الجمهورية الجزائرية الديمقراطية الشعبية

PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA UNIVERSITY EL-OUED

DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

وزارة التعليم العالي والبحث العلمي

MINISTRY OF HIGHER EDUCATION AND

SCIENTIFIC RESEARCH

جامعة الشهيد حمه لخضر الوادي

ECHAHID HAMMA LAKHDAR UNIVERSITY OF EL-OUED

كلية علوم الطبيعة والحياة

Faculty of Natural and Life Sciences

قسم البيولوجيا الخلوية والجزيئية

Department of Cellular and Molecular Biology

MASTER'S DISSERTATION

For the Award of the Academic Master's Degree in Biological Sciences

Specialization: Applied Biochemistry

THEME

In Vitro and In Silico Investigation of the Interaction Between Vitamin D and α-Amylase: Insights into Their Potential Antidiabetic Synergy.

Submitted by:

Oeullabi sondous **Haroun nour el-islame**

President	**Dr .**	**M.C.A**	**El-Oued University**
Examiner	**Dr .**	**M.A.A**	**El-Oued University**
Supervisor	**Dr. Adaika Aicha**	**M.C.A**	**El-Oued University**
Co-Promoter	**Dr. Djarallah Marwa**		

University year: 2025/ 2026

REMERCIMENT

We would like to express our sincere gratitude and deepest appreciation to our supervisor, Dr. Adaika Aicha, for her continuous support, valuable guidance, and constant encouragement throughout the completion of this work.

Her patience, kindness, and dedication played a major role in helping us overcome many difficulties and challenges during this academic journey. She was always willing to help, guide, and support us whenever we needed, and she never hesitated to share her knowledge and advice with us.

We are truly grateful for all the efforts and time she devoted to helping us accomplish this dissertation in the best possible way. Beyond her scientific guidance, her understanding, encouragement, and positive attitude gave us confidence and motivation to continue despite moments of stress and fatigue.

It was truly an honor for us to work under her supervision, and no words can fully express our appreciation and respect for everything she has done for us. May Allah bless her, reward her abundantly, and grant her continued success in her career and life.

REMERCIMENT

With sincere appreciation and heartfelt gratitude, we dedicate these words to Dr. Marwa DJarallah, whose support and kindness accompanied us throughout this academic journey.

Your encouragement, valuable advice, and constant willingness to help have greatly contributed to giving us the confidence and motivation needed during the preparation of this dissertation. Your generosity in sharing your knowledge and your positive spirit have left a lasting and meaningful impression on both our work and ourselves.

This achievement would not have been the same without your guidance and support. We are deeply grateful for every effort you made, every piece of advice you offered, and every moment you devoted to helping us move forward.

Please accept our sincerest expressions of gratitude and respect, along with our warmest wishes for your continued success, happiness, and prosperity in all your future endeavors.

Dedication

بسم الله الرحمن الرحيم

﴿وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ﴾

إلى نفسي أولًا . . . لأنكِ كافحتِ بصمت، وصبرتِ كثيرًا، وآمنتِ بحلمكِ حتى النهاية، أهديكِ هذا الإنجاز الذي استحققتهِ بكل فخر.

إلى من لا ينفصل اسمي عن اسمه، ذلك الرجل العظيم الذي علّمني أن الدنيا كفاح، وغرس في روحي مكارم الأخلاق والقيم النبيلة...

إلى والدي الغالي، أطال الله في عمره وحفظه لي.

إلى من جعل الله الجنة تحت أقدامها، إلى من كان دعاؤها سر نجاحي، وحنانها بلسم جراحي، وقدوتي ومعلمتي الأولى وصديقة أيامي...

إلى أمي الحنونة، حفظها الله وأدامها نعمة في حياتي.

إلى من يشبه وجودهم الطمأنينة، إلى الوجوه التي لم أحتج يومًا لشرح تعبي أمامها فكانت تفهمني من صمتي، إلى من كانوا القوة حين ضعفت، والنور حين ازدحم الطريق بالتعب، حفظكم الله لي دائمًا، فأنتم أجمل ما منحني الحياة...

إخوتي الأحبة: منى، عبد الرؤوف، سمير، بلقيس، عبد البارئ، وعبد المهيمن.

إلى من كانت الأخت قبل الصديقة، ورفيقة البدايات التي لم تبخل يومًا بدعمها ووقوفها بجانبي صديقتي وسندي الدائم نور الاسلام، شكرًا لكِ على وجودك معي منذ البداية وإلى الان ♡

ولا أنسى صديقتي رميصاء، رفيقة البدايات التي كانت سندًا لي في هذا المشوار، شكرًا لكِ على وجودكِ الصادق.

إلى كل من ساندني بدعوة صادقة أو كلمة طيبة أو موقف جميل، أهديكم خالص الشكر والامتنان.

سندس

Dedication

إلى كل من كان له أثر جميل في حياتي، إلى الذين منحوني القوة والأمل في أصعب اللحظات...

إلى أمي الغالية،

أول وطنٍ عرفته، وأول قلبٍ آمن بي دون شروط، إلى من كانت تخفي تعبها كي لا أضعف، وتزرع الطمأنينة في قلبي بكلماتها ودعواتها في كل مرة أكاد أستسلم فيها، إلى كانت فرحتها بنجاحي أكبر من فرحتي أنا ...حفظكِ الله لي نعمة لا تزول

إلى أبي الغالي،

الرجل الذي علمني أن الطريق الصعب يستحق المحاولة، والذي كان دعمه الصامت أعظم قوة دفعتني للاستمرار، فكنتَ دائمًا السند والأمان والفخر

إلى إخوتي ابراهيم وياسين وعادل وأخواتي احلام وحكيمة ويمينة الذين جعلوا الأيام الثقيلة أخف، وكانوا بالقرب مني في لحظات القلق قبل الفرح، شكرًا لأنكم كنتم الجزء الجميل من هذه الرحلة

إلى صديقتي وحبيبتي سندس،

التي كانت جزءًا جميلًا من أيامي، ورفيقةً خففت عني الكثير من لحظات التعب والقلق، والتي كانت دائمًا قريبة مني في أكثر الأوقات التي كنت أحتاج فيها إلى الدعم والطمأنينة، فكان وجودها وحده كافيًا ليجعل الأيام الثقيلة أخف، واللحظات الصعبة أهون.

الى نفسي . . . .

إلى تلك النسخة مني التي عاشت أيامًا طويلة بين التعب والقلق والضغط،

التي حاولت كثيرًا، وتعثرّت أحيانًا، لكنها لم تتوقف يومًا عن المحاولة،

إلى نفسي التي سهرت، وصبرت، وتحملت لحظات الإنهاك والخوف من الفشل،

ثم نهضت في كل مرة وكملت الطريق رغم كل شيء.

أفخر بكِ لأنكِ وصلتِ، ولم تستسلمي.

نور الاسلام

Abstract

Résumé

الملخص

Abstract

This study aimed to investigate the synergistic effect between vitamin D and Centaurium erythraea extract as a promising natural strategy for reducing insulin resistance and its associated metabolic disorders through a combined In Vitro and In Silico approach. Particular emphasis was placed on evaluating the ability of this combination to modulate oxidative stress, inflammation, and glycation, which are considered major contributors to the development of insulin resistance and its complications.The study included the evaluation of antidiabetic activity through α-amylase inhibition assay, in addition to antioxidant, anti-inflammatory, antibacterial, BSA interaction, and anti-gastric ulcer activities. The obtained results revealed low IC50 values, particularly for the combination, reflecting higher biological activity compared to the individual compounds and confirming a clear synergistic effect between vitamin D and the plant extract. The BSA interaction assay also demonstrated a significant protective effect against protein oxidation and structural alterations associated with inflammation and glycation.In parallel with the experimental findings, molecular docking studies revealed low and negative ΔG values, indicating stable molecular complexes and high binding affinity toward the targeted proteins, especially for the combination. Furthermore, Swiss ADME and Pro Tox-II analyses demonstrated suitable pharmacokinetic and physicochemical properties with relatively low toxicity, supporting the therapeutic potential of this combination.Overall, the findings of this study suggest that the combination of vitamin D and Centaurium erythraea extract represents a promising natural approach that may contribute to reducing insulin resistance and improving metabolic balance through its antioxidant, anti-inflammatory, anti-glycation, and cytoprotective effects.

Résumé

Cette étude vise à explorer l'effet synergique entre la vitamine D et l'extrait de Centaurium erythraea en tant qu'approche naturelle prometteuse pour réduire l'insulinorésistance et les troubles métaboliques qui y sont associés, à travers une approche combinée In Vitro et In Silico. Une attention particulière a été accordée à l'évaluation de la capacité de cette combinaison à moduler le stress oxydatif, l'inflammation et la glycation, considérés comme des facteurs majeurs impliqués dans le développement de l'insulinorésistance et de ses complications.

