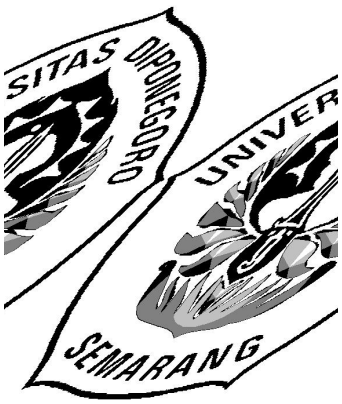


The Effects of Gorgonian *Isis hippuris* on Histologic Grade Score of Adenocarcinoma Mammae Tissue in C3H Strain Mice

RESEARCH FINAL REPORT

Submitted to fulfill the assignment
and fit-out requisite in passing
Undergraduate Education Program
Faculty of Medicine

By

Anisatusholihah
G2A005017

FACULTY OF MEDICINE
DIPONEGORO UNIVERSITY
SEMARANG
2009

APPROVAL SHEET

RESEARCH FINAL REPORT

The Effects of Gorgonian *Isis hippuris* on Histologic Grade Score of Adenocarcinoma Mammae Tissue in C3H Strain Mice

This Research Final Report was prepared and written by:

ANISATUSHOLIHAH
G2A005017

It was presented and examined by The Assessor Team of Research Final Report, Faculty of Medicine, Diponegoro University on 24th August 2009 and was revised afterwards.

Semarang, 26th August 2009

Head of Examiner,	Examiner,
dr. Awal Prasetyo, M.Kes, Sp. THT-KL	dr. Ika Pawitra Miranti, M.Kes, Sp. PA
NIP. 132 163 893	NIP. 131 875 465

Supervisor,

dr. Neni Susilaningsih, M. Si

NIP. 131 832 234

TABLE OF CONTENTS

THE LIST OF TABLE

THE LIST OF ATTACHMENT

The Effects of Gorgonian *Isis hippuris* on Histologic Grade Score of Adenocarcinoma Mammae Tissue in C3H Mice Strain

Anisatusholihah[1], Neni Susilaningsih[2]

ABSTRACT

Background: Breast cancer is the most common cancer in women worldwide. Gorgonian *Isis hippuris* is marine organism which its secondary metabolits compounds was predicted to have a role in cytotoxic activity that can be developed to be anticancer. In-vitro research proved anticancer activity of *Isis hippuris*. The aim of this study was to find out the effects of Gorgonian *Isis hippuris* extract in graded doses on histologic grade score of adenocarcinoma mammae tissue in C3H mice strain.

Methods: This experimental study utilized post test only control group design. Twenty C3H mice strain were divided into 4 groups. Treatment groups (P1, P2, and P3) received 500 mg of food pellets that contained 0.15 mg; 1.5 mg; and 15 mg of *Isis hippuris* extract. The control group (C) received no treatment. Treatment was routinely done for 3 weeks. Then all groups were inoculated with adenocarcinoma mammae and the treatment were continued. After another three weeks, all mice were terminated and operated to remove their tumors. Histologic grade score of adenocarcinoma mammae tissues were observed and scored according to *Elston and Ellis modification of the Scarff-Bloom-Richardson*.

Results: The highest mean of histologic grades score of adenocarcinoma mammae was 5.32 (SD 0.61) on C group while the lowest was 4.02 (SD 0.08) on P3 group. Kruskal Wallis Test presented significant differences with p value was 0.008 ($p<0.05$). There were significant differences between C group and P1 group ($p=0.009$), C group and P2 group (0.012); and also C group and P3 group ($p=0.009$). However, there were no significant differences among treatment groups.

Conclusion: Gorgonian *Isis hippuris* extract could decrease histologic grade score of adenocarcinoma mammae tissue in C3H strain mice. Gorgonian *Isis hippuris* extract could decrease histologic grade score of adenocarcinoma mammae tissue in C3H mice at any doses were used in this experiment.

Keyword: Gorgonian *Isis hippuris*, Histologic Grade Score, Adenocarcinoma Mammae

[1]Student of Faculty of Medicine, Diponegoro University, Semarang
[2]Lecturer of Histology Department, Diponegoro University, Semarang

Pengaruh Pemberian Ekstrak Gorgonian *Isis hippuris* Terhadap Skor Derajat Histologis Jaringan Adenokarsinoma Mammae Mencit C3H

Anisatusholihah[1], Neni Susilaningsih[2]

ABSTRAK

Latar Belakang: Kanker payudara merupakan keganasan yang paling sering dialami wanita di dunia. Gorgonian *Isis hippuris* merupakan biota laut dengan kandungan senyawa metabolit sekunder yang memiliki sifat toksisitas dan berpotensi sebagai zat antikanker. Penelitian in-vitro telah membuktikan aktivitas antikanker dari *Isis hippuris*. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian pemberian ekstrak Gorgonian *Isis hippuris* terhadap skor derajat histologis jaringan adenokarsinoma mammae mencit C3H.
Metode: Penelitian eksperimental ini menggunakan *Post Test Only Control Group Design*. Dua puluh ekor mencit strain C3H dibagi menjadi 4 kelompok. Kelompok perlakuan (P1, P2, dan P3) diberi pakan perlakuan sebanyak 500 mg yang masing-masing mengandung ekstrak *Isis hippuris* sebanyak 0,15; 1,5; dan 15 mg. Kelompok kontrol (C) hanya menerima pakan standar. Perlakuan dilakukan selama tiga minggu. Kemudian seluruh mencit diinokulasi sel kanker dan perlakuan dilanjutkan kembali selama tiga minggu berikutnya. Skor derajat histologis adenokarsinoma mammae dinilai dengan metode *Elston and Ellis modification of the Scarff-Bloom-Richardson*.
Hasil: Nilai rerata tertingggi skor derajat histologis adenokarsinoma mammae adalah 5.32 (SD 0.61) pada kelompok kontrol (C) sedangkan yang terendah 4.02 (SD 0.08) pada kelompok perlakuan P3. Uji Kruskal Wallis menunjukkan perbedaan bermakna dengan nilai p=0.008 (p<0.005). Terdapat perbedaan bermakna antara kelompok C dan P1 (p=0.009); antara C dan P2 (p=0.012); dan antara C dan P3 (p=0.009). Dalam kelompok perlakuan tidak terdapat perbedaan bermakna antar masing-masing kelompok dosis bertingkat.
Kesimpulan: Ekstrak Gorgonian *Isis hippuris* dapat menurunkan skor derajat histologis jaringan adenokarsinoma mammae mencit C3H. Semua dosis yang digunakan dalam penelitian ini dapat menurunkan skor derajat histologis.

Kata Kunci: Gorgonian *Isis hippuris*, skor derajat histologis, adenokarsinoma mammae.

[1)]Mahasiswa Fakultas Kedokteran Universitas Diponegoro
[2)]Staf Pengajar Bagian Histologi Fakultas Kedokteran Universitas Diponegoro

CHAPTER 1

INTRODUCTION

1.1. Background

Breast cancers which begin in breast tissue, are a group of diseases that cause cells in the body to change and grow out of control.[1-2] Breast is the most common site of cancer in women.[3-6] In Indonesia from 2004 to 2006, breast cancer is the most common type of cancer followed by cervical cancer.[7] Chemotherapy has become one of the important components for breast cancer therapy. Since there are high costs, unsatisfying, and multi-drug resistant for several types of breast cancers, finding alternative anticancer becomes necessary.[1-2]

The discovery of Indonesia marine organisms biomedical potency gave an alternative in development of cancer therapy.[8-9] One of Indonesia marine potency sources that is potential enough to be explored is Gorgonian *Isis hippuris*.[10-12] Gorgonian *Isis hippuris* has many secondary metabolites compound including *polyoxygenated steroids, sesquiterpene, hydrocarbon, phenol and fatty acids.*[12-21] Those compounds were predicted to have a role in cytotoxic activity which can be developed to be anticancer.[10-21]

Previous studies demonstrated the effect of *Isis hippuris* extract againsts the growth of leukemia cell line L1210.[10] Besides, in vivo toxicity experiments were done in graded doses based on the half maximum inhibitory concentration (IC_{50}) values.[22] This in vivo experiment about anticancer

activity of Gorgonian *Isis hippuris* extract was a continuation of those researches.

Cancer cell has uncoordinated cell growth and proliferative activity.[23] Histologic grade in breast cancer essentially describes proliferation and differentiation in breast cancer.[24] Pathologists closely observe three features when determining grade of breast cancer: tubule formation, nuclear pleomorphism, and mitotic count. Within the last decades, histologic grade has become widely accepted as a powerful indicator of prognosis in breast cancer.[25-26]

1.2. Research Problem

What are the effects of Gorgonian *Isis hippuris* extract on histologic grade score of adenocarcinoma mammae tissue in C3H mice strain?

1.3. Research Purposes

1.3.1. General Purpose

To find out the effects of Gorgonian *Isis hippuris* extract on histologic grade score of adenocarcinoma mammae tissue in C3H mice strain.

1.3.2. Specific Purposes

a. To compare the effects of Gorgonian *Isis hippuris* extract on histologic grade score of adenocarcinoma mammae tissue in C3H strain mice between treatment groups and control group.

b. To determine the most effective dose among three experimented doses of Gorgonian *Isis hippuris* extract.

1.4. Research Benefits

a. Providing information about the effects of Gorgonian *Isis hippuris* on breast cancer.

b. As a basic development of adenocarcinoma mammae alternative therapy.

c. As a preliminary study for further research and study about medical use of Gorgonian *Isis hippuris.*

CHAPTER 2

LITERATUR REVIEW

2.1. Gorgonian *Isis hippuris*

Gorgonian is the most familiar ordo of the *octocorallians* sub-class. Its class is *Anthozoa* and it is included in *Cnidaria (coelenterata)* phylum. Gorgonian colonies can take the form of whips, fans or bushy shrub-like colonies. They are often brightly colored and common in shallow water. Gorgonian *Isis hippuris* is soft coral, commonly called "sea fans" because the colony has many branches and it grows as fan. These marine invertebrates animals have an axial skeleton composed of an organic substance called gorgonin (a tanned collagen) and the thick layer, *coenenchyme*, protects its frame.[27-28]

Gorgonians are common and conspicuous members of reefs' faunas, which can be found in Andamas Sea, Philipina, Taiwan, Indonesia, Papua Nugini, and Ryukyu Archipelago. In Indonesia, based on research, these marine organisms can be found in some regions, such as Jepara Sea, Karimunjawa Archipelago, Krakatau Archipelago, Makassar Sea, and Flores Sea.[10]

Gorgonian *Isis hippuris* has many secondary metabolites compounds and it was predicted that Gorgonian *Isis hippuris* has a role in their defense mechanism so it also has toxicity.[10-11] High cytotoxic effect of Gorgonian *Isis hippuris* can be developed to be anticancer. Analysis results profound that

Gorgonian *Isis hippuris* extract contains at least 18 compounds which many of them are hydrocarbon, phenol and fatty acid.[10-11,16-18]

Previous studies on *Isis hippuris* resulted the isolation of a series novel metabolites, including *polyoxygenated steroids* and *suberosane-type sesquiterpene*.[16-18] *Polyoxygenated steroids* which are isolated from Gorgonian *Isis hippuris* have been determined based on its side chain into *hippurins* or *hippuristanols* having a spiroketal, *gorgosterol* or *gorgosteroid* possessing a cyclopropane, *hippuristerones* and *hippuristerol* containing a 3-keto function.[16-19] Some *hippuristanols* and *suberosane-type sesquiterpene* were reported to have significant cytotoxicity againts several cancer cell line.[19]

Five new *suberosane sesquiterpenes*: *suberosenol A, suberosenol B, suberasanone, suberosenol A acetat,* and *suberosenol B acetat,* along with the known *sesquiterpene subergorgic acid,* have been isolated from the Gorgonian *Isis hippuris*. *Suberosanone* exhibited potent cytoyoxicity toward several cancer cell lines. *Suberosenol A, suberasanone, and suberosenol B* acetat were found to exhibit potent cytotoxicity toward P-388, A549 (human lung adenocarcinoma), and HT-29 (human collon adenocarcinoma) cancer cell lines.[13,16]

Hippuristanols are steroids with spiroketal ring in the side chain. Two bioactive sterols, *hippuristanol* and *2a¹ 7a¹dihydroxy hippuristanol* showed cytotoxicity againts fibrosarcoma respectively. *Hippuristanol* was also reported to be active againts P-388 cell (lymphocytic leukemia in mice).[12]

Hippuristanols collected showed the ability to slow down, possibly prevent virus replication and may promise a cancer treatment. *Hippuristanol* is an inhibitor of eukaryotic translation initiation. It prevents eIF4A (eukaryotic initiation factor 4A) protein from binding to mRNA, which carries the code to make proteins from DNA to specific sites of protein synthesis in the cell.[29]

Gorgosterols, hippuristerol, and hippuristerones are other steroids that have been isolated from Gorgonian *Isis hippuris* which reported also have cytotoxicity againts cancer cell line although not significant. Recent studies resulted the isolation of novel *hippuristerones A-L*, *hippuristerol A-F*, and identified *gorgosterol*. Another bioactive compound that have been isolated from Gorgonian *Isis hippuris* is *gorgost-5-en-3ß,7ß,12ß-triol-11ß,15S-diasetat*, showed selective inhibition againts KB-C2 cell line (multidrugs resistant cancer cell).[14-15,20]

2.2. Mammary Glands

The mammary glands in the breasts are parts of reproduction system in women. The mammary glands are placed in the subcutaneous tissue overlying the pectoralis major and minor muscles. Female breast extends transversely from lateral border of sternum to the midaxillary line and vertically from second through sixth ribs. Breasts size and shape are determined by genetic, ethnic, and dietary factors. Inactive female breasts weight are 150-200 grams and active female breasts weight are 400-500 grams.[30]

The arterial supply of breast derives from *lateral thoracic arteries*, *thoracoabdominal arteries*, *posterior intercostal arteries*, *medial mammary arteries* and *anterior intercostal arteries*. The venous drainages of breast mainly flow to the *axillary vein*, and some drainages flow to the *internal thoracic vein*. Lymph passes from the nipple, areola, and lobules of the gland to the subareolar lymphatic plexus. Most lymph from the periphery quadrant drains to the axillary lymph nodes, while most lymph from central quadrant drains to parasternal lymph nodes or to opposite breast. The nerve of breast derived from anterior and lateral cutaneous branches of the forth until sixth *intercostal nerves*. Those are the vasculatures and nerves of the breast.[31]

The mammary glands are compound tubuloalveolar glands that consist of 15 to 20 lobes radiating out from the nipple and are separated from each other by adipose and collagenous connective tissue. The lactiferous ducts give rise to buds that form lobules of glandular tissue and converge toward the niple. These ducts, 2 - 4,5cm long, emerge independently in the nipple, which has 15-25 openings, each of them is about 0,5mm in diameter. Deep to areola, each duct has dilated portion, the lactiferous sinus, which is a small droplet of milk accumulate in the nursing mother.[32]

2.3. Adenocarcinoma Mammae

2.3.1. Epidemiology and Risk Factor

Breast cancer is the most common cancer in women worldwide.[3-6] The incidence of breast cancer continuous to increase, but mortality has stayed the same. It will be the number one of four leading causes projected of death globally in 2030.[33]

Indonesia's 2007 health profile reported that breast cancer is the most common type of cancer followed by cervical cancer.[7] Estimation of breast cancer incidence of Indonesia is 26 per 100.000 women.[4] In Central Java, based on hospital report programme, breast cancer is the most common cancer about 3.593 cases (43,91% from 8.182 cancer cases). The most cases occur in Semarang approximately 1.205 cases (33,53%).[34]

The primary risk factors that have been identified are sex, family history, genetics, age, previous medical history, hormones, a high-fat diet, alcohol intake, obesity, and environmental factors such as tobacco use and radiation.[1-2]

According to histologic representation, breast cancer can be classified as non invasive breast cancers or carcinoma in situ and invasive (infiltrative) breast cancers.[25] These are incidences of histologic diagnosis:

Table 1. Incidences of histologic diagnosis[25]

Type	*Incidence*
In Situ Carcinoma	**15%-30%**
Ductal carcinoma in situ	80%
Lobular carcinoma in situ	20%
Invasive carcinoma	**70%-85%**
Ductal carcinoma	59%
Lobular carcinoma	4%
Tubular/cribriform carcinoma	4%
Mucinous carcinoma	7%
Medullary carcinoma	8%
intracystic carcinoma	5%
other diagnoses	13%

2.3.2. Sign, Symptom and Treatment

Early-stage breast cancer typically produces no symptom when the tumor is small and most treatable. When breast cancer has grown to a size that can be felt, the most common physical sign is a painless mass. Few common signs and symptoms appear, include breast pain or heaviness and persistent changes to the breast, such as thickening, swelling, redness, and nipple abnormalities such as spontaneous discharge, erosion, inversion, or tenderness.[1-2]

Many factors which are considered in making an accurate diagnosis and treatment decisions are the type of breast cancer, size of tumor, stage, hormone receptor status, lymph node involvement, and

the tumor grade. A pathologist will take a sample of tissue from tumor, and examine it under a microscope. Tumor cells that look most like normal cells are given a low grade, while those that look the most abnormal are given a high grade. High-grade tumors are fast-growing, spreading (metastatic), and aggressive. Knowing tumor grade helps a doctor to decide the best treatments.[35]

Taking into account tumor size, stage, and hormone receptor status, lymph node involvement, and the tumor grade, treatment may involve lumpectomy or mastectomy with removal of some of the axillar lymph nodes (to obtain accurate information on stage of disease). It may also involve radiation therapy, chemotherapy (before or after surgery), hormone therapy (tamoxifen, aromatase inhibitors), or targeted biologic therapy. Targeted therapy with trastuzumab (Herceptin®) or lapatinib (Tykerb®) is sometimes used in women whose cancer tests positive for HER2/neu. Two or more methods are often used in combination. Numerous studies have shown that long-term survival rates after lumpectomy plus radiation therapy are similar to survival rates after mastectomy for women whose cancer has not spread to the skin, chest wall, or distant organs. Tumor grade can be used to know the spreading.[1-2,6]

Cancer medication in advanced stage is not only very difficult but also the result is very unsatisfied. Medication for advanced stage cancer patient needs sophisticated technology, skills, and high costs.

So, alternative therapy which is cheaper than that and available in abundant amount is very necessary.[1-2]

2.4. Carcinogenesis

Carcinogenesis involves damage-induced genetic mutations that produce cancers.[38] Many of the most powerful biological regulators of cell growth and proliferation are encoded by unstable mRNAs, which are targeted for rapid degradation by the cell. The loss of rapid degradation of these growth-promoting mRNAs can result in oncogenic transformation of the cell.[37]

There are several ways in which translational control is relevant to cancer. Firstly, the effciency of expression of key proteins involved in cell growth regulation, proliferation or cell death may be controlled at the translational level by changes in the activity of components of the protein synthesis machinery.[38-39] Secondly, mutations that lead to changes in the structure of individual mRNA species may alter the rates at which the proteins encoded by these mRNAs are produced.[37,39] Thirdly, disruptions in the regulation of signalling pathways that result in a loss of constraint on the synthesis of growth-promoting proteins (or impair the synthesis of growth-inhibitory or pro-apoptotic proteins) may alter the balance of production of key cellular components. Finally, infection of cells with tumour-associated viruses can result in interference with normal cellular controls on translation that may contribute to the transformed phenotype.[39]

Carcinogenic agents include mutagenic carcinogens, non-mutagenic carcinogens, irradiation, viruses (tumorigenic viruses), transforming retroviruses and DNA tumor viruses encode oncogenes, and genetic predisposition.[38] Carcinogenesis is typically resulted from a series of mutations that affect regulation of proliferation. These are the process of carcinogenesis; (1) inactivation of a tumor suppressor gene (TSG) results in cell proliferation, (2) mutation inactivates a DNA repair gene, (3) mutation of a proto-oncogene generates an oncogene, (4) mutation inactivates more cancer suppressor genes, resulting in cancerous proliferation.[38]

These are mutations lead to characteristics of cancer cells: [37-38]

a. Loss of normal cell differentiation or inability to undergo normal cell death (apoptosis).

In normal condition, cell divides and differentiates, passing through many steps. Growth factors that are known to stimulate transitions are indicated. Aberrant differentiation due to cancer can be caused by malignant cells that arise at any stage during the process of differentiation or cells are normal but they differentiate into different kind of cells.

b. Unregulated cell proliferation.

Cell proliferation is controlled by growth factors that bind to receptors on the cell surface that connect to signaling molecules (signal transduction pathway) that convey message from receptor to the nucleus where transcription factors bind to DNA, turning on or off the production of proteins that cause cells to continue dividing. Mutations in any of the genes encoding

these types of proteins can affect proliferation and many examples have been found in cancer cells. For example, several mutations affecting different proteins that regulate one part of the cell cycle (G1 to S phase) have been consistently seen in many tumors.

c. Genomic instability.

Cancer cells exhibit genomic instability. Chromosomal rearrangements and duplications are often seen in the karyotype of cancer cells. Cells normally will stop in the cell cycle if DNA is damaged. Several proteins have been identified that act to halt the cell cycle until DNA damage is repaired.

2.5. Immune Responses to Tumors

Tumors express antigens that are recognized by the immune system. The effector mechanisms of both cell-mediated immunity and humoral immunity have been shown to kill tumor cell in vitro. Effector of cell-mediated immunity are T-cells, Natural Killer cells, and Macrophages. Whereas cell-mediated immunity are more dominant, our body also produce antibody to respond tumor antigen.[38]

The T-cells response is the most important host response for the control of growth of antigenic tumor cells, it responsible for both direct killing of tumor cells and the activation of other component of the immune system. The principal mechanism of tumor immunity is killing tumor cells by $CD8^+$ CTL_s. Cytotoxic T Lymphocytes may perform a surveillance function by recognizing and killing potentially malignant cells hat express peptides

derived from mutant cellular proteins or oncogenic viral proteins and presented as foreign peptides in association with class I Major Histocompatibility Complex (MHC) molecules. CTL_s are distinguished by expressing the $CD8^+$ coreceptor on their surfaces. $CD8^+$ cells responses specific for tumor antigens may require cross presentation of the tumor antigens by Antigen Presenting Cells (APC_s). Most tumor cells are not derived from APC_s so stimulation of helper T cells is needed to promote the differentiation of $CD8^+$ T cells. The second major class of T-cells, the T-helper cells, express a coreceptor called CD4, which binds to class II MHC molecules. CD4 strengthens the interaction between T-cell receptor and the antigenic complex on Antigen Presenting Cells (APC_s). When antigenic triggering, these T-cells further secrete cytokines, such as tumor necrosis factor (TNF) and interferon-?)(IFN-?), that can increase tumor cell class I MHC expression and sensitivity to lysis by CTL_s. IFN-?pmay also activate macrophages to kill tumor cells.[38,40]

NK cells can kill a wide range of tumor targets in vitro. The cytolytic potential of NK cells is largely contained by off signals delivered via families of inhibitory receptors that bind to class I molecules on potential target. Cytolysis of NK cells is mediated by the release of cytotoxic factors and the use of perforins to puncture holes in the target cell membrane.[40]

Macrophages are important in tumor immunity as antigen presenting cells to stimulate the immune response to mediate tumor lysis. Their mechanisms include the release of lysosomal enzymes, cytokine TNF,

reactive oxygen intermediate, and nitric oxide. Macrophages are activated by Macrophages Activating Factors (MAF) which are secreted by T-cells.[40]

Tumor-bearing hosts may produce antibodies against various tumor antigens. Antibodies may kill tumor cells by activating complement or by antibody dependent cell-mediated cytotoxicity. Complement-antibody bind to the tumor cell membrane and promote attachment of complement components that create pores in the membrane, resulting cell disruption. An alternative mechanism is Antibody Dependent Cell-mediated Citotoxicity (ADCC), in which antibodies, form an intercellular bridge by binding via the variable region to a specific determinan on target cell.[38]

2.6. Histologic Grade

Within the last decades, histologic grade has become widely accepted as a powerful indicator of prognosis in breast cancer. Pathologists closely observe three features when determining a cancer grade: the frequency of cell mitosis (rate of cell division), tubule formation (percentage of cancer composed of tubular structures), and nuclear pleomorphism (change in cell size and uniformity). Each of these features is assigned a score ranging from 1 to 3 (1 indicates slower cell growth and 3 indicates faster cell growth). The scores of each of the cells' features are then added together for a final sum that will be ranged from 3 to 9. The Scarff-Bloom-Richardson (SBR) system is the most common type of cancer grade system used today.[25-26]

In Europe, the Elston-Ellis modification of the SBR grading system (Nottingham grading system) is preferred and is becoming increasingly popular in the US. This modification provides somewhat more objective criteria for the three component elements of grading and specifically addresses mitosis counting in a more rigorous fashion. For example hyperchromatic nuclear and apoptotic cells which are counted in the original SBR system are excluded in the Elston-Ellis modification and the area being assessed is specifically defined in square millimeters. These modifications have enhanced reproducibility of grading among pathologists and to a considerable extent have fostered acceptance of grading by clinicians.[25-26]

Table 2. Histologic grade score of adenocarcinoma mammae according to *Elston and Ellis modification of the Scarff-Bloom-Richardson*[25-26]

Score / Appearance	1	2	3
Tubule formation	>75%	10-75%	<10%
Nuclear pleomorphism	Small, uniform cell	Moderate increase in size and variation	Marked variation
Mitosis count	0-7	8-14	>15

The following is total score that indicates the grade of adenocarcinoma mammae:

Grade I (*well differentiated*) : 3-5

GradeII (*moderate differentiated)* : 6-7

Grade III (*poorly differentiated*) : 8-9

Histologic grade has been shown to be useful predictors of prognosis for patients stratified by stage of diseases. Increasing tumor grade has been associated with several factors that are related to an increased risk for recurrence after conservation therapy. In patients with relatively favorable stage I carcinomas treated by lumpectomy without radiotherapy, the tumor recurred sooner and with greater frequency in high grade carcinomas.[26]

Table 3. Histologic grade of adenocarcinoma mammae according to Scarff-Bloom-Richardson and survival rates[26]

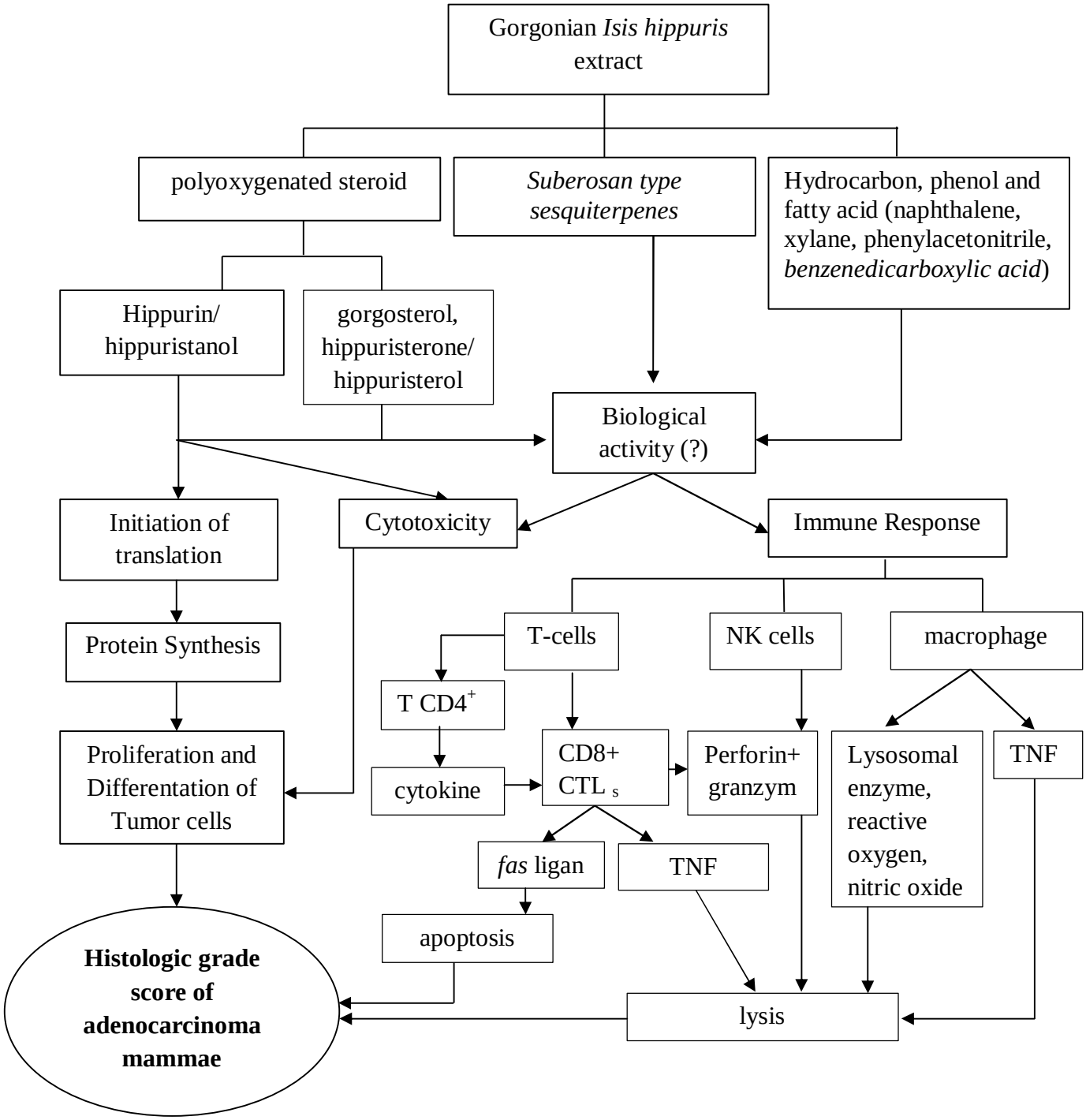
Grades	Description	Score	5 yr. survival	7 yr. survival
Grade 1 (lowest)	Well-differentiated breast cells; cells generally appear normal and are not growing rapidly; cancer arranged in small tubules.	3,4,5	95%	90%
Grade 2	Moderately-differentiated breast cells; have characteristics between Grade 1 and Grade 3 tumors.	6,7	75%	63%
Grade 3 (highest)	Poorly differentiated breast cells; Cells do not appear normal and tend to grow and spread more aggressively.	8,9	50%	45%

CHAPTER 3

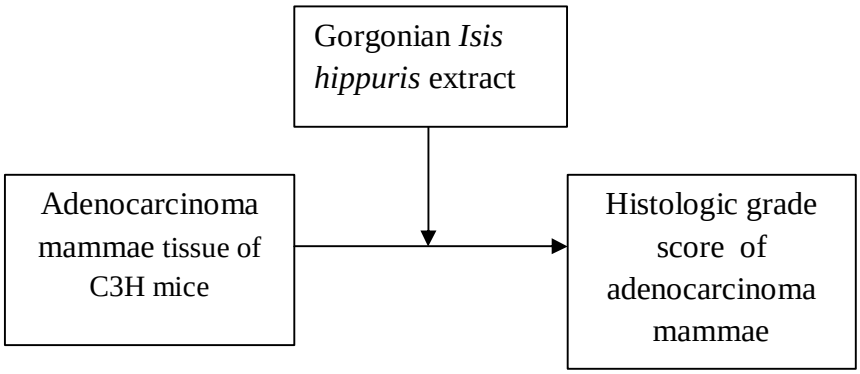
THEORITICAL FRAMEWORK,

CONCEPTUAL FRAMEWORK, AND HYPOTHESIS

3.1. Theoritical Framework



3.2. Conceptual Framework



3.3. Hypothesis

a. Gorgonian *Isis hippuris* extract have effects to decrease histologic grade score of adenocarcinoma mammae tissue in C3H mice strain.

b. The most effective dose among three experimented doses of Gorgonian *Isis hippuris* extract is 15 mg (the highest dose).