STUDY OF GONIOTHALAMIN AND BIOACTIVE GLASS 45S5 (GTN-BG) ON OSTEOSARCOMA (SAOS-2) AND BREAST ADENOCARCINOMA (MCF-7) CELLS

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by

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LIST OF ABBREVIATIONS

^{1}H	Deuterium
^{13}C	Carbon-13
3D	Three-dimensional
3T3	Normal mouse fibroblast
A549	Human adenocarcinoma alveolar basal epithelial cells
AIF	Apoptosis-inducing factor
ANOVA	Analysis of variance
Apaf-1	Apoptotic protease-activating factor-1
ATCC	American Type Culture Collection
BET	Brunauer, Emmel and Teller
BG	Bioactive glass
C	Carbon
Ca	Calcium
$Ca(NO_3)_2.4H_2O$	Calcium nitrate tetrahydrate
Ca^{2+}	Calcium ion
CaO	Calcium oxide
CDK	Cyclin-dependent kinase
CDKI	CDK inhibitor
cDNA	Complementary DNA
CHOP	C/EBP homologous protein
CO_2	Carbon dioxide
D10	Cumulative 10% point of diameter
D50	Median diameter
D90	Cumulative 90% point of diameter
DCFH-DA	2, 7-dichlorodihydrofluorescein diacetate

DCM	Dichloromethane
DD	Death domain
DDIT3	DNA damage-inducible transcript
DISC	Death-inducing signalling complex
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOX	Doxorubicin hydrochloride
DUSP	Dual-specificity phosphatase
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
EST	Expressed sequence tag
FBS	Fetal bovine serum
FESEM	Field emission scanning electron microscopy
FITC	Fluorescein isothiocyanate
FPKM	Fragments per kilobase million
FTIR	Fourier transform infrared spectroscopy
G0	Gap G0 growth phase
G0/G1	Checkpoint occurs between the G0 and G1 phases
G1	Gap G1 growth phase
G2	Gap G2 growth phase
G2/M	Checkpoint occurs between the G2 and M phases
GO	Gene ontology
GTN	Goniothalamin
GTN-BG	Combination of GTN and BG
H	Hydrogen
H_20	Water

HA	Hydroxyapatite
HMSC	Human bone marrow-derived mesenchymal stem cells
HT29	Human colorectal adenocarcinoma cells
IAP	Inhibitor of apoptosis protein
IC_{25}	Inhibitory concentration 25%
IC_{50}	Half maximal inhibitory concentration
IC_{75}	Inhibitory concentration 75%
ICP-OES	Inductively coupled plasma optical emission spectrometry
IR	Infrared
JNK	C-Jun N-terminal kinase
K	Potassium
K^+	Potassium ion
KBr	Potassium bromide
M	Mitotic phase
MAPK	Mitogen-activated protein kinase
MCF-7	Human breast adenocarcinoma cells
MDA-MB-231	Triple-negative breast cancer cells
MDBK	Normal kidney cells
MLKL	Mixed lineage kinase domain-like
MOMP	Mitochondrial outer membrane permeabilization
Mg	Magnesium
Mg^{2+}	Magnesium ion
mRNA	messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na	Sodium
Na^+	Sodium ion

$Na_2Ca_2(Si_2O_7)$	Disodium dicalcium disilicate
Na_2O	Sodium oxide
NaNO3	Sodium nitrate
NGS	Next-generation sequencing
NMR	Nuclear magnetic resonance
n	Sample size
ns	Not significant
O_2	Oxygen
OD	Optical density
OH	Hydroxyl group
Omi/HtrA2	High-temperature requirement A2
OS	Osteosarcoma
P	Phosphorus
P_2O_5	Phosphorus pentoxide
PB	Presto blue
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PI	Propidium iodide
PLK5	Polo-like kinase 5
PO_4^{3-}	Phosphate ion
PS	Phosphatidylserine
QC	Quality control
RIN	RNA integrity number
RIPK	Receptor-interacting serine-threonine kinase
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPM	Revolution per minute

RPMI 1640	Roswell Park Memorial Institute 1640
S	Synthesis phase
Saos-2	Human osteosarcoma cells
SBF	Simulated body fluids
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SEM-EDS	Scanning electron microscopy-elemental analysis
SI	Selectivity index
Si	Silicon
SiO_2	Silicon dioxide
Si-OH	Silanol group
Smac/DIABLO	Second mitochondria-derived activator of caspases/direct IAP-associated binding protein with low pI
T	Treated sample
TEOS	Tetraethyl orthosilicate
TEP	Triethyl phosphate
TNFRSF	Tumour necrosis factor receptor superfamily
UACC	Human breast carcinoma cells
USA	United States of America
UT	Untreated sample
UV	Ultraviolet
WHO	World Health Organization
XRD	X-ray diffraction

LIST OF SYMBOLS

α	Alpha
β	Beta
δ	Delta
θ	Theta
~	Approximately
*	Asterisk
–	Dash
=	Equals
>	Greater than
-	Hyphen
<	Less than
≤	Less-than or equal to
/	Or
±	Plus-minus
®	Registered trademark
×	Times
™	Trade mark
%	Percentage

LIST OF UNITS

°C	Degree celcius
µg	Microgram
µL	Microliter
µm	Micrometer
µM	Micromolar
Å	Ångström
cells/mL	Cells per millilitre
cm^{-1}	Reciprocal wavelength
cm^2	Centimeter square
cm^3/g	Centimeter cubic per gram
Da	Dalton
g	Gram (weight per unit mass)
g	Gravity
hr	Hour
Hz	Hertz
kcal/mol	Kilocalorie per mole
kV	Kilovolt
M	Molar
m^2/g	Meter square per gram
mA	Milliampere
mg/mL	Milligram per mililiter
MHz	Megahertz
min	Minute
mL	Milliliter
mm	Millimeter

mM	Millimolar
nm	Nanometer
nM	Nanomolar
psi	Pounds per square inch
U/mL	Units per mililiter
v/v	Volume/volume
w/v	Weight/volume
w.t%	Weight percentage

KAJIAN GONIOTALAMIN DAN KACA BIOAKTIF 45S5 (GTN-BG) TERHADAP SEL OSTEOSARKOMA (SAOS-2) DAN ADENOKARSINOMA PAYUDARA (MCF-7)

ABSTRAK

Pembangunan rawatan kanser alternatif adalah penting disebabkan oleh keterbatasan kemoterapi konvensional yang boleh membahayakan sel sihat semasa menyasarkan sel kanser. Produk semula jadi yang berasal daripada tumbuhan, dengan mekanisme yang pelbagai dan ketoksikan yang rendah, mempunyai potensi untuk digunakan dalam rawatan kanser, dan gabungan produk ini dengan bahan bioaktif boleh meningkatkan keberkesanan rawatan. Dalam kajian ini, aktiviti sitotoksik stiril-lakton tumbuhan, iaitu goniotalamin (GTN) telah diuji terlebih dahulu dalam beberapa titisan sel kanser manusia dan didapati mempunyai julat kesitotosikan yang cukup besar terhadap osteosarkoma (Saos-2), adenokarsinoma payudarara (MCF-7), karsinoma payudara (UACC-732), adenokarsinoma alveolar epitelium basal (A549) dan sel adenokarsinoma kolorektal (HT29), tetapi kurang ketoksikan terhadap sel tunjang mesenkima daripada sumsum tulang manusia (HMSC). GTN menunjukkan ketoksikan terpilih terhadap sel kanser dengan nilai SI yang tinggi (>2) bagi setiap titisan sel kanser yang diuji berbanding doksorubisin (DOX). Potensi peningkatan kesan antikanser GTN telah diteliti dengan cara menggabungkannnya dengan kaca bioaktif 45S5 (BG 45S5) daripada sintesis sol-gel, yang mempunyai sifat bioaktif, bioserasi dan terbiodegradasi. Gabungan GTN-BG didapati lebih berkesan daripada GTN dalam menghalang pembiakan sel Saos-2 dan MCF-7 disebabkan oleh persekitaran mikro yang lebih baik, disokong dengan pembebasan produk pelarutan ionik seperti ion Ca^{2+}, Na^{+} dan Mg^{2+} daripada BG. Bagi kedua-dua rawatan GTN dan

GTN-BG, beberapa ciri apotosis telah dikesan semasa pemerhatian dilakukan di bawah mikroskop fasa, termasuk pengecutan sel, sel membulat dan pembetukan bleb pada membran. Didapati bahawa apoptosis tercetus melalui laluan reseptor kematian ekstrinsik berikutan pengaktifan caspase 8 yang kebanyakannya telah dikesan dan lebih tinggi dalam GTN-BG berbanding dengan GTN. Pengikatan ligan kematian mencetuskan pengaktifan caspase 8, yang kemudiannya mengaktifkan efektor caspase 3/7. Keputusan ini disokong oleh pemprofilan transkriptom. Beberapa kelompok *TNFRSF*, termasuk gen *TNFRSF1A*, *TNFRSF16* dan *TNFRSF14*, didapati meningkat parasnya dalam sel Saos-2 yang dirawat dengan GTN-BG. Rawatan GTN-BG juga mendorong ekspresi gen *DUSP4*, *DUSP6*, *PLK5* dan *CHOP* yang boleh menyebabkan penghentian kitaran sel pada fasa G2/M, seterusnya mengakibatkan apoptosis. Sebagai kesimpulan, aktiviti antiproliferatif dan apoptosis GTN dipertingkatkan dengan gabungan BG.

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ABSTRACT

Developing alternative cancer treatment is crucial due to the limitations of conventional chemotherapy, which can harm healthy cells while targeting cancer cells. Plant-derived natural products, with their diverse mechanisms and low toxicity, hold promise as cancer treatments, and their combination with bioactive materials may improve treatment effectiveness. In this study, the cytotoxic activity of a plant styryl-lactone, goniothalamin (GTN) was screened first in several human cancer cell lines and was found to possess a substantial range of cytotoxicity against osteosarcoma (Saos-2), breast adenocarcinoma (MCF-7), breast carcinoma (UACC-732), adenocarcinoma alveolar basal epithelial (A549) and colorectal adenocarcinoma (HT29) cells, but less toxicity towards human bone marrow-derived mesenchymal stem (HMSC) cells. GTN demonstrated selective toxicity towards cancer cells with a high SI value (>2) for each examined cancer cell line compared to doxorubicin (DOX). The potential enhancement of GTN's anticancer effects was explored by combining it with a sol-gel-derived bioactive glass 45S5 (BG 45S5) that possesses bioactive, biocompatible, and biodegradable properties. The combination of GTN-BG was found more potent than GTN in inhibiting the proliferation of Saos-2 and MCF-7 cells due to a better microenvironment provided with the release of ionic dissolution products such as Ca^{2+}, Na^{+} and Mg^{2+} ions from the BG. For both GTN and GTN-BG treatments, several apoptotic features were detected when observed under a phase microscope, including cell shrinkage, rounded cells, and membrane blebbing. It was found that

apoptosis was triggered through the extrinsic death receptor pathway as the activation of caspase 8 was mainly detected and significantly higher in GTN-BG compared to GTN. The binding of a death ligand triggers the activation of caspase 8, which later activates the effector's caspase 3/7. These results were supported by transcriptome profiling. Several *TNFRSF* members, including *TNFRSF1A*, *TNFRSF16*, and *TNFRSF14* genes, were upregulated in Saos-2 cells treated with GTN-BG. GTN-BG treatment also induced the expression of *DUSP4*, *DUSP6*, *PLK5* and *CHOP* genes that may contribute to cell cycle arrest at the G2/M phase, subsequently resulted in apoptosis. To conclude, the antiproliferative and apoptotic activities of GTN were enhanced with the combination of BG.

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is the leading cause of death in the world, accounting for nearly 10 million deaths in 2020, or nearly one out of every six deaths. Osteosarcoma (OS) is a type of primary malignant bone tumour that emerges from the mesenchymal tissue, which contains spindle-shaped stromal cells capable of producing bone-like tissues (Zhao *et al.*, 2021). OS is the most common type of bone malignancy and a leading cause of cancer death, particularly in children and adolescents, with approximately 3.4 cases per million diagnosed globally (Misaghi *et al.*, 2018). On the other view, breast cancer is a malignant tumour that begins in the breast tissues and can invade adjacent tissues or spread to other body parts. In 2020, breast cancer was the leading cause of cancer-related death among women accounting for approximately 15.5% of all cancer deaths and 24.5% of all cancer cases worldwide (Sung *et al.*, 2021). With an estimated 2.3 million cases and 685,000 deaths in 2020, it has surpassed lung cancer as the most commonly diagnosed cancer and the fifth leading cause of cancer death worldwide (Soerjomataram & Bray, 2020; Sung *et al.*, 2021).

Natural products derived from plants are a promising source for developing new and improved anticancer therapies because of their diverse chemical structure and relatively low toxicity (Talib *et al.*, 2022). Despite the ease with which modern synthetic drugs can be obtained, researchers continue to examine plants in search of new therapeutic compounds. Various bioactive compounds derived from plant extracts have been experimentally investigated to expand clinical understanding of their biological effects (Fernandes *et al.*, 2017; Talib *et al.*, 2022). Alkaloids from vinca, taxol, epipodophyllotoxin, camptothecin and their semisynthetic or synthetic

derivatives are just a few examples of the many compounds isolated from plant species that have the ability to prevent or treat some types of cancer cells and are used as anticancer drugs (Fernandes *et al.*, 2017).

Goniothalamus is one of the largest genera of palaeotropical *Annonaceae*, with more than 160 species dispersed throughout tropical southeast Asia, with Indochina and Western Malaysia serving as the centre of diversity (Saunders, 2003). Goniothalamus species have yielded numerous compounds, including styryl-pyrone derivatives, alkaloid derivatives, and styryl-lactones (Seyed *et al.*, 2014). Goniothalamin (GTN), a natural styryl-lactone extracted from *Goniothalamus sp*., has been shown to have anticancer effects on a variety of cancer cells, including leukaemia, breast, lung, oral, cervical, colon, ovarian, pancreatic, and prostate cancer cells, with less toxicity to normal cells. Most GTN-treated cancer cells have reportedly undergone diverse forms of cell death, most notably apoptosis (Seyed *et al.*, 2014).

Bioactive glass (BG) was first discovered by Larry Hench in 1969 with an original BG composed of 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5 in weight percentages (wt.%) using the traditional melt method at high temperature (Hench, 2006). The sol-gel method, on the other hand, has the advantages of low processing temperatures and ease of control over textural properties. Furthermore, it has fewer components than melt-derived glasses and may have higher bioactivity and absorbability due to the increased surface area (Zheng & Boccaccini, 2017). It has a wide variety of biomedical uses, from bone regeneration to skin regeneration, as a result of its high biocompatibility and bioactivity (Miguez-pacheco *et al.*, 2014; Jones *et al.*, 2016).

The concept of combining plant-derived compounds with modern biomaterials, such as BG, is aimed at improving therapeutic strategies. This combination can lead

to various outcomes, including cases where there are no interactions and the therapeutic effect is additive. Additionally, optimal combinations may occur where each substance enhances the effectiveness of the other, potentially reducing the required drug dosage. However, negative interactions between BG dissolution products and tested compound can impede the desired combined therapeutic effect, highlighting the need for a systematic evaluation of their combined effects (Schuhladen *et al.*, 2019).

1.2 Problem statements

The problem statement for this study has been divided into three main categories, each intricately linked to the rationale behind conducting this research: -

1) The treatment for cancer includes chemotherapy which uses drugs to kill fast-growing cancer cells throughout the body but also destroys some healthy cells. Thus, researchers worldwide are continuously looking for better strategies to improve cancer treatment. Any compound with a strong range of anticancer activity and less toxicity toward normal cells could be considered a promising alternative to the present chemotherapeutic drug.

While numerous studies have examined the cytotoxic properties of GTN in cancer cell lines, less attention has been paid to its selectivity in human cancer cell lines as opposed to normal human cells. Therefore, in the first part of this study, the cytotoxic activity of GTN was tested on several human cancer cell lines, including osteosarcoma Saos-2, breast adenocarcinoma MCF-7, breast carcinoma UACC-732, adenocarcinoma alveolar basal epithelial A549 and colorectal adenocarcinoma HT29 cells. The human mesenchymal stem cells (HMSC) were selected as the control normal human cells due to its ability to differentiate into multiple cell types and its homing

capacity in almost all human cancer cell lines (Nombela-Arrieta *et al.*, 2011; Caplan, 2017; Krueger *et al.*, 2018). Two important parameters were measured; the half maximal inhibitory concentration (IC_{50}) to study the efficacy of GTN in killing cancer cells and the selectivity index (SI) to examine the safety of GTN as a drug candidate to normal cells. Doxorubicin (DOX) was selected as the present chemotherapeutic drug for comparison with the tested GTN compound. This finding may provide additional evidence of the potential biological properties of GTN, which is being considered as a future alternative anticancer treatment.

2) Instead of using single anticancer agents or monotherapy, combining them with another substance that has an additive or synergistic effect on killing cancer cells may be more effective. For many years, research on GTN has been focused on its anticancer effects as a single treatment. However, limited studies were conducted to study the potential combination of GTN with any substance in order to enhance the GTN's inhibitory effects on cancer cells.

Thus, this study was designed to explore the combination of GTN with bioactive glass (BG) on targeted cancer cells, paving the way for the current trend in combining phytochemicals with synthesised bioactive materials. Not many studies tend to elaborate on the combined effect of phytotherapeutics compounds with BG; hence, there is a crucial need to embark on this endeavour in creating powerful phytochemicals as transcending for cancer treatment. BG was selected to be combined with GTN as it is known to be bioactive, biocompatible and biodegradable. Therefore, there is no need for a second surgical procedure to remove it from the body. The conventional melting method for BG synthesis requires a high temperature greater than 1300°C, resulting in dense structures with a low specific surface area that may reduce

bioactivity. The sol-gel method overcame these drawbacks by significantly lower temperature, increased porosity and surface area, and enhanced bioactivity (Peltola *et al.*, 1999; Sepulveda *et al.*, 2001). Bioactivity is related to a material's surface area, which affects glass dissolution and apatite formation. Due to its high biocompatibility and bioactivity, it has a wide range of biomedical applications, ranging from bone regeneration to skin regeneration (Miguez-pacheco *et al.*, 2014; Jones *et al.*, 2016).

In this study, the combined effects of GTN and synthesised sol-gel BG (GTN-BG) on the proliferation of Saos-2 and MCF-7 cells were examined quantitatively and qualitatively using several assays, including MTT assay, PrestoBlue (PB) assay, morphological observation and scratch-wound assay using Incucyte® Live-Cell analysis. Besides, assessing BG degradation in culture media is essential for predicting any effects that may contribute to Saos-2 and MCF-7 cell growth inhibition. Therefore, the extracellular changes in the growth media of Saos-2 and MCF-7 cells, including pH and ion release patterns, were also investigated. The inhibitory patterns of GTN-BG in both cell lines were compared to a single GTN treatment to detect any significant difference between the treatments applied. Thus, any potential improvement in the inhibitory effects of the combined treatment on the tested cancer cells could be demonstrated.

3) Defects in cell death pathways contribute to cancer development and can cause drug resistance, making cancer treatments less effective. Thus, cancer treatment aims to boost the ability to kill cancerous cells and reduce their chances of proliferating and survival (Pistritto *et al.*, 2016). There is growing evidence that the apoptotic signalling pathway by anticancer drugs is a key target for developing novel anticancer drugs (An *et al.*, 2019). The ability to discover and develop novel anticancer

drugs that can increase the sensitivity of cancer cells to undergo apoptosis can be achieved by identifying the mechanisms underlying apoptosis, as well as its effector proteins and the genes responsible for it (Goldar *et al.*, 2015).

GTN exerted its anticancer activity on various cancer cells through the regulation of the cancer cell cycle, induction of apoptosis by oxidative stress, and activation of caspases (Seyed et al., 2014). The non-apoptotic cell death mechanism was also observed in human triple-negative breast cancer MDA-MB-231 cells induced by GTN *via* caspase-independent pathway (Khaw-On et al., 2019). To date, no research has been done to examine the mechanism of cell death induced by GTN-BG in any cancer cells since this is the first proposed combination. For Saos-2 cells, not even the GTN's potential anticancer effect has been investigated.

Therefore, the underlying mechanisms associated with the inhibitory effects in the treated cells were further explored in this study. The mode of cell death in the treated cells, whether apoptosis or necrosis, was determined by annexin V-FITC/PI using a flow cytometer. In addition, the release of reactive oxygen species (ROS), the effects on the cell cycle, activation of initiator caspases 8 and 9, and executioner caspase 3/7 were also evaluated. Besides, the gene expression study was also performed to further explain the mechanism of action in response to this treatment. Thus, this study provides insight into the underlying molecular mechanisms of cell death, specifically by apoptosis in cells treated with GTN-BG. It will be useful for further evaluation in preclinical and clinical settings for cancer treatment.

1.3 Hypothesis

It is hypothesised that the combined treatment of GTN-BG could result in enhanced inhibition of cell proliferation in both Saos-2 and MCF-7 cells, as well as the induction of apoptosis when compared to the single treatment of GTN.

1.4 Research objectives

1) To assess the cytotoxicity and selectivity of GTN on selected cancer cells.
2) To synthesise and characterise BG 45S5 using the sol-gel method.
3) To examine the combined effects of GTN and BG 45S5 (GTN-BG) on the proliferation of Saos-2 and MCF-7 cells.
4) To study the mechanism of cell death in GTN-BG-treated Saos-2 and MCF-7 cells.
5) To investigate the gene expression of GTN-BG treated Saos-2 cells.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

2.1.1 An overview of cancer

Cancers are a category of illnesses distinguished by their insensitivity to signals that inhibit cell division, independence from signals that promote growth, unregulated replication, evasion of apoptosis, persistent angiogenesis, and the ability to spread to other tissues or metastasis (Hanahan & Weinberg, 2000; Schiliro & Firestein, 2021). The inability of cancerous cells to respond to normal cell cycle activation signals results in uncontrolled growth and proliferation of altered cells. It is lethal if cancer cells continue to proliferate and spread to other tissues, which accounts for 90% of cancer-related fatalities (Hanahan & Weinberg, 2000; Abbas & Rehman, 2018). Both external (including chemicals, radiation, tobacco, and infectious organisms) and internal (including inherited mutations, hormones, immunological conditions, and random mutations) factors can contribute to the development of cancer (Mathur *et al.*, 2015).

Cancer is the leading cause of mortality globally, with its occurrence steadily increasing. Around 19.3 million new cancer cases and nearly 10.0 million cancer-related deaths were observed in 2020 (excluding nonmelanoma skin cancer) occurred worldwide (Sung *et al.*, 2021). Approximately 2.3 million new cases (11.7%), female breast cancer has surpassed lung cancer as the most diagnosed cancer, followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach cancer (5.6%). With an estimated 1.8 million deaths (18%), lung cancer remained the major cause of

cancer-related death. This was followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and female breast cancers (6.9%) (Sung *et al.*, 2021). In general, the incidence of both sexes was 2- to 3-fold higher in developed countries than in developing countries, whereas male mortality varied 2-fold, but female mortality varied little. However, mortality rates for female breast and cervical cancer were noticeably higher in developing countries than in developed countries (Sung *et al.*, 2021). Global cancer cases are projected to surge by 47% to reach 28.4 million in 2040. Developing countries are expected to experience a more substantial increase (64% to 95%) compared to developed countries (32% to 56%), primarily due to demographic changes (Sung *et al.*, 2021). The slower population growth in developed countries is attributed to factors like economic development, urbanisation, family planning, and cultural shifts, with cancer prevalence being closely linked to their ageing population, as cancer is more common among older individuals (Kadambi *et al.*, 2021; Götmark & Andersson, 2023).

Various cancer treatments are available such as surgery, chemotherapy, radiation therapy, and immunotherapy. Treatment must be chosen by considering multiple factors such as the site and stage of cancer, metastatic area, and patient history such as age, gender, health status, etc. Depending on the condition of the patient, cancer treatments may be applied alone or in combination (Yildizhan *et al.*, 2018).

2.1.2 Osteosarcoma

Osteosarcoma (OS) is a form of malignant bone cancer that emerges from the mesenchymal tissue, which contains spindle-shaped stromal cells capable of producing bone-like tissues (Zhao *et al.*, 2021). It accounts for 20% of all primary malignant bone cancer cases worldwide and is the most common type in adolescents

(Messerschmitt *et al.*, 2009; Arndt *et al.*, 2012). In terms of age, the prevalence of OS follows a bimodal distribution pattern, during the adolescent development phase, which is between 10 and 14 years old when it first peaks, and it then peaks again after the age of 60 (Mirabello *et al.*, 2009). OS is commonly found in the metaphysis of long tubular bones, such as the proximal humerus, distal femur, and proximal tibia; however, OS is rarely found in the lumbar, pelvic, and sacral regions (Luetke *et al.*, 2014).

Clinically, the disease's onset is characterised primarily by local pain and swelling, with occasional joint dysfunction. Growth pain and trauma symptoms are perplexing, but the degree of malignancy is high (Isakoff *et al.*, 2015). Notably, nearly 10-20% of patients have measurable metastatic disease prior to onset, with the lungs being the most common site, followed by the bones and, on rare occasions, the lymph nodes. The remaining 80-90% of patients are thought to have subclinical or micrometastases, which cannot be detected accurately using current diagnostic methods (Zhao *et al.*, 2021).

In the 1970s, amputation was the standard treatment for OS, even though the 5-year survival rate after amputation was less than 20% (Ferrari & Palmerini, 2007). In the 1980s, advances in surgical techniques, research on effective chemotherapy drugs, and preoperative and postoperative chemoradiation adjuvant therapy, among other significant developments, contributed to the enhancement of the OS treatment modality. For instance, limb salvation gradually replaced the traditional amputation method. Notably, the combined limb salvage treatment supported by neoadjuvant chemotherapy achieved great clinical success by allowing patients to live for at least five years without enduring the pain of amputation. Additionally, the survival rate increased to almost 80% (Ferrari & Palmerini, 2007; Anderson, 2016).

The present comprehensive treatment for OS patients involves preoperative and postoperative chemotherapy along with surgical intervention. Treatment outcomes for OS have exhibited enhancement compared to the past. Nevertheless, in recent times, there have been occurrences of chemotherapy resistance, particularly impacting patients with lung metastases. Thus, developing new and effective medications and implementing innovative treatment strategies is necessary. In the current research landscape, the optimal combination and strategy of different treatment methods are also focal points (Zhao *et al.*, 2021).

2.1.3 Breast cancer

Breast cancer is a malignant tumour that begins in the breast tissues and can invade adjacent tissues or spread to other body parts. Ductal carcinoma, the most prevalent form of breast cancer, arises from cells lining the ducts. Those that form in the lobule-lining cells are called lobular carcinoma, while others begin in other tissues (Shah & Raytthatha, 2021). The major breast cancer risk factors are family history, increasing age, high body mass index or obesity, physical inactivity, high-fat diet, tobacco use, hormonal menopause therapy or oral contraceptives and reproductive factors including early menarche, shorter breastfeeding periods, late menopause and low parity (Britt *et al.*, 2020; Shah & Raytthatha, 2021). There is a significant association between parity and luminal breast cancer, with parous women having a 34% reduced risk of luminal A and a 29% reduced risk of luminal B compared to nulliparous women (Li *et al.*, 2021).

In 2020, breast cancer accounted for the majority of cancer-related deaths among women accounting for approximately 15.5% of all cancer deaths and 24.5% of all cancer cases worldwide (Sung *et al.*, 2021). With an estimated 2.3 million cases

and 685,000 deaths in 2020, It has superseded lung cancer as being the most prevalent cancer diagnosed and the fifth leading cause of cancer death worldwide, and the cases are expected to reach 4.4 million in 2070 (Soerjomataram & Bray, 2020; Sung *et al.*, 2021).

Breast cancer is classified into three major tumour subtypes based on oestrogen or progesterone receptor expression and *ERBB2* gene amplification. The three subtypes have different risk profiles and treatment approaches. Depending on the patient's tumour subtype, anatomic cancer stage, and personal preferences, the best course of treatment may be surgery, chemotherapy, immunotherapy, or hormonal therapy (Waks & Winer, 2019). Chemotherapy kills rapidly proliferating cancer cells and healthy cells as well. Thus, researchers around the world researchers all over the world are constantly looking for better breast cancer treatment strategies. Any compound with strong anticancer activity with fewer side effects could be a promising chemotherapeutic drug replacement. Furthermore, the effectiveness of anticancer agents could be increased by combining them with another substance that has an additive or synergistic effect on killing cancer cells, rather than just using either alone.

2.1.4 Lung cancer

Lung cancer continues to be a major global health concern with high morbidity and mortality rates. It is the second most often diagnosed cancer and the leading cause of cancer-related deaths in men, accounting for 18% of total cancer fatalities in 93 countries. Besides, it is the third most frequent malignancy in women, after breast and colorectal cancer, and the second major cause of death after breast cancer (Sung *et al.*, 2021; Kim & Lee, 2022).

While cigarette smoking remains the predominant risk factor for lung cancer, various environmental exposures, including biomass fuels, asbestos, arsenic, and radon, also play significant roles as risk factors, with exposure levels varying considerably worldwide (Leiter *et al.*, 2023). Lung cancer can be categorised into two main histologic classes with distinct growth and spreading patterns: small-cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). Treatment options for lung cancer encompass surgery, radiation therapy, chemotherapy, and targeted therapy, and the choice of therapy depends on various factors, such as the type and stage of cancer (Lemjabbar-alaoui *et al.*, 2015).

2.1.5 Colorectal cancer

Colorectal cancer, which includes cancer of the colon or rectum, represents a major global health concern, ranking as the third most frequently diagnosed and second most deadly cancer worldwide (Sawicki *et al.*, 2021). In 2020, colorectal cancer (CRC) accounted for approximately 9.4% of cancer-related deaths, but its global incidence is projected to more than double by 2035, primarily affecting the older population in less developed countries (Hossain *et al.*, 2022). Colorectal cancer involves abnormal growth of glandular epithelial cells in the colon or rectum, influenced by environmental and genetic factors, and its risk increases with age, especially in individuals with long-standing ulcerative colitis and Crohn's disease (Triantafillidis *et al.*, 2009; Hossain *et al.*, 2022).

Treatment for colon cancer depends on the stage at diagnosis, the size and location of the tumour, and the patient's overall health. Standard treatment options include surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy (Dekker *et al.*, 2019). The ideal treatment for colorectal cancer involves complete

tumour and metastases removal through surgical intervention. For patients with unresectable lesions or who cannot undergo surgery, the goal is to shrink the tumour and control its spread using radiotherapy and chemotherapy. Sometimes, these treatments may be used before or after surgery to maximize tumour reduction and stabilisation (Messersmith, 2019). Although surgery and chemotherapy have been the primary treatments, the outlook for colorectal cancer has been disappointing, especially in patients with metastatic lesions. Nevertheless, targeted therapy offers an optional approach that holds promise in potentially extending overall survival for colorectal cancer patients (Xie *et al.*, 2020).

2.2 *Goniothalamus* spp. and bioactive compounds

Goniothalamus, with the scientific classification as depicted in Table 2.1, is one of the largest palaeotropical plant genera in the family Annonaceae. It is recognised by various regional names, including Gajah Beranak, Penawar Hitam (Malaya), Ki Cantung (Indonesia), Limpanas Putih (Brunei), and Chin Dok Diao (Thailand) (Aslam *et al.*, 2016). This genus comprises approximately 160 species of trees and shrubs widely distributed in lowland and submontane tropical forests in southeast Asia, with a centre of diversity in western Malaysia (Saunders, 2003). The species is distinguished by solitary, axillary, and pendent flowers, two whorls of petals with inner petals smaller than outer petals, the inner petals connivent and forming a distinct dome over the stamens and carpels acting as a pollination chamber, and stamens with apical connectives. The fruits are invariably apocarpous, with monocarps that are either sessile or stipitate (Saunders, 2002, 2003). The image of leaf and flower of *Goniothalamus macrophyllus* as depicted in Figure 2.1. Some *Goniothalamus sp* such as *G. macrophyllus, G. giganteus, G. scortechinii, G. tapis Miq., G. amuyon*

Merr, *G. laoticus* and *G. uvaroides King* has been used traditionally to treat various ailments such as body pains, swelling, fever, cold, stomachache, headache, postpartum remedy, abortion, rheumatism, rheumatism and tympanites (Aslam *et al.*, 2016).

Table 2.1: Scientific classification of *Goniothalamus.* Adapted from (Hook.f. & Thomson, 1855; Wikipedia, 2022).

Kingdom:	Plantae
Clade:	Angiosperms
Order:	Magnoliales
Family:	Annonaceae
Subfamily:	Annonoideae
Genus:	*Goniothalamus*

A)

B)

Figure 2.1: The image of *Goniothalamus macrophyllus* plant is illustrated with **A)** leaf and **B)** flower. Adapted from (Khaytarova, 2022).

Approximately 40 *Goniothalamus* species have been investigated, and many cytotoxic molecules, especially styryllactone derivatives, acetogenins and aporphine alkaloids, have been isolated (Aslam *et al.*, 2016). The species in this genus have gained significant interest from many research groups and are considered to be a source of drugs for the treatment of cancer and bacterial infections (Wiart, 2007).

Examples of styryl lactones bioactive compounds extracted from *Goniothalamus* are goniothalamin, altholactone, ardiopetalolactone, goniopypyrone, goniodiol, goniofufurone, goniofupyrone, goniofufurone, goniopypyrone, goniotriol, digoniodiol 8-acetylgoniotriol, deoxygoniopypyrone A, howiinol, gonodiol-8-monoacetate, 5-isogoniothalamin oxide and 5-Acetyl goniothalamin (Aslam *et al.*, 2016).

2.2.1 Goniothalamin and its biological activities

Goniothalamin (GTN), a styryl-lactone of medicinal plant *Goniothalamus sp.* was reportedly found in the bark of *G. andersonii*, *G. macrophyllus Miq*., *G. malayanus*, *G.velutinus* and *G. tapisoides* (Seyed *et al.*, 2014; Aslam *et al.*, 2016), root of *G. tapis*, *G. umbrosus* and *G. woodii* (Aslam *et al.*, 2016) and leaves of *G. tamirensis* and *G. griffithi* (Aslam *et al.*, 2016). It was first isolated in 1972 and since then, the substance has been extracted, isolated, and characterised (Jewers *et al.*, 1972; Seyed *et al.*, 2014).

Briefly, plant materials such as leaves, stem bark or roots were ground to powder, extracted in methanol or ethanol. Then, the initial extracts were partitioned with hexane, chloroform or dichloromethane and ethyl acetate. Various chromatographic techniques were used to isolate bioactive compounds such as Vacuum Liquid Chromatography (VLC), column chromatography and Preparative

Thin Layer Chromatography (Prep-TLC). Spectroscopic methods were also used to clarify the structures of bioactive compounds, including 1-dimensional and 2-dimensional nuclear magnetic resonance (1D, 2D NMR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Ultraviolet (UV) and mass spectrometry. The plant extract was also subjected to spectrophotometric fingerprinting (attenuated total reflectance Fourier transform infrared spectroscopy, ATR-FTIR) and chromatographic fingerprinting (high-performance liquid chromatography, HPLC) analyses with reference markers (Blázquez *et al.*, 1999; Seyed *et al.*, 2014).

GTN has been shown to possess a wide range of biological functions, including anti-inflammatory, immunosuppressive, and anticancer properties (Seyed *et al.*, 2014). Table 2.2 summarises previous research on the cytotoxic properties and mode of action of GTN in various cancer cell lines.

Table 2.2: Previous reports on anticancer activities of GTN and its derivative against various human cancer cell lines

Previous studies	Cancer cell lines	Note
(Ali *et al.*, 1997)	MCF-7, HGC-27, Hela, PANC-1	Cytotoxic properties with IC_{50} values of ≤3 μg/mL. The ultrastructural features of MCF-7 showed induction of necrotic mode of cell death with swollen mitochondria containing flocculent, irregularly clumped chromatin deposited along the nuclear membrane and ruptured nuclear and mitochondrial membranes.
(Inayat-Hussain *et al.*, 1999, 2010)	Jurkat-T	Activation of Caspases 3 and 7, DNA damage and oxidative stress.
(Inayat-Hussain *et al.*, 2003)	HL-60	Externalisation of phosphatidylserine, loss of mitochondrial transmembrane potential, activation of caspase 9, 3 and 7.
(Rajab *et al.*, 2005)	HL-60, CEM-SS	Induction of DNA damage.
(Lin & Pihie, 2003)	Caov-3	Increased bax, suppressed Bcl-2 protein
(de Fátima *et al.*, 2006, 2008)	786-0	Anti-proliferative activity involved distinct signalling pathways, apoptosis, and autophagy as dominant responses.
(Chen *et al.*, 2005)	MDA-MB-231	Disrupts intracellular redox balance, induces cdc25C degradation, G2/M cycle arrest.
(Wattanapiromsakul *et al.*, 2005)	COR-L23, LS-174T	Cytotoxic properties

Table 2.2: Continued.

Previous studies	Cancer cell lines	Note
(Zhou *et al.*, 2005)	SGC-7901	The derivatives 10-nitro-goniothalamin and 10-amino-goniothalamin gave selective inhibition concentration of 1.10 and 1.14 µg/mL, respectively.
(Vendramini-Costa *et al.*, 2010)	UACC-62, MCF-7, NCI-460, OVCAR-03, PC-3, HT-29, 786-0, U251, NCI-ADR/RES	*In-vitro* screening of GTN both in racemic and in its enantiomeric; I-1 and (S)-1.Racemic, I and (S)-goniothalamin showed lower TGI values against 786-0 and NCI-ADR/RES. Racemic has displayed similar activity profile as I-1 and (S)-1 against NCI-ADR/RES, 786-0, NCI-460, PC-03, and U251 but, in analogy to I-1, it displayed higher potency against UACC-62, MCF-7, OVCAR-03, and HT-29 than (S)-1.
(Al-Qubaisi *et al.*, 2011, 2013)	HepG2	Inhibition of DNA synthesis, G2/M cell cycle arrest, induction of caspase 3.
(Kuo *et al.*, 2011)	HCC	ROS accumulation, DNA double-strand breaks, TP53 and/or PMAIP1 gene transactivation, TP53 and/or PMAIP1 protein translocation to mitochondria, the release of cytochrome c from mitochondria, and caspase cleavage.
(Chiu *et al.*, 2011)	NSCLC	DNA damage and reduction in the activity level of two major migration-associated matrix metalloproteinases, MMP-2 and MMP-9.

Table 2.2: Continued.

Previous studies	Cancer cell lines	Note
(Yen *et al.*, 2012)	Ca9-22	ROS induction, DNA damage, and mitochondria membrane depolarization.
(Alabsi *et al.*, 2012)	HT29	Cytotoxic with IC_{50} value of 3.8±1.10 µg/mL.
(Alabsi *et al.*, 2012, 2013)	Hela	Apoptosis as the mode of cell death, DNA fragmentation, DNA damage, caspase 9 activation, and a large increase in the sub-G1 and S cell cycle.
(Petsophonsakul *et al.*, 2013)	HL-60, U937	Apoptosis *via* intrinsic and extrinsic pathways. Expression of caspase 8, 9 and Smac/Diablo.
(Orlikova *et al.*, 2013)	K562	NF-κB inhibition activity and subsequent anti-inflammatory and anti-carcinogenic activity.
(Semprebon *et al.*, 2014)	NCI-H460	Induction of DNA damage by the downregulation of *BIRC5* gene without changing the mRNA levels of the TP53 and BAX genes.
(Khaw-On *et al.*, 2019)	MDA-MB-231	The non-apoptotic cell death mechanisms of necroptosis and anoikis.
(Braga *et al.*, 2020)	MCF-7	Utilising pH-Sensitive acetylated dextran (Ac-Dex) nanoparticles improved the anticancer activity and selectivity of GTN.

The cytotoxicity of GTN has been shown in earlier studies to affect a variety of cancer cell lines, including leukemic cells (Inayat-Hussain *et al.*, 2003, 2010; Rajab *et al.*, 2005; Orlikova *et al.*, 2013), liver cancer cells (Kuo *et al.*, 2011; Al-Qubaisi *et al.*, 2013), breast cancer cells (Ali *et al.*, 1997; Chen *et al.*, 2005; Wattanapiromsakul *et al.*, 2005) lung cancer cells (Wattanapiromsakul *et al.*, 2005; Chiu *et al.*, 2011; Semprebon *et al.*, 2014), oral cancer cells (Yen *et al.*, 2012), cervical cancer (Alabsi *et al.*, 2013), colon cancer cells (Wattanapiromsakul *et al.*, 2005; Alabsi *et al.*, 2012), ovarian cancer cell line (Lin & Pihie, 2003), pancreatic cancer (Ali *et al.*, 1997) and prostate cancer cells PC-3 (Vendramini-Costa *et al.*, 2010).

In contrast, the normal liver Chang cell (Al-Qubaisi et al., 2013), normal kidney cell (MDBK) (Lin & Pihie, 2003) and normal mouse fibroblast (3T3) (Alabsi et al., 2012) were reported to be less toxic to GTN. Multiple types of cell death, most notably apoptosis, were reported in most GTN-treated cancer cells (Alabsi et al., 2012; Al-Qubaisi et al., 2013). The regulation of the cancer cell cycle and induction of apoptosis by oxidative stress and activation of caspases was how GTN exerted its anticancer activity on various cancer cells (Seyed *et al.*, 2014). However, the non-apoptotic cell death mechanism was also observed in human MDA-MB-231 breast cancer cells induced by GTN *via* caspase-independent pathway by expressions of *rip1*, *rip3*, and *mlkl* genes (Khaw-On *et al.*, 2019).

The possibility of combining GTN with other biomaterials to improve their efficacy has recently gained attention. The incorporation of pH-Sensitive acetylated Dextran (Ac-Dex) nanoparticles improved GTN's anticancer activity and selectivity. Cellular uptake and morphology studies show that GTN@Ac-Dex NPs rapidly internalise into MCF-7 cells and cause cell death (Braga et al., 2020).

2.3 Bioactive glass (BG)

2.3.1 History

Larry Hench invented the first bioactive glass in 1969 at the University of Florida. Following a conversation with a US Army colonel on a bus ride, Hench began searching for a material that could bond to the bone. The colonel, who had just returned from the Vietnam War, inquired as to whether materials could be designed to withstand the hostile environment of the human body. All available implant materials at the time, including bioinert metals and polymers, caused fibrous encapsulation instead of forming a stable interface or bond with tissues (Jones, 2013).

Hench was intrigued by the topic of the colonel, even in the sense of its social consequences and came out later with two simple hypotheses; i) a foreign body reaction triggered by metals and synthetic polymers was due to components were entirely different from those that makeup living tissues, ii) the body does not reject a material capable of forming a bone-like hydroxyapatite coating on its surface, as hydroxyapatite is the major mineral component of ordinary bone tissue (Hench *et al.*, 1971; Hench, 2006).

These lead to the development of a degradable glass in the Na_2O–CaO–SiO_2–P_2O_5 system, high in calcium content and with a composition close to a ternary eutectic in the Na_2O–CaO– SiO_2 diagram (Hench, 2006). The original bioglass (45S5) composition is as follows: 45% silica (SiO2), 24.5% calcium oxide (CaO), 24.5%sodium oxide (Na_2O), and 6% phosphorous pentoxide (P_2O_5) in weight percentage (Krishnan & Lakshmi, 2013). It is referred to 45S5, which was later named Bioglass® and trademarked by the University of Florida. Since then, it has undergone extensive study as a biomaterial for repairing and replacing bone tissue (Jones, 2013).

A bioactive material is a material that stimulates a particular biological response at its interface, forming a bond between the material and the tissue (Asthana & Bhargava, 2014). The interest in bioactive materials is mainly due to their capacity to spontaneously produce an apatite layer when in contact with phosphate-containing physiological fluids (Hedge *et al.*, 2017). When a BG is implanted, it gradually dissolves, and the release of ions promotes the formation of a carbonated hydroxyapatite layer on its surface. This dissolution mechanism is aided by the low connectivity of the SiO_2 network due to the presence of sodium and calcium as network modifiers, resulting in the formation of non-bridging silicon-oxygen bonds (González *et al.*, 2003).

Briefly, surface cations Na and Ca are first exchanged with H^+ from the biological fluid, forming Si-OH bonds. Phosphate ions may also be released if they are present initially. Then, as the pH rises, more Si-OH bonds are formed due to the hydrolysis of Si-O-Si bonds, and they repolymerise, resulting in a glass surface depleted in Na and Ca cations. Then, the hydrolysis of Si-O-Si bonds due to a pH increase results in the formation of more Si-OH bonds, and they repolymerise, producing a glass surface devoid of Na and Ca cations. The migration of Ca^{2+} and PO_4^{3-} ions to the surface resulted in the formation of an amorphous layer of calcium phosphate, which then crystallised into biomimetic hydroxyapatite (Hench *et al.*, 1971; Gunawidjaja *et al.*, 2010).

2.3.2 Synthesis of bioactive glass 45S5

The original BG was developed by Hench using a conventional high-temperature melting process (Hench, 2006; Jones, 2013). Some BGs have been synthesised using this method, including 45S5, 13-93 and 46S6 with and without

doping other elements; Sr, Zn or Mg. *In vitro* and *in vivo* studies both confirmed the bioactivity of these BGs. However, volatile compounds such as P_2O_5 may evaporate and result in different compositions as this method requires high temperatures of more than 1300°C. In addition, the obtained glasses usually have dense structures with low specific surface area values, which may decrease the bioactivity of the BG (Sepulveda *et al.*, 2001; Oudadesse *et al.*, 2011; Jones, 2013).

The drawbacks of the melt-derived method in synthesising BG were overcome by soft chemistry strategies using the sol-gel method in the 1990s. Sol-gel technology enables the synthesis of bioactive glasses of similar composition at a much lower temperature than the conventional melting method (Zheng & Boccaccini, 2017). This procedure begins with the synthesis of a solution (sol) composed of metal-organic and metal salt precursors, is followed by the formation of a gel *via* chemical reaction or aggregation, and finally, thermal treatment for drying, organic removal, and in some cases, crystallisation and cooling treatment (Li *et al.*, 1991; Zheng & Boccaccini, 2017)

The gel-like texture describes the solvent being trapped inside the network. In the case of pure silica, the hydrolysis and poly-condensation of tetraethyl orthosilicate (TEOS) triggers the formation of primary colloidal nanoparticles, which later agglomerate to form a 3D, highly connected network in acidic conditions or spherical secondary particles in basic conditions (Vichery & Nedelec, 2016). Because glasses produced in both acidic and basic media are made of coalesced nanoparticles, they present a lot of interparticular interstices (Lin *et al.*, 2009). Therefore, the glasses synthesised by this sol-gel process have a higher porosity and surface area compared to those produced from the melting method (Sepulveda *et al.*, 2001). It has also been suggested that glasses made from sol-gel processing have increased bioactivity. This