L'étude a inclus l'évaluation de l'activité antidiabétique par le test d'inhibition de l'α-amylase, ainsi que des activités antioxydante, anti-inflammatoire, antibactérienne, de l'interaction avec la BSA et de l'activité anti-ulcéreuse gastrique. Les résultats obtenus ont montré de faibles valeurs d'IC50, particulièrement pour le mélange, traduisant une activité biologique plus élevée par rapport aux composés individuels et confirmant un effet synergique clair entre la vitamine D et l'extrait végétal. Le test d'interaction avec la BSA a également révélé un effet protecteur important contre l'oxydation des protéines et les altérations structurales associées à l'inflammation et à la glycation.Parallèlement aux résultats expérimentaux, les études de Molecular Docking ont révélé des valeurs de ΔG faibles et négatives, indiquant la formation de complexes moléculaires stables et une forte affinité de liaison avec les protéines cibles, notamment pour le mélange. De plus, les analyses Swiss ADME et ProTox-II ont montré des propriétés pharmacocinétiques et physicochimiques favorables avec une toxicité relativement faible, soutenant ainsi le potentiel thérapeutique de cette combinaison.Dans l'ensemble, les résultats de cette étude suggèrent que l'association de la vitamine D et de l'extrait de Centaurium erythraea représente une approche naturelle prometteuse pouvant contribuer à la réduction de l'insulinorésistance et à l'amélioration de l'équilibre métabolique grâce à ses effets antioxydants,anti inflammatoires, anti-glycation et cytoprotecteurs

الملخص

تهدف هذه الدراسة إلى استكشاف التأثير التآزري بين فيتامين D ومستخلص القنطريون الأحمر كاستراتيجية طبيعية واعدة للتقليل من مقاومة الإنسولين والاضطرابات الأيضية المرتبطة بها، وذلك من خلال مقاربة تجمع بين الدراسات In Vitroو .In Silico وقد تم التركيز على تقييم قدرة هذا المزيج على التأثير في الإجهاد التأكسدي، الالتهاب، والغلكزة باعتبارها من أهم العوامل المساهمة في تطور مقاومة الإنسولين ومضاعفاتها.

شملت الدراسة تقييم النشاط المضاد للسكري من خلال اختبار تثبيط إنزيمα-Amylase ، إضافة إلى النشاط المضاد للأكسدة، والمضاد للالتهابات، والمضاد للبكتيريا، واختبار التفاعل مع بروتينBSA ، إلى جانب النشاط الواقي من القرحة المعدية. أظهرت النتائج قيماً منخفضة لـ IC50 خاصة في المزيج، مما يعكس فعالية بيولوجية مرتفعة مقارنة بالمركبات الفردية ويؤكد وجود تأثير تآزري واضح بين فيتامين D والمستخلص النباتي. كما بين اختبار BSA قدرة مهمة على حماية البروتينات من الأكسدة والتغيرات البنيوية المرتبطة بالالتهاب والغلكزة وبالتوازي مع النتائج التجريبية، كشفت دراسات Molecular Dockingعن قيم سالبة ومنخفضة لـΔG ، مما يدل على استقرار المعقدات الجزيئية وارتفاع ألفة الارتباط بالبروتينات المستهدفة، خاصة في حالة المزيج.

كما أظهرت تحاليلSwiss ADME و Pro Tox-II خصائص دوائية مناسبة مع سمية منخفضة نسبيًا، مما يعزز الإمكانات العلاجية لهذا المزيج.

بصفة عامة، تشير نتائج هذه الدراسة إلى أن الجمع بين فيتامين D ومستخلص القنطريون الأحمر يمثل مقاربة واعدة قد تساهم في الحد من مقاومة الإنسولين وتحسين التوازن الأيضي من خلال تأثيراته المضادة للأكسدة، والالتهاب، والغلكزة، إضافة إلى دوره الوقائي على البروتينات والخلايا.

List of Figures

List of Figures

List of Figures

List of Figures

List of Figures

List of Table

Table of contents

Table of Contents

Table of Contents

Nomenclature

WHO: World Health Organization

UNESCO: United Nations Educational, Scientific and Cultural Organization

C. erythraea: Centaurium erythraea

SG: Secoiridoid Glycosides

DLD1: DLD-1 human colon adenocarcinoma cell line

DLD1-TxR: DLD-1 Taxol-resistant cell line

MeOH: Methanol

EtOH: Ethanol

ANS: Antipyretic

ANTIDIAB: Antidiabetic activity

CHOL: Choleretic

ETHNOMED: Ethnomedicine / Ethnomedicinal use

COSM: Cosmetic use

APP: Appetite stimulation

WHO: World Health Organization

UNESCO: United Nations Educational, Scientific and Cultural Organization

C. erythraea: Centaurium erythraea

SG: Secoiridoid Glycosides

DLD1: DLD-1 human colon adenocarcinoma cell line

DLD1-TxR: DLD-1 Taxol-resistant cell line

MeOH: Methanol

EtOH: Ethanol

STZ: Streptozotocin

ROS: Reactive Oxygen Species

SOD: Superoxide Dismutase

CAT: Catalase

GSH-Px: Glutathione Peroxidase

IDF: International Diabetes Federation

DM = Diabetes Mellitus

T1DM = Type 1 Diabetes Mellitus

T2DM = Type 2 Diabetes Mellitus

IFG = Impaired Fasting Glucose

NAFLD = Non-Alcoholic Fatty Liver Disease

Nomenclature

PCOS = Polycystic Ovary Syndrome

IR = Insulin Resistance

HOMA-IR = Homeostasis Model Assessment of Insulin Resistance

β-cells = Pancreatic Beta Cells

AGE = Advanced Glycation End-products

ROS = Reactive Oxygen Species

DAG = Diacylglycerol

TG = Triglycerides

NF-κB = Nuclear Factor kappa B

JNK = c-Jun N-terminal Kinase

PI3K = Phosphoinositide 3-Kinase

Akt = Protein Kinase B

MAPK = Mitogen-Activated Protein Kinase

IRS = Insulin Receptor Substrate

GLUT4 = Glucose Transporter type 4

PTPs = Protein Tyrosine Phosphatases

PTP1B = Protein Tyrosine Phosphatase 1B

SHP-1 = Src Homology region 2-containing Phosphatase 1

SHP-2 = Src Homology region 2-containing Phosphatase 2

EGFR = Epidermal Growth Factor Receptor

PDGFR = Platelet-Derived Growth Factor Receptor

IGF-1R = Insulin-like Growth Factor 1 Receptor

ER = Endoplasmic Reticulum

TNF-α = Tumor Necrosis Factor alpha

IL-1α = Interleukin-1 alpha

IL-6 = Interleukin-6

IL-10 = Interleukin-10

MCP-1 = Monocyte Chemoattractant Protein-1

Treg = Regulatory T cells

SOD = Superoxide Dismutase

CAT = Catalase

GSH-Px = Glutathione Peroxidase

VDR = Vitamin D Receptor

PTH = Parathyroid Hormone

Nomenclature

CYP2R1 = Vitamin D 25-hydroxylase

CYP27B1 = 1α-hydroxylase

CYP27A1 = Sterol 27-hydroxylase

CYP24A1 = 24-hydroxylase

DBP = Vitamin D Binding Protein

VDRE = Vitamin D Response Element

PDIA3 = Protein Disulfide Isomerase Family A Member 3

COX = Cyclooxygenase

COX-1 = Cyclooxygenase-1

COX-2 = Cyclooxygenase-2

COX-3 = Cyclooxygenase-3

RTK = Receptor Tyrosine Kinase

WHO: World Health Organization

ADA: American Diabetes Association

C. erythraea: Centaurium erythraea

ROS: Reactive Oxygen Species

SOD: Superoxide Dismutase

CAT: Catalase

GSH-Px: Glutathione Peroxidase

STZ: Streptozotocin

NF-κB: Nuclear Factor kappa B

JNK: c-Jun N-terminal Kinase

PI3K: Phosphoinositide 3-Kinase

Akt: Protein Kinase B

MAPK: Mitogen-Activated Protein Kinase

IRS: Insulin Receptor Substrate

PTH: Parathyroid Hormone

VDR: Vitamin D Receptor

DBP: Vitamin D Binding Protein

CYP2R1: Cytochrome P450 2R1

CYP27B1: Cytochrome P450 27B1

CYP27A1: Cytochrome P450 27A1

EGFR: Epidermal Growth Factor Receptor

PDGFR: Platelet-Derived Growth Factor Receptor

Nomenclature

IGF-1R: Insulin-Like Growth Factor 1 Receptor

Shc: Src Homology 2 domain-containing protein

Gab-1: GRB2-associated-binding protein 1

Cbl: Casitas B-lineage lymphoma proto-oncogene

mTORC1: Mechanistic Target of Rapamycin Complex 1

mTORC2: Mechanistic Target of Rapamycin Complex 2

FOXO1: Forkhead Box O1

PDK1: Phosphoinositide-dependent kinase-1

RAAS: Renin-Angiotensin-Aldosterone System

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance

BMI: Body Mass Index

TNF-α: Tumor Necrosis Factor alpha

IL-1α: Interleukin-1 alpha

IL-6: Interleukin-6

IL-10: Interleukin-10

MCP-1: Monocyte Chemoattractant Protein-1

COX: Cyclooxygenase

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

COX-1: Cyclooxygenase-1

COX-2: Cyclooxygenase-2

COX-3: Cyclooxygenase-3

IFG: Impaired Fasting Glucose

NAFLD: Non-Alcoholic Fatty Liver Disease

PCOS: Polycystic Ovary Syndrome

T1DM: Type 1 Diabetes Mellitus

T2DM: Type 2 Diabetes Mellitus

AGEs: Advanced Glycation End-products

AGE: Advanced Glycation End-product

MS: (as written in text: “MandS”, likely Multiple Sclerosis context)

AGEs: Advanced Glycation End Products

ROS: Reactive Oxygen Species

BSA: Bovine Serum Albumin

DNA-AGEs: DNA Advanced Glycation End Products

RCS: Reactive Carbonyl Species

Nomenclature

SOD: Superoxide Dismutase

CAT: Catalase

GSH-Px: Glutathione Peroxidase

STZ: Streptozotocin

ALT-711: Alagebrium (AGE breaker compound)

DJ-1: Protein deglycase DJ-1

PI3K: Phosphoinositide 3-Kinase

Akt: Protein Kinase B

mTOR: Mammalian Target of Rapamycin

IRS: Insulin Receptor Substrate

MAPK: Mitogen-Activated Protein Kinase

ERK: Extracellular Signal-Regulated Kinase

Grb2: Growth factor receptor-bound protein 2

SOS1: Son of Sevenless 1

Raf: Rapidly Accelerated Fibrosarcoma kinase

MEK: MAPK/ERK Kinase

GLUT4: Glucose Transporter Type 4

TNF-α: Tumor Necrosis Factor alpha

IL-6: Interleukin 6

RBP4: Retinol Binding Protein 4

DAG: Diacylglycerol

PKC: Protein Kinase C

HSA: Human Serum Albumin

PIP2: Phosphatidylinositol 4,5-bisphosphate

PIP3: Phosphatidylinositol 3,4,5-trisphosphate

PDK1: Phosphoinositide-dependent kinase-1

AMPK (not mentioned but related—skip if strict)

ADME: Absorption, Distribution, Metabolism, Excretion

kcat: Catalytic constant

Km: Michaelis constant

General Introduction

General Introduction

Insulin resistance is considered one of the major metabolic disorders involved in the development of Type 2 Diabetes Mellitus (T2DM). It is characterized by a reduced cellular response to insulin, particularly in muscle, liver, and adipose tissues, leading to disturbances in glucose and lipid metabolism as well as impairment of energy homeostasis. As this condition persists, compensatory insulin secretion progressively increases, eventually causing pancreatic β-cell exhaustion and the development of chronic metabolic complications affecting several vital organs. Insulin resistance is closely associated with oxidative stress and chronic low-grade inflammation, where excessive accumulation of reactive oxygen species (ROS) activates several inflammatory pathways such as NF-κ B, MAPK, and JNK, which contribute to the impairment of insulin signaling and aggravation of metabolic dysfunction.

In addition, advanced glycation end products (AGEs) play a central role in the progression of insulin resistance and diabetic complications. Their accumulation alters protein structure and function and promotes interaction with RAGE receptors, leading to enhanced oxidative stress and inflammatory mediator production. Consequently, cellular dysfunction increases and insulin resistance worsens. Therefore, the search for natural compounds capable of reducing oxidative stress, inhibiting inflammation, and protecting proteins from oxidation and glycation has become an important focus in antidiabetic research.

In this context, medicinal plants have attracted considerable attention due to their richness in biologically active compounds, particularly polyphenols known for their antioxidant and anti-inflammatory properties. Centaurium erythraea is considered one of the promising medicinal plants because it contains several bioactive compounds such as flavonoids, phenolic acids, stilbenes, and lignans, which possess strong free radical scavenging activity, antioxidant potential, and protein-protective effects. These compounds are also known for their ability to modulate oxidative stress-related pathways and improve metabolic balance.

On the other hand, the role of vitamin D is no longer limited to calcium and phosphorus homeostasis. Recent studies have demonstrated its important involvement in metabolic regulation and insulin sensitivity improvement through the presence of vitamin D receptors (VDR) in insulin-sensitive tissues and pancreatic β-cells. Vitamin D also contributes to reducing oxidative stress and inflammation while regulating the expression of genes associated with insulin signaling. Moreover, several studies suggest that combining vitamin D with polyphenol-rich plant extracts may produce a synergistic effect capable of enhancing antioxidant, anti-inflammatory, and cytoprotective activities against insulin resistance-related disorders.

Based on these considerations, the present work aims to investigate the interaction between vitamin D and α-amylase using both in vitro and in silico approaches, in addition to evaluating the biological properties of Centaurium erythraea extract, particularly its antioxidant, anti-inflammatory, antibacterial, and protein interaction activities. This study also aims to explore the potential synergistic effect between vitamin D and the plant extract in reducing oxidative stress and mechanisms associated with insulin resistance through the integration of experimental findings and molecular computational analyses to better understand the possible biological effects of this combination.

Part I: Bibliographical Synthesis

Chapter I: Bibliographic Study of Centaurium erythraea, Diabetes Mellitus and α-Amylase Enzyme

I. Medicinal plants and diabetes:

I.1. Phytotherapy:

Phytotherapy refers to the use of therapeutic practices based on the use of medicinal plants for the purpose of preventing and treating various diseases. It is generally classified among alternative medicines according to the World Health Organization (WHO) **(UNESCO, 1960).**

From an etymological perspective, the term phytotherapy originates from two Greek roots:

phyton, meaning plant, and Therapeia, meaning treatment **(Limonier, 2018).**

From a scientific standpoint, phytotherapy can be defined as a therapeutic discipline based on the use of plants, plant parts, or plant-based preparations, administered either internally or externally, with the aim of preventing or treating certain functional disorders as well as various pathological conditions **(Wichtl et Anton, 2003).**

I.1.1 The advantages of phytotherapy:

Phytotherapy presents numerous advantages that explain its increasing use **(Grenez, 2019).**

I.1.1.1 From a public health perspective:

it is generally associated with low iatrogenicity. Moreover, it does not induce drug dependence requiring with draw all upon discontinuation of treatment.

I.1.1.2 From an ecological and environmental perspective:

plants are natural entities whose constituents are metabolized and subsequently reintegrated into the environment. In contrast, drugs derived from the chemical industry may exhibit potential environmental toxicity.

I.1.1.3 From an economic perspective:

plant-based medicines are generally less expensive than conventional drugs. However, it should be noted that they are typically not reimbursed by social security systems **(Grenez, 2019).**

I.1.2 Different Types of Phytotherapy:

Several types of plant-based therapies can be distinguished **(Zaiband, 2016):**

I.1.2.1 Gemmotherapy:

it is based on the use of embryonic plant tissues, particularly buds, young shoots, and rootlets, which are rich in active compounds.

I.1.2.2 Phytotherapy:

it involves the use of different parts of plants (roots, leaves, flowers, or the whole plant), administered in various galenic forms adapted for therapeutic use.

I.1.2.3 Aromatherapy:

it consists of the use of essential oils obtained through various extraction processes, particularly distillation, and characterized by a high concentration of active compounds **(Vernex, 2011**).

I.1.2.4 Pharmaceutical phytotherapy:

it relies on plant-derived preparations obtained by extraction, generally diluted in ethyl alcohol or other solvents. These extracts are standardized and dosed to ensure a rapid and sustained therapeutic effect. They are available in various pharmaceutical form such as syrups, drops, or capsules **(Strang, 2006).**

I.1.3 Medicinal Plants and Active Compounds:

Medicinal plants are defined as plant-based drugs in which at least one part possesses pharmacological properties **(Boulfous et al., 2012).** They contain one or more active compounds capable of preventing, alleviating, or treating diseases **(Benghanem et Amara, 2012).**

More broadly, a medicinal plant is any plant that exerts a therapeutic effect on the human body without being toxic at normal doses. It is estimated that around 35,000 plant species are used worldwide for medicinal purposes, representing the largest reservoir of biodiversity exploited by humans. Despite the growing influence of modern healthcare systems, medicinal plants continue to mean an important therapeutic demand **(Boulfous et al., 2012).**

In addition, these medicinal plants may also be used for nutritional, condiment, or hygienic purposes **(Jean-Y, 2010).**

I.1.4 Pharmaceutical Properties:

Centaurium erythraea is well known for its medicinal virtues, mainly due to its richness in bioactive compounds. These include alkaloids (notably gentianine), xanthone derivatives

(Valentao et al., 2002), and bitter secoiridoids **(Piatczak et al., 2005, 2006).** The plant also contains other natural compounds such as triterpenes **(Aquino et al., 1985),** tannins, and phenolic acids **(Hatjimanoli et al., 1977),** in addition to waxes and resins.

Numerous scientific studies have confirmed its therapeutic properties. Since ancient times, the plant has been recognized for its wound-healing effects. Gentianine, an alkaloid present in the plant, exhibits strong anti-inflammatory activity.

Moreover, the methanolic extract of Centaurium erythraea has demonstrated a hepatoprotective effect in intoxicated rats **(Mroueh et al., 2004).** In certain cases of diabetes, the plant is used to stimulate pancreatic activity **(Hamdi Pacha et al., 2007).** Furthermore, gentiopicrine, one of its major constituents, has been identified as having antimalarial properties.

A study conducted by Jbilou revealed that the methanolic extract of Centaurium erythraea was highly effective against larvae of Tribolium Castaneum, achieving a mortality rate of 63% after only 10 days of exposure. This suggests a potential vermifuge effect of the plant.

Additionally, Centaurium erythraea exhibits several other biological activities, including antibacterial **(Kumarasamy, 2003-2004),** antipyretic **(Lacroix et al., 1973; Berkan et al., 1991),** antioxidant **(Valentao et al., 2003),** and diuretic effects **(Haloui et al., 2000).**

I.1.5 Galenic Forms of Medicinal Plants:

Galenic forms are designed to facilitate the administration of al active compounds present in medicinal plants **(Mansour, 2015).** Phototherapeutic products are prepared from fresh plants, dried plants, or plant extracts.

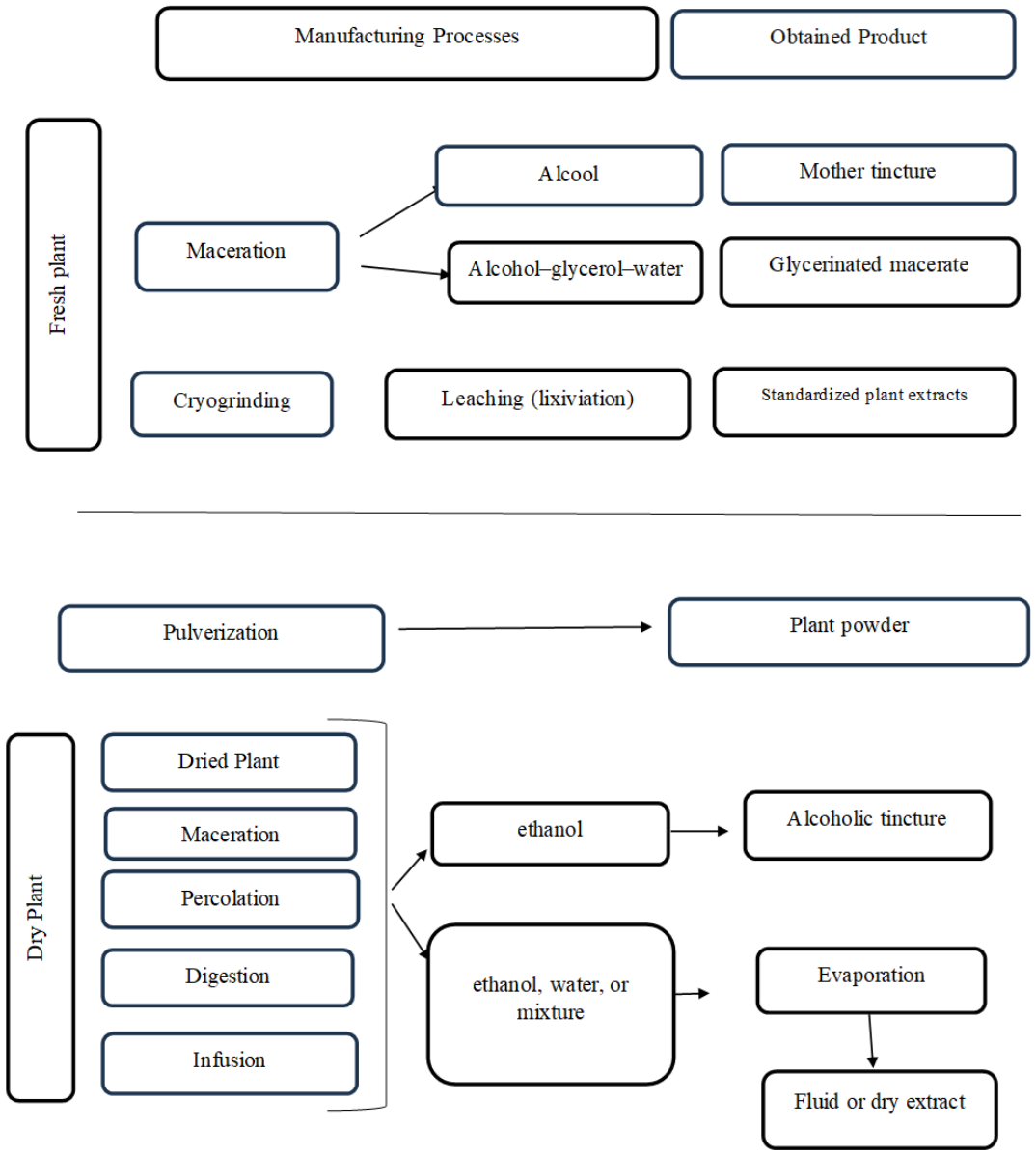


Figure 01: Processing methods of medicinal plants (Gibellin, 2003; Mansour,2015).

I.2. Plant description:

Centaurium erythraea is an annual or biennial herbaceous plant that can reach a height of 20–60 cm. This species belongs to the Gentianaceae family, and the genus Centaurium includes about 28 species **(El Menyiy, N et al., 2021).**

It has a slender, quadrangular stem. The cauline leaves are entire, pale green, and ovate in shape. These leaves are opposite, sessile, and show three main veins. The basal leaves are larger, elongated, and arranged in a basal rosette.

The inflorescence is dense and corymb-like (corymb), bearing grouped flowers. The calyx consists of five narrow, pointed sepals, while the corolla is tubular and dark pink, with

five deep lobes. The flower also contains five stamens, whose anthers become spirally twisted at maturity **(Baba Aissa, F et al., 1999),** forming characteristic clusters at the top of the stems.

The fruits are cylindrical capsules that exceed the calyx in length. They contain parietal placentas bearing numerous very small reddish seeds.

Figure02: the partie of Centaurium erythraea (Lorrain, M et al.,1978)

Table 01: Toxonomic data

Kingdom	Plantae
Division	Magnoliopsida
Order	Gentianales
Family	Gentianaceae
Genus	Centaurium
Species	C. erythraea
Common Name	Small centaury
French	Centaurium erythraea (Rafn.; Erythraea centaurium)
English	Common centaury or American centaury
Arabic	Merarand el h'nech, goustt el haïa

I.2.1 Chemical Composition:

The plant contains a variandy of bioactive compounds, including:

- **Glycosides:** Erythrocentaurin, gentiopicrin, Erythaurin
- **Alkaloids:** erythrizine, gentianine

Essential oils (present at 0.08–0.2%), including:

- Citral (50%)
- Geraniol (30%)
- Nerol (7%)
- Citronellol (4%)
- Thymol (0.2%)

Other active substances: lemon essence, sesquiterpenes, and.

Acids: ascorbic acid, oleanolic acid

Flavonoids: luteolin, rutin, kaempferol, cosmozein, apigenin, apiin, quercandin

Other constituents: phytosterols, resins **(Zubtsova I.V et al.,2020).**

I.2.2 Biological Effects of Centaurium Erythraea

Centaurium erythraea is widely recognized in traditional medicine and is listed in the pharmacopoeia of 23 countries, and was named Medicinal Plant of the Year in 2004 **(Tahraoui A, Z. H et al., 2010).** It is mainly used to stimulate gastrointestinal motility and prevent gastric lesions **(Cunha, A.P et al., 2003; Tuluce, Y et al., 2011).**

The aerial parts (herba Centauri) are used as herbal tea or extracts, showing multiple pharmacological activities such as analgesic, anti-inflammatory, and antidiabetic effects **(Hänsel, R et al., 2010; Berkan T et al., 1991; Haloui M et al., 2000; Hamza N et al., 2011).** Studies also report anticancer activity against colon cancer cells, including resistant lines, mainly due to xanthone compounds like eustomin.

Additionally, extracts show antibacterial, antioxidant, and antimicrobial properties **(Kumarasamy, Y et al., 2003; Chou O et al., 2021).** Its medicinal effects are mainly attributed to phenolic compounds, especially secoiridoid glycosides, used in treating digestive disorders and loss of appetite **(Hänsel, R et al., 2010; Grünwalld et al., 2000).**

I.2.3 Traditional Uses:

According to German herbalists, small centaury was traditionally recommended for treating melancholy and as a nervous system sedative. In Egypt, the plant is used to manage hypertension and kidney stones. In Algeria, it is employed as a febrifuge, tonic, and choleretic agent **(Baba Aissa, F, 1991; Baba Aissa, F, 2000).** A decoction of the whole plant has been used to treat indigestion, jaundice, wounds, ulcers, urinary retention, colic, and diabetes

mellitus (Gjoshe Stefkov et al., 2014), and it is also applied as a lotion to reduce hair loss **(Douira, J. B et al., 2002).**

Beyond its ethnomedicinal uses, Centaurium erythraea is also incorporated into certain cosmetic preparations due to its soothing and astringent properties **(Stoiko Liliya, D. I. et al., 2017).**

Table 02: Biological Effects of Centaurium Erythraea

Traditional Use	Part Used	Preparation Method	References
Antipyretic	Leaves and whole plant	Infusion and decoction	(Di Novella, R et al,2013; Guarrera, P.M,2005)
Diabetes	Whole plant	Infusion and decoction	(Gaspar, N et al.,2002; Benkhnigue, O et al.,2014)
Anti-bacterial	Aerial parts	Not specified	(Kumarasamy, Y et al.,2003)
Digestive	Leaves and flowers	Decoction	(El-Hilaly, J et al.,2003)
Kidney diseases	Leaves and flowers	Decoction	(El-Hilaly, J et al.; 2003)
Anti-coagulant	Aerial parts	Not specified	(Ageland, A et al.,2003)
Dyspepsia and loss of appandite	Aerial parts	Not specified	(Menkovi´c, N et al.;2011)
Skin diseases	Flowers	Maceration	(Bouyahya, A et al.,2017)
Anti-inflammatory	Aerial parts	Infusion	(Chou O et al.,2021)
Anti-oxidants	Aerial parts	Infusion	(Chou O et al.,2021)
Anti-cancer	Aerial parts	Infusion	(Trifunović-Momčilov, M et al.,2017)

I.2.4 Natural plant products and their biological activities:

Plants are capable of producing a wide variant of natural compounds with numerous industrial applications, particularly in the fields of food, cosmetics, and dermatology. They currently play a significant role in therapeutic applications, with more than 100,000 identified substances **(Haioun et Hamoudi, 2015).**

Natural plant-derived products are generally classified into two main categories: primary Metabolism and secondary Metabolism.

I.2.4.1 Primary metabolism

Primary metabolism are organic molecules found in al plant cells, where they play an essential role in maintaining the plant's survival **(Donatien, 2009).**

These compounds are considered constitutive and permanent, as they are directly involved in the fundamental pathways of cellular metabolism, making them indispensable for cell survival **(Elkolli, 2017).**

They are classified into four main categories:

- nucleic acids;
- carbohydrates, which serve as an energy source and contribute to cell wall structure;
- lipids, involved in energy storage and the formation of cellular membranes;
- amino acids, which are the primary building blocks for protein synthesis.

I.2.4.2 Secondary Metabolism

Most plant species distributed worldwide possess therapeutic properties due to the presence of bioactive compounds that act directly on biological systems. These plants are widely used in both conventional medicine and phytotherapy, as they offer several advantages that are not always provided by synthetic drugs **(Ticli, B et al,1993)**

From primary metabolism, plants have evolved complex networks of secondary biosynthetic pathways responsible for the production of a wide diversity of secondary metabolites. This large chemical diversity is mainly generated through three major biosynthetic routes. Each pathway involves a limited number of key metabolites, which are subsequently converted into numerous derivatives through a series of enzymatic reactions. In some cases, the synthesis of a single compound, such as benzophenanthridine alkaloids, may require up to 20 enzymatic steps

(Ticli, B et al,1993)

I.2.4.3 Polyphenols

Polyphenols represent an important group of plant secondary Metabolism. The main classes of this family include phenolic acids, flavonoids **(Oliveira L.D.L.D. et al,2014)** and tannins **(Crozier A et al.,2009)**

I.2.4.4 Flavonoids

Flavonoids are widely distributed plant secondary metabolism belonging to the large polyphenol family, which comprises nearly 8,000 natural compounds **(Stalika et al.,2007).** Structural, al flavonoids share a common carbon skeletal composed of fifteen carbon atoms. This structure consists of two aromatic rings (A and B) linked by a three-carbon chain (C3), forming a central heterocyclic ring (C) **(Tapas et al.,2008)**.

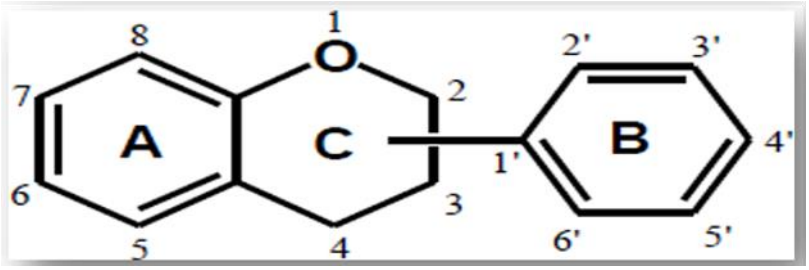


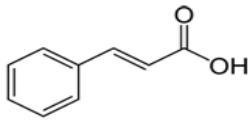
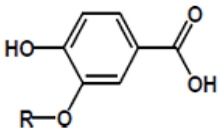
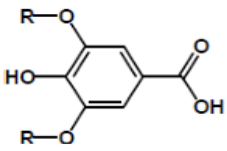
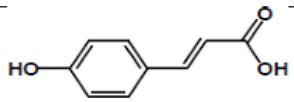
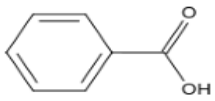
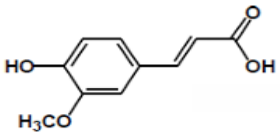
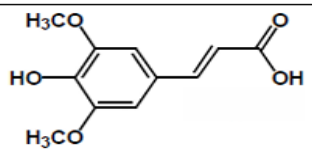
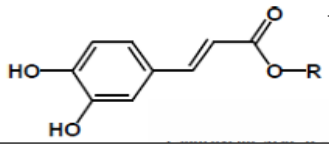
Figure03: Basic Structure of Flavonoids (Di Carlo G et al.,1999)

The main classes of flavonoids include flavonols, flavones, flavanones, flavan-3-ols, isoflavones, and anthocyanins **(sadasivan S et al.,2003)**

I.2.4.5 Phenolic Acids:

Phenolic acids are widely distributed in several agricultural and medicinal plants **(Psotova J et al.,2003).** They belong to the non-flavonoid polyphenol group and can be classified into two main categories: benzoic acid derivatives and cinnamic acid derivatives **(Psotova J et al.,2003).**

Table03: Structures and classification of some phenolic acids (Tsao R et al.,2010).

class	structur	Phenolic acid
Cinnamic acid		Vanillic acid (R=OCH3) Protocatochnic
		
		Gallic acid (R=H) Syringic acid (R=COH3)
		p-coumaric acid
Benzoic		
		Ferulic
		Sinapic
		Caffeic (R=H) Chlorogenic (R=5-

Fruits and vegetables contain high levels of free phenolic acids, whereas in cereals and seeds these compounds are predominantly present in bound forms. These phenolic acids can only be released through acid or alkaline hydrolysis, or enzymatic processes. They exhibit several biological activities, including antioxidant, antimicrobial, anti-inflammatory, and chelating effects **(Mandal S.M et al.,2010)**

I.2.4.6 Tannins:

are plant-derived polyphenolic compounds widely distributed in almost all plant parts, including bark, wood, leaves, fruits, and roots **(Berthod A et al.,1999).** They exhibit a broad spectrum of biological activities such as antioxidant,

anti-inflammatory, antifungal, antitumor, antiviral, and antidiarrheal effects **(Brunandon J et al.,1999)**

tannins are classified as plant secondary metabolism into two main groups: hydrolysable tannins and condensed tannins **(Saric T et al.,2015).** Hydrolysable tannins have a molecular weight ranging from 500 to 5000 Da and are characterized by strong antioxidant activity as well as an astringent effect **(Arapites P.,2012; Shahat A.A et al.,2013).** Condensed tannins, also known as proanthocyanidins, are high-molecular-weight polymers that can reach up to 30,000 Da.

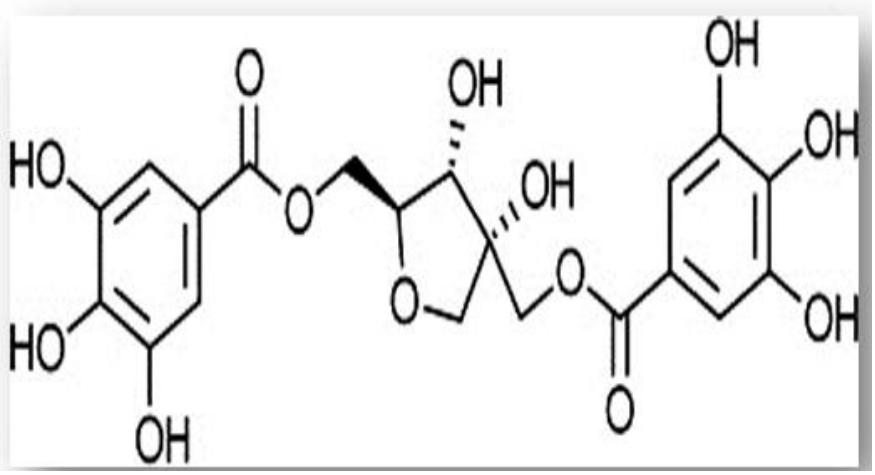


Figure04: Structure of tannins (Oroian M et al,.2015)

I.2.4.7 Terpenoids:

Terpenoids are a class of natural compounds built from isoprene units containing five carbon atoms (C5H8). They are classified according to the number of isoprene units into several groups.

Monoterpenoids consist of two isoprene units and include compounds such as geranyl pyrophosphate, eucalyptol, limonene, citral, camphor, and pinene. Sesquiterpenoids are composed of three isoprene units, including artemisinin, bisabolol, and farnesol. Diterpenoids contain four isoprene units, such as retinol and retinal. Triterpenoids are formed from six isoprene units, including lanosterol and squalene. Finally, tetraterpenoids contain eight isoprene units and include acyclic compounds such as lycopene, monocyclic compounds such as gamma-carotene, and bicyclic compounds such as alpha- and beta-carotenes **(Kashani A.H et al.,2012).**

I.2.4.8 The alkaloids:

are nitrogen-containing plant compounds with complex structures and high molecular weight, including various bases and quaternary ammonium salts with heterocyclic rings. Along

with coumarins, they are key secondary Metabolism widely found in the rutaceae family, especially in the genus **(Süntar I et al.,2020).**

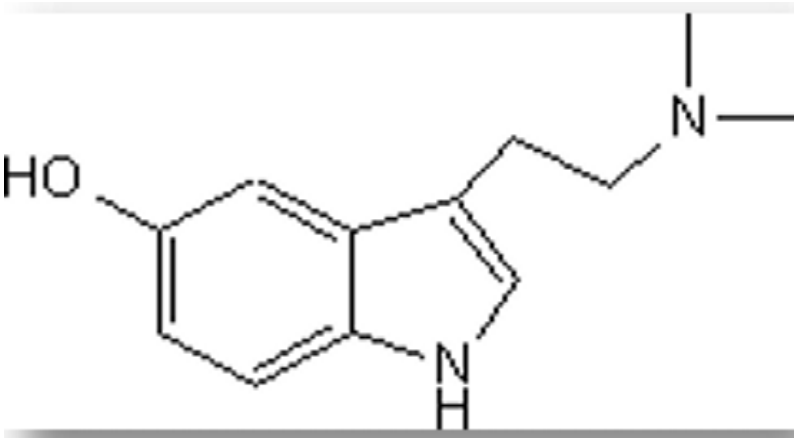


Figure05: General structure of an alkaloid (Meradji Amira Nesrine et Ouchtati Samah.,2024)

II. General overview of diabetes:

Blood glucose refers to the concentration of glucose in the blood, which is the main energy source of the body. Glucose has two origins:

- An exogenous source: dietary carbohydrates.
- An endogenous source: hepatic glucose produced through two metabolic pathways, glycogenolysis and gluconeogenesis.

In the fasting state, blood glucose is about 5.5 mM (1 g/L). After a carbohydrate-rich meal, it temporarily rises to 1.2–1.3 g/L. After a 24-hour fast, it decreases to around 0.6–0.7 g/L. The maintenance of blood glucose homeostasis mainly depends on insulin and glucagon.

An increase in blood glucose rapidly stimulates insulin secretion while simultaneously inhibiting glucagon secretion **(Brindisi, 2007).**

Insulin, the only hypo-glycemic hormone, is secreted by pancreatic β-cells in the islets of Langerhans **(Bouglé et al., 2009).** It acts on the liver, muscle, and adipose tissue by promoting glucose uptake and storage as glycogen, while inhibiting hepatic gluconeogenesis and glycogenolysis **(Carpeau et al., 1996).**

Diabetes mellitus is a chronic disease characterized by insufficient insulin secretion or improper use of insulin by the body **(WHO, 2016).**

It can also be defined as a multifactorial metabolic disorder affecting carbohydrate, lipid, and protein metabolism, resulting from both genetic and environmental factors that often act together **(Klein, 2009).**

II.1 Etiological classification of diabetes mellitus

Diabetes mellitus includes a group of metabolic disorders mainly characterized by chronic hyper-glycemia.

Since 1997, the American Diabetes Association (ADA) has proposed a new classification system for diabetes **(Alberti et al., 1998),** which has been adopted by the World Health Organization (WHO) to date. This classification has replaced the former terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus.

II.1.1 Type 1 diabetes

Globally, type 1 diabetes, also known as insulin-dependent diabetes, accounts for approximately 15% of all diabetes cases. It can occur at any age, but it most commonly appears during childhood or early adulthood, which is why it is often referred to as juvenile diabetes.

Its onset is associated with an autoimmune process leading to the destruction of pancreatic β-cells, influenced by both genetic and environmental factors **(Québec, 2000).**

II.1.2 Type 2 diabetes

Type 2 diabetes, non-insulin-dependent diabetes, is characterized by the body's inability to respond properly to insulin produced by the pancreas. In the long term, this condition may lead to damage, dysfunction, and failure of various organs **(Observatoire Régional de la Santé Réunion, 2015**).

II.1.3 Gestational diabetes

According to the World Health Organization (WHO), gestational diabetes is a disorder of glucose tolerance characterized by hyper-glycemia of variable severity, first detected or diagnosed during pregnancy, regardless of the required treatment or postpartum outcome **(Collège des enseignants d'endocrinologie, 2003).**

II.1.4 The Type 2 Diabetes

Type 2 diabetes is defined as a chronic metabolic disorder that has historically been classified as adult-onset diabetes due to its primary occurrence in individuals older than 50 **(Grimaldi, 2004).** However, contemporary data show an increasing prevalence of this condition among younger populations, particularly children and adolescents **(International Diabetes Federation, 2006).**

A major challenge of this type is that its symptoms are often subtle or less evident than those of type 1 diabetes, allowing the disease to progress gradually and silently. As a result,

clinical diagnosis is frequently delayed for years after onset, with many cases being identified only after the appearance of associated complications **(Tayeb, 2017).**

II.2 Epidemiology:

Type 2 diabetes represents about 90% of all global diabetes cases (Villar and Zaouïa, 2010) and is considered a global epidemic due to its rapid increase. This rise is mainly associated with modern lifestyle changes such as unhealthy diets, sedentary behavior, and increased life expectancy. The disease is no longer limited to the elderly, as it increasingly affects young adults **(Boudiba et Zerguini, 2008).**

Historically, diabetes became a major public health issue in the 19th century and is now a leading cause of death worldwide, responsible for about 3.8 million deaths annually. It also significantly increases the risk of cardiovascular complications and causes a major economic burden due to long-term treatment costs **(Arbouche et al., 2012).**

Figure 06: Estimated prevalence of diabetes, 2025 (Hirst, 2013).

II.2.1 Worldwide

The International diabetes Federation (IDF) classifies diabetes as a worldwide pandemic posing a serious threat to public health. Current statistics highlight a deeply concerning situation. In its most recent Atlas, the IDF indicates that the global prevalence of diabetes reached 9.3%, corresponding to approximately 463 million individuals living with the disease in 2018. Notably, type 2 diabetes accounts for nearly 90% of these cases.

At the European level, reported prevalence rates vary between 3% and 5% **(Bosschaert et al., 2006).** In France specifically, data from 2007 revealed a prevalence rate of 3.95% **(Odile et al., 2008).**

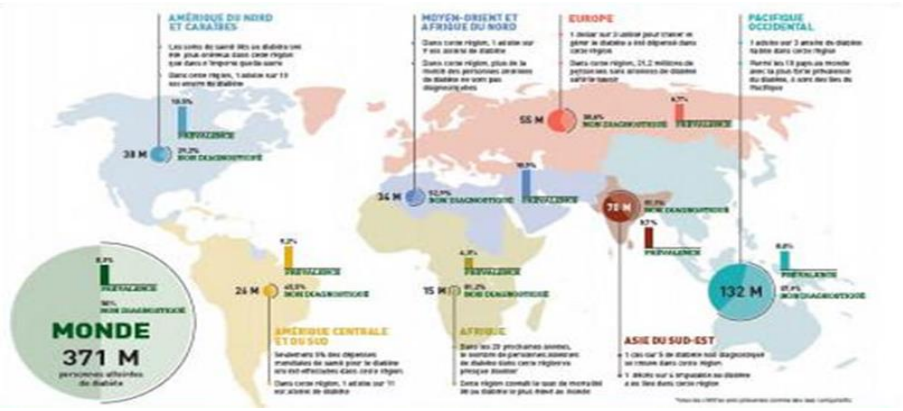


Figure 07: Global distribution of diabetes (International Diabetes Federation,2012)

Across Asia, the burden of diabetes appears even more pronounced. India records over 40.9 million individuals affected by the disease, placing it at the forefront, followed closely by China with 39.8 million cases, while Japan reports approximately 7 million people living with diabetes **(IDF, 2006).**

In North America, the overall prevalence reaches 8.4%. Within this region, the United States reports a rate exceeding 5% **(Buysschaert et al., 2006),** whereas in Canada, the prevalence was estimated at 7.1% for the period 2004–2005 **(PHAC, 2007)**

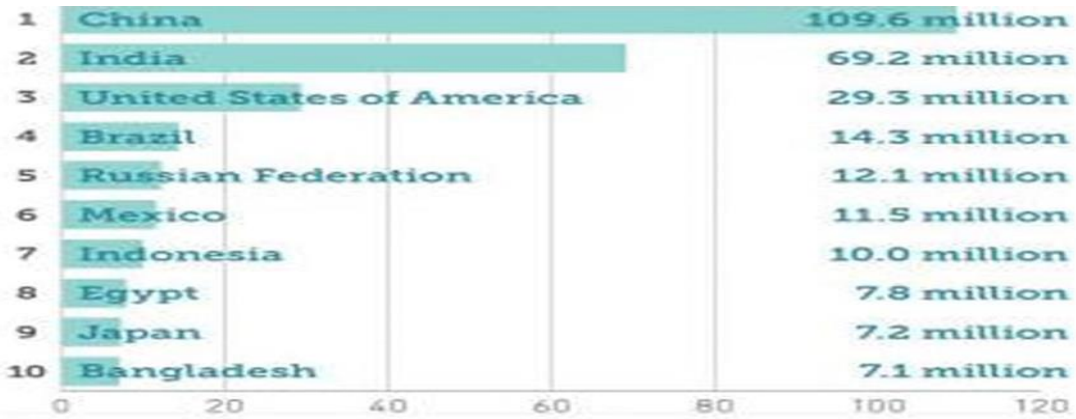


Figure08: Ranking of the top 10 countries by number of people with diabetes (Chabani.2020)

II.3 Physiopathology of Type 2 Diabetes:

The progression of type 2 diabetes is described in five stages (Weir and Bonner-Weir, 2004). It begins with a compensation stage where β-cells increase insulin secretion through hyperplasia and hypertrophy to overcome insulin resistance. In the second stage (adaptation failure), β-cells can no longer compensate, leading to hyper-glycemia and delayed insulin secretion **(Curry et al., 1968**).

The third stage (decompensation) is characterized by severe insulin resistance and glucotoxicity, creating a vicious cycle that worsens hyper-glycemia. In the fourth stage (stable decompensation), insulin secretion is reduced but still sufficient to delay ketoacidosis, which results from insulin deficiency and ketone body accumulation. Studies show a ~50% reduction in β-cell mass in type 2 diabetes due mainly to increased apoptosis rather than reduced neogenesis **(Butler et al., 2003; Bonner-Weir, 2000).**

The final stage (severe decompensation) involves extensive β-cell loss, where endogenous insulin is insufficient, making external insulin necessary to prevent ketoacidosis and ensure survival.

II.4 Relationship between Centaurium erythraea and diabetes:

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyper-glycemia due to defects in insulin function and disturbances in carbohydrate, lipid, and protein metabolism, leading to long-term complications. Its prevalence is increasing globally.

Persistent hyper-glycemia promotes oxidative stress through excessive production of reactive oxygen species (ROS), which are normally regulated by antioxidant enzymes such as SOD, CAT, and GSH-P x **(Robertson, 2004; Saxena et al., 1993; Kakkar et al., 1995).**

Streptozotocin (STZ) is commonly used to induce experimental diabetes via oxidative damage to pancreatic β-cells **(Kim et al., 2003; Szkudelski, 2001).** Due to limitations of synthetic drugs, interest in medicinal plants has increased, as many show hypo-glycemic activity and low toxicity **(Holman et Turner, 1991; Valiathan, 1998; Eddouks et al., 2005).**

Centaurium erythraea is traditionally used in diabetes treatment and exhibits antidiabetic, antioxidant, anti-inflammatory, and enzyme inhibitory effects (α-amylase and α-glucosidase), along with diuretic properties **(Flück, 1973; Bellakhdar et al., 1991; Kumarasamy et al., 2003; Loizzo et al., 2008; Haloui et al., 2000).** However, its protective role against oxidative stress in diabetes is still not fully studied, and this research aims to evaluate its antihyperglycemic and antioxidant effects and its ability to protect pancreatic tissue in STZ-induced diabetic rats.

III. α-Amylase

III.1 Definition:

α-Amylase is a glycosidase enzyme (EC 3.2.1.1) that catalyses the hydrolysis of α-1,4 glycosidic bonds in polysaccharides such as starch and glycogen, producing simpler sugars including glucose, maltose, and soluble maltodextrins of various molecular sizes **(Elleuche et Antranikian, 2013).**

This enzyme is widely distributed among living organisms, including animals, plants, and microorganisms, where it plays an essential role in the digestion and metabolism of carbohydrates. α-Amylase consists of a long chain of amino acids forming its characteristic protein structure. Due to its efficiency in starch hydrolysis, this enzyme has been used for many years in several industrial applications, particularly in the food industry **(Farooq et al., 2021; Dehkordi, 2012).**

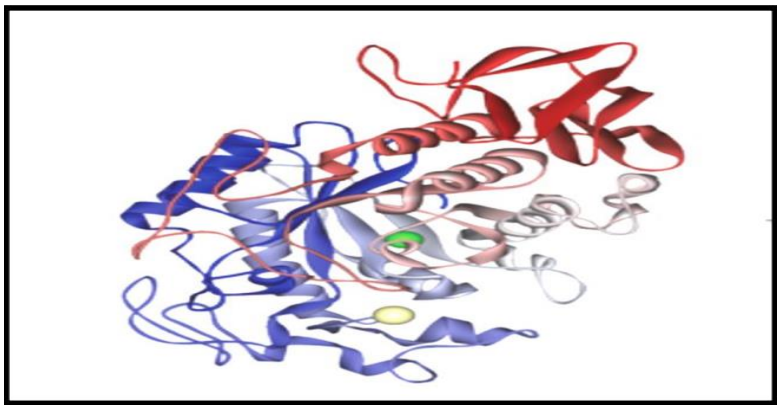


Figure 09: Structure of α-amylase (Subash, 2017)

III.2 Nomenclature

According to Olempska (2004), α-amylase classified within the hydrolase family. This enzyme primarily acts on polysaccharides and oligosaccharides by randomly hydrolyse the

α-amylase-D-glucose glycosidic bonds present in carbohydrate chains, such as starch and glycogen.

Table 04: Nomenclature of alpha-amylase

Terme(α)	Coded name	Accepted name	another name	Systematic name
Related to the initial anomic configuration that is linked to the carbone of the suger	EC (3,2,1,1)	α-amylase	Glycogensis α-amylase Endo amylase Maxilase; Take-amylaseA	1-4-α-D-glucan glucanohydrolase

III .3 type alpha amylase.

In the human body, α-amylase is mainly produced in two sites: the salivary glands, where it is referred to as salivary α-amylase (S-amylase), and the pancreas, where it is known as pancreatic α-amylase (P-amylase). The synthesis of these two forms is regulated by two distinct genes, Amy1 and Amy2A, both located on chromosome 1.

Salivary α-amylase (S-amylase) exists in two main forms: type A, which is a glycosylated protein with a molecular weight of approximately 62 K-Da, and type B, which is a non-glycosylated form with a molecular weight of about 55 K-Da **(Sahraoui et Youmbai, 2021).**

III.4 Role of α-amylase

The digestion of starch, the main Dietary source of carbohydrates, begins in the oral cavity through the action of salivary α-amylase. This enzyme hydrolyses internal α (1→4) glycosidic bonds in starch, producing smaller carbohydrate fragments. However, it cannot cleave terminal bonds or those located near branching points.

Once food reaches the acidic environment of the stomach, salivary α-amylase becomes inactivated and its activity stops. Starch digestion is then continued and completed in the small intestine through the action of pancreatic α-amylase and other enzymes. These enzymes further hydrolyse starch into smaller products such as maltose, maltotriose, and dextrins containing α (1→6) branch points.

Finaly, brush-border enzymes in the small intestine convert these oligosaccharides into monosaccharides, which are then absorbed into the bloodstream **(Voand et Voand, 2005).**

III.5 Structure of α-Amylase

α-Amylases are enzymes that catalyse the hydrolysis of starch (a polysaccharide). They belong to the endo-amylase family, which breaks down polysaccharide chains into shorter oligosaccharides by cleaving α-D-(1→4) glycosidic bonds **(Souza et Magalhães, 2010).**

According to Saboury, these enzymes are classified as metalloenzymes, as they require calcium ions to maintain their structural stability, catalytic efficiency, and proper mode of action. Human α-amylase consists of a single polypeptide chain of 512 amino acids, with a molecular weight of approximately 57.6 K-Da **(Whitcomb et al., 2007).**

The protein structure is composed of three main domains: A, B, and C **(Zhang et Xiao, 2016):**

- **Domain A:** The largest domain, characterized by a specific structure consisting of eight β-strands surrounded by eight α-helices.
- **Domain B:** Connected to domains A and C via a disulfide bond; it contains the calcium-binding site, which plays a key role in stabilizing the enzyme's active site.
- **Domain C:** Exhibits a β-sheand structure and is linked to domain A by a simple polypeptide chain; it is involved in the specific recognition of the substrate.
- Ca+: Located between Domain A and Domain B, it plays a crucial role in maintaining
- the stability of the enzyme through four amino acids: (His, Glu, Asp, Asn)

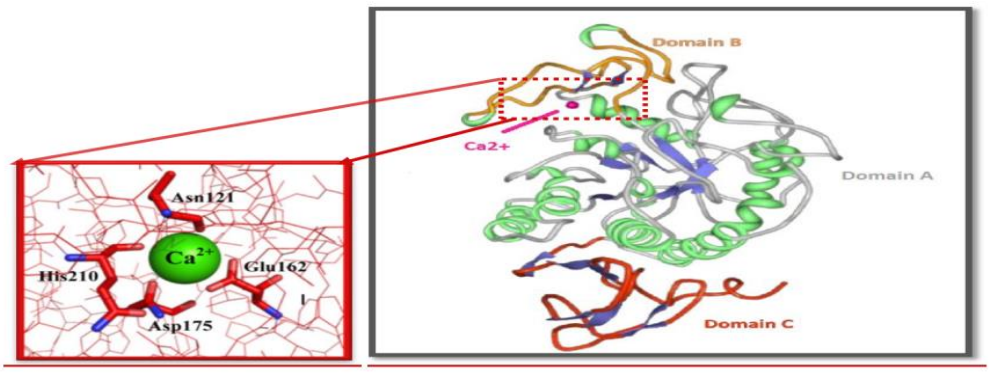


Figure 10: Structural domains of alpha-amylase (Zhang and Xiao, 2016 et benabdelmalek and al,.2009)

III.6 Characteristics of α-Amylase

III.6.1 Molecular Weight

The molecular weight of α-amylase varies depending on its source and the producing organism, generally ranging between 40,000 and 90,000 Daltons (Schomburg et Salzmann,

1991). Yeast-derived α-amylases, however, exhibit a narrower range, with molecular weights typically between 40,000 and 70,000 Daltons (Panchal, 1990).

III.6.2 Temperature

The production of α-amylase requires specific thermal conditions involving two main phases: an initial phase for microbial growth, followed by a phase that ensures maximum enzyme yield (Jujjavarapu et Dhagat, 2018).

Enzyme production increases with rising temperature until it reaches an optimal level. Beyond this point, productivity declines, mainly due to moisture loss in the substrate, which negatively affects microbial growth and consequently reduces enzyme production (Jujjavarapu et Dhagat, 2018).

III.6.3 pH

pH is a critical factor influencing both the stability and activity of α-amylase, as enzymes are highly sensitive to changes in acidity or alkalinity.

The optimal pH varies among microorganisms, depending on the nature of the substrate and the fermentation conditions employed.

III.7 Activators and Inhibitors of alpha-amylase

Enzymatic activity can be regulated by molecules known as effectors, which may act as activators or inhibitors. These compounds influence the enzyme either directly or indirectly by interacting with its active site **(Garrandt et Grisham, 2000).**

Inhibitors are generally molecules with a chemical structure similar to that of the substrate; however, they either fail to produce a reaction or react at a significantly slower rate compared to the natural substrate. The study of inhibitor effects is a valuable approach for elucidating the catalytic mechanism of enzymatic reactions, understanding enzyme specificity, and obtaining physicochemical information about the active site **(Garrandt et Grisham, 2000)**.

Furthermore, Cu^{2+}, Fe^{2+}, and Hg^{2+} ions act as competitive inhibitors due to their structural resemblance to molecules involved in the reaction. In contrast, Ca^{2+} and Mg^{2+} ions function as activators of alpha-amylase, as they contribute to the structural stability of the enzyme by associating with elements of the active site **(Mercier, 1985)**.

Table 05: Organic activators of salivary α-amylase

Inorganic Activators	Characteristic	References
Calcium	Allosteric activator, providing resistance to denaturation at extreme pH and temperature.	**(Mercier, 1985).**
Carbonate	Increases thermostability of α-amylases when in the form of calcium carbonate.	**(Knight, 1967).**
Chloride ion	These ions have a significant activating effect at high concentration.	**(Mercier, 1985)**

Table 06: Organic inhibitors of salivary α-amylase

Organic inhibitors	Characteristic	References
Maltose	It is the degradation product of starch by amylase, which causes feedback inhibition of the enzyme.	(Mercier, 1985)
Acarbose	It is a sugar analogue. The inability of digestive enzymes to hydrolyse these molecules is due to the presence of an amine bridge, considered the key factor of its inhibitory effect. It is the most widely used for the treatment of type 2 diabetes	(Kadema et al., 1984). (Romane et al., 2012)

Table 07 Other organic and inorganic activators and inhibitors of α-amylases

Inorganic Activators	organic activators	Inorganic inhibition	Organic inhibition
Bromide Nitrate Iodide Phosphate	Acetylcholine Pancreozymin Albumin	Iron Mercury Silver Copper	D-glucose Citrate Oxalate

III.8 Enzymatic Kinetics of α-Amylase

III.8.1 Definition

Enzymatic kinetics focuses on the study of biochemical reaction mechanisms catalysed by enzymes by analysing their reaction rates. It helps elucidate the catalytic properties of enzymes and understand the mechanisms involved in regulating their activity. In 1913, Leonor Michaelis and Maud Menten proposed a simplified model describing the behavior of so-called Michaelian enzymes **(Vignais, 2001).**

III.8.3 Different Types of Enzyme Inhibition

III.8.3.1 Competitive Inhibition

Competitive inhibitors are compounds that structurally resemble the enzyme's substrate. This similarity allows them to compete with the substrate for binding to the active site, or to bind to another site causing conformational changes in the active site, thereby affecting enzymatic activity **(Moussard, 2002).**

III.8.3.2 Non-Competitive Inhibition

Non-competitive inhibitors can bind both to the free enzyme and to the enzyme–substrate complex, as their binding site is distinct from the active site. This interaction leads to the formation of (EI) or (ESI) complexes, both of which inhibit the enzymatic reaction **(Murray et al., 2002).**

III.8.3.3 Uncompetitive Inhibition

Uncompetitive inhibitors do not bind to the free enzyme but only to the enzyme–substrate complex (ES). This results in the formation of an (ESI) complex, which effectively blocks enzymatic activity **(Murray et al., 2002).**

III.9 Mechanism action of α-Amylase

According to Lim and Oslan, α-amylase catalyses the endo-hydrolysis of glycosidic bonds in its substrate, such as starch. Three amino acid residues are involved in this process

Glutamic acid (Glu) plays a key role in promoting the nucleophilic attack on the C1 carbon of the sugar.

As for aspartate (Asp), two residues are involved:

- The first Asp acts as a nucleophile and also helps maintain glutamic acid in the proper protonated state required for enzymatic activity.
- The second Asp interacts with the hydroxyl groups (OH-2 and OH-3) of the substrate through hydrogen bonds, causing distortion of the substrate structure.

The mechanism occurs in two main steps:

- In the first step, the substrate binds to the enzyme's active site, allowing glutamic acid (Glu) to donate a proton to the glycosidic bond at the anomeric oxygen. This is followed by a nucleophilic attack by Asp on the C1 carbon, leading to the formation of an intermediate and modification of the substrate end.
- In the second step, the enzyme returns to its initial state and reaction products are released. A water molecule participates in breaking the covalent bond, with the release of the glycone part. Then, water attacks the active site to cleave the covalent interaction between glucose, Asp, and the C1 carbon, restoring the enzyme for another catalytic cycle **(Lim et Oslan, 2021).**

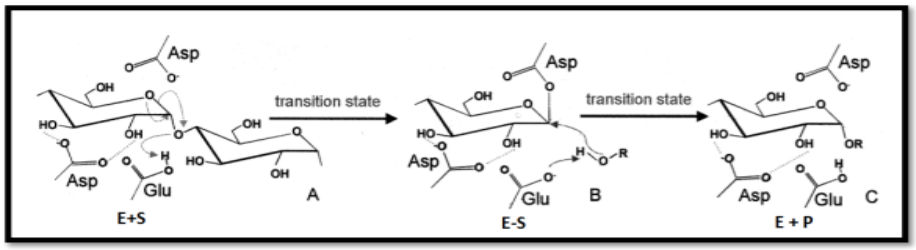


Figure 11: Mechanism of α-amylase (Lim et Oslan, 2021)

Chapter II: Insulin resistance and vitamin D

I. The Insulin:

Insulin is a crucial hormone involved in metabolism and the survival of various cell types. Skeletal muscle, adipose tissue, and the liver are the primary insulin-responsive tissues. In diabetes, these tissues become resistant to insulin, which results in impaired regulation of glucose metabolism **(Saltiel et Olefsky, 1996).** This condition is referred to as systemic insulin resistance. Beyond its metabolic role, insulin also affects the survival and function of other cell types. Among them, podocytes rely on insulin signalling for their viability. Studies have demonstrated that podocytes are insulin-sensitive **(Coward et al., 2005; Hale et Coward, 2013)**, and that deletion of the insulin receptor in podocytes in mice leads to a condition similar to diabetes nephropathy (**Hale et Coward, 2013; Welsh et al., 2010).**

I.1 The insulin receptor:

belongs to the family of tyrosine kinase receptors (RTKs) and functions as a dimer, unlike most RTKs, as it exists in a dimerized form even in the absence of a ligand (except IGFR). It is initially synthesised as a single polypeptide chain that undergoes cleavage, glycosylation, folding, and dimerization. Two isoforms of the receptor, A and B, exhibit different affinities for insulin and insulin-like growth factors **(Lee et Pilch, 1994).** Structural, the receptor is composed of two extracellular α-subunits and two β-subunits linked by disulfide bonds. The α-subunits contain cysteine-rich and fibronectin type 3 domains and are responsible for ligand binding, while the β-subunits consist of extracellular, transmembrane, and intracellular regions. The intracellular domain contains multiple tyrosine residues that can be phosphorylated, as well as an ATP-binding site essential for its kinase activity **(Lee et Pilch, 1994; De Meyts, 2004).**

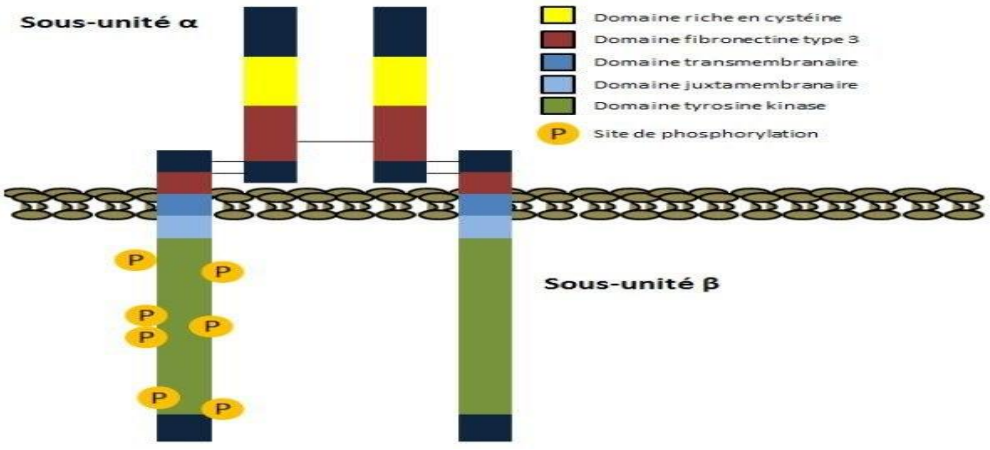


Figure 12: Structure and activation of the insulin receptor (adapted from De Meyts et al., 2004).

I.2 Insulin signalling:

is initiated when insulin binds to the α-subunits of its receptor, leading to receptor autophosphorylation on tyrosine residues and activation of downstream signalling **(Du et Wei, 2014)**. The activated receptor recruits and phosphorylates insulin receptor substrates (IRS1–4), mainly IRS1 and IRS2, and can also activate other adaptor proteins such as Shc, Gab-1, and Cbl, triggering additional signalling pathways **(Boucher et al., 2014; E. Xu et al., 2014**).

Insulin mainly activates two major pathways: PI3K/Akt and MAPK. The PI3K/Akt pathway regulates glucose metabolism, