin on HeLa cells using the MTT assay. Results demonstrated that the combination therapy significantly inhibited cell proliferation in a dose-dependent manner with the IC_{50} 69.85 ± 4.145µg/ml and p-value less than 0.05. These findings highlight the potential of the Bai-CDs combination as a new therapeutic strategy for cervical cancer, providing enhanced bioavailability and targeted anti-cancer effects while minimizing adverse outcomes associated with conventional chemotherapy.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer remains one of the leading causes of death worldwide and poses a significant challenge to improving global life expectancy. As estimated by the World Health Organization (WHO) in 2019, cancer is the second leading cause of death before the age of 70 years in 112 of 183 countries and ranks fourth in a further 23 countries (Sung et al., 2021). According to the WHO, cervical cancer is the fourth most common cancer in women, and there are 660,000 new cases of cervical cancer and 350,000 deaths of cervical cancer in the year 2022 (WHO, 2024). As for now, chemotherapy is the most widely used and promising cancer treatment. Other available major cancer treatments are hormone therapy, surgery, immunotherapy, radiotherapy, laser therapy, combination therapy, and targeted therapy (Ramos et al., 2021). The number of people living with cancer has increased as a result of better treatment options and higher overall survival.

Nevertheless, the commonly used chemotherapy medications not only effectively destroy cancer cells but can also harm healthy cells and have adverse effects, which could lower patients' quality of life (QOL) and psychological health. It does cause side effects such as nephrotoxicity, hepatotoxicity, neurotoxicity, cardiotoxicity, hematological toxicities and other toxicities such as ototoxicity, gastrointestinal toxicity, and gonadal toxicity (Van Den Boogaard et al., 2022). Chemotherapy also leads to other minor side effects including nausea, hair loss, nerve damage, vomiting, loss of appetite, constipation, and bleeding (Ullah et al., 2020).

Therefore, it is crucial to develop new alternative treatments, such as natural products, that lessen the negative effects of chemotherapy and improve the survival and quality of life of patients without compromising therapeutic efficacy to achieve a better cancer treatment with fewer side effects than chemotherapy. For instance, natural compounds containing flavonoids could induce apoptosis, cell cycle arrest in cancer cells and show potential for use as supplementary cancer therapies (Rahman et al., 2019).

Studies in recent years emphasize the potential of natural products in drug development, with plant-based sources showing promising effects in cancer therapies (Naeem et al., 2022; Rahman et al., 2019). *Oroxylum indicum* (*O. indicum),* commonly known as the beko plant, is a medicinal herb widely distributed in Asia, particularly in countries such as Malaysia, India, and Thailand. It has been traditionally used for its dual purposes such as both food and medicine. *O. indicum* is rich in secondary metabolites, including flavonoids, alkaloids, tannins, glycosides, saponins, phenols, and quinones, with baicalein being the most dominant and abundant active flavonoid. This plant exhibits a wide range of pharmacological properties, such as anti-cancer, anti-inflammatory, antioxidant, anti-bacterial, analgesic, hepatoprotective, nephroprotective, anti-diabetic, anti-helminthic, immunomodulatory, anti-hyperlipidemic, and gastroprotective activities. Recent study has shown that compounds like baicalein can significantly affect cellular functions, such as inhibiting the attachment of cancer cells, suggesting its potential for cancer therapy (Khanzada et al., 2021).

Baicalein (5,6,7-trihydroxyflavone) is the major flavonoid that can be found in almost all parts of *O. indicum* plant (Nik Salleh et al., 2020). Baicalein is also can be obtained from the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora* (Hu et al., 2022). Baicalein exerts multiple pharmacological effects including anti-inflammatory, antioxidant, and anti-viral properties, as well as protection against cardiovascular illness and anti-cancer applications (Arshad Husain Rahmani et al., 2022). Baicalein is known for a wide spectrum of therapeutic properties, including anti-microbial, anti-inflammatory, anti-oxidative, immunomodulatory, and anti-angiogenesis effects. In the process of carcinogenesis, flavonoid interferes with multiple signal transduction pathways and limit proliferation, angiogenesis, and metastasis or increase apoptosis. Wahab et al. (2021) reported that baicalein-rich fraction extracted from *O. indicum* leaves was found to attenuate the growth and survival of cervical cancer cells greater than crude extract with less baicalein content by down- regulating the expression of E6 and E7 oncoproteins that play a key role in human papillomavirus (HPV)-associated cervical carcinoma tumorigenesis (Wahab et al., 2021). Despite having enormous prospects for anti-cancer use, low bioavailability such as low aqueous solubility, rapid metabolism and poor oral absorption limit its applications (Rahmani et al., 2022).

In response to these bioavailability challenges, nanoparticles (NPs) have gained attention for their potential to enhance the drug delivery and therapeutic effectiveness of bioactive compounds. NPs are chemical substances or materials that are manufactured and used at a very small scale such as 1–100 nm in at least one dimension (Sameer et al., 2023). Based on structural makeup, nanoparticles

are divided into four groups such as organic or dendrimers, inorganic, carbon-based, and composite. Among all these nanoparticles, carbon nanomaterials are potential drug delivery agents, they serve as a vehicle for the delivery of anti-cancer drugs to the cells site. Carbon nanomaterials enhance drug pharmacological activity through controlled release, target-specific delivery, and prolonged drug action, offering a promising alternative to address the limitations and side effects of conventional cancer treatments (Mohammed et al., 2022).

One of the carbon nanoparticles is carbon nanodots or simply carbon dots (Sameer et al., 2023). CDs are carbon-based nanomaterials in sizes of less than 10 nm, they are quasi-spherical in shape, and used in a wide range of applications in the area of novel drug delivery in cancer, ocular diseases, infectious diseases, and brain disorders (Kaurav et al., 2023). CDs are less toxic to cells than metallic quantum dots since they are more biocompatible and have higher stability due to their hydrophilic nature (Sameer et al., 2023). NPs-mediated anti-cancer drug delivery include high therapeutic efficacy, reduced toxicity, site-specific delivery and cost-effectiveness (Malhan et al., 2024). CDs are proven to increase water solubility and bioavailability of drugs based on a study in which anti-cancer agents such as doxorubicin combined with carbon nanodots on liver cancer cells has shown greater inhibition of cancer cells (Xia et al., 2019). Therefore, the combination of baicalein which lacks bioavailability and CDs which consist of increased water solubility and bioavailability is expected or assumed could also increase the bioavailability of baicalein for drug delivery (Su et al., 2020).

1.2 Problem statement

Cancer continues to be the country's second-greatest cause of death overall and the leading cause of death for people under the age of 85 (Siegel et al., 2024). According to the statistics, cervix uteri cancer has been reported in 1913 cases in females and it holds rank number three based on the year 2022 (Global Cancer Observatory, 2024). Cervical cancer is an extremely complex sequence of disease conditions progressing gradually with a generalized loss of growth control. Additionally, there were only limited choices of cervical cancer treatments for patients for many decades which included surgery, radiation therapy, and chemotherapy as single treatments or in combination (Ramey et al., 2018). The main treatments include chemotherapy, radiotherapy, surgery, immunotherapy and bone marrow transplantation are highly efficient in treating cancer. However, treatments such as chemotherapy and radiotherapy have adverse side effects on the patient such as fatigue, loss of appetite, diarrhea, nausea, and dyspnea (Palagudi et al., 2024). To kill tumour cells, chemo- and radiotherapy are frequently used that mainly target DNA, but these therapies also elevate DNA damage in surrounding healthy tissue, causing toxicities and accelerated aging. To address this issue, researchers are exploring natural compounds that do not harm the normal cells at the same time treats the cancer cells. Baicalein which is a flavonoid that can be isolated form *O. indicum* consist of multiple anti-cancer effects via modulating multiple signalling pathways which involves cell growth, survival, proliferation, invasion, migration, angiogenesis, metastases, chemo- and radio-resistance and suppresses cancer development and progression (Nik Salleh et al., 2020).

However, despite its promising anti-cancer properties, low bioavailability has

significantly incapacitated the effectiveness of baicalein in clinical use (Zhou et al., 2017). This is to say that only a very small amount of the compound is usually absorbed and utilized by the body upon administration, which reduces its ability to reach higher, therapeutic levels in cancerous tissues. Hence, the full circle of its anti-cancer potential is not met, and one must develop strategies that enhance its absorption, distribution, and overall bioavailability to ensure that treatment outcomes are realized efficiently (Wen et al., 2023). Therefore, it is combined with CDs which are known for their potential for increased bioavailability, and high solubility which could efficiently improve baicalein's function by improving its delivery and efficacy of anti-cancer agents. Thus, this project aims to test the anti-proliferative effect of baicalein in combination with CDS on HeLa cells to determine its therapeutic effects towards HeLa cancer cells.

1.3 Rationale of the study

It has been proven by Wahab et al., that *O. indicum* has anti-proliferative activity and strongly induced apoptosis in treated cervical cancer cell lines via E6 and E7 repression (Wahab et al., 2021**).** Therefore, baicalein isolated from *O. indicum* can be further exploited as a potential anti-cancer candidate for cervical cancer treatment. However, the baicalein lacks bioavailability which prevents baicalein from reducing its anti-proliferative potential. Therefore, carbon nanodot, which is less than 10nm in size with specific folate targeting ligand, has a large drug loading capacity and most importantly it does not cause any toxic effects to the cells was used. Thus, baicalein is combined with carbon nanodots as a carrier to evaluate their combined anti-proliferative effect against cancer cells.

1.4 Objectives

General Objective:

The main objective of this study is to screen the anti-proliferative effects of baicalein and carbon nanodots combination on HeLa cells.

Specific objectives:

1. To determine the anti-proliferative effect of baicalein, carbon nanodot and cisplatin alone on HeLa cells using the MTT assay
2. To measure the anti-proliferative effects of baicalein and carbon nanodots combination (Bai-CDs) on HeLa cells using the MTT assay.

CHAPTER 2

LITERATURE REVIEW

2.1 Cervical cancer

Cervical cancer is the fourth most common malignant disease in low-income countries and women worldwide where there were around 660,000 new cases and in the same year, about 94% of the 350,000 deaths occurred (WHO, 2024). Human papillomavirus (HPV) is a common sexually transmitted infection where all sexually active people will be infected at some point in their lives, usually without symptoms which affect the skin, genital area and throat. HPV is accountable for 90–100% of cervical cancer cases among women, especially those <35 years old (WHO, 2024). Usually, the immune system will clear the HPV from the body but persistent infection with high-risk HPV causes the growth of abnormal cells which leads to cancer. This persistent HPV infection which left untreated tends to cause 95% of cervical cancer in women. Normally it takes 15-20 years for abnormal cells to become cancer but in an immunocompromised person, it only takes 5-10 years to become cancer (WHO, 2024). Cervical cancer in the early stages is often asymptomatic and may be diagnosed during a routine screening or pelvic examination. The usual symptoms include heavy or abnormal vaginal bleeding, in particular following intercourse, vaginal discharge that may be watery, mucoid, or purulent and malodorous, however it is rarely seen in isolation of other symptoms. In advanced disease, patients present with symptoms such as lower limb oedema, flank pain, as well as pelvic or lower back pain. In addition to this, bowel and/or bladder-related complaints such as changes in pressure or the passage of

urine and/or feces through the vagina indicate invasion of the bladder and rectum respectively (Liu et al., 2023).

Diagnosis initiated through pelvic examination in patients with any symptoms of cervical cancer and involves visualisation of the cervix and vaginal mucosa and biopsy if an abnormality is seen. The cervix might appear normal when the disease is micro-invasive or in the endocervical canal while large tumors on the other hand may appear to completely replace the cervix and metastatic lesions may be identified through enlarged palpable lymph nodes. If the Pap smear result is suggestive of a high-grade precancerous lesion (HSIL), or recurrent low- grade cytology (LSIL), then a colposcopy will be performed for definitive diagnosis and any questionable lesions will be biopsied for further analysis. If a precancerous lesion is confirmed by colposcopy findings or biopsy, a therapeutic procedure called large loop excision of the transformation zone (LLETZ) will be performed to excise the precancerous cells and prevent cancer (Burmeister et al., 2022). The stage of cervical cancer is an important prognostic marker and is determined clinically, based on tumor size and degree of pelvic extension and imaging (Burmeister et al., 2022).

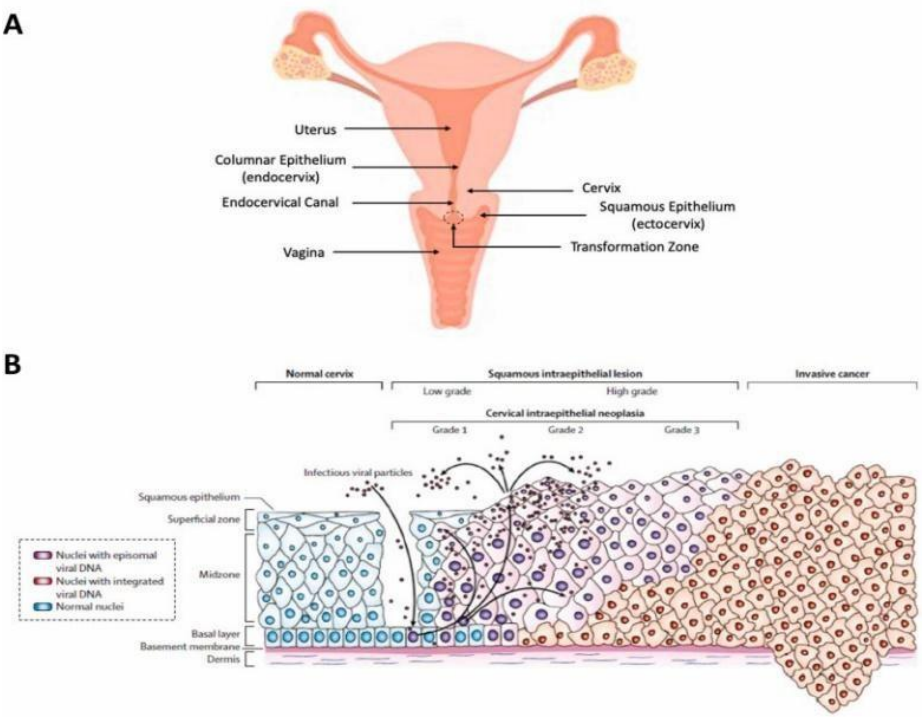

Figure 2. 1 Anatomical location and progression of cervical cancer. A) Diagram of female reproductive organs. B) Illustration of HPV's role in cervical cancer development (Adapted from Burmeister et al., 2022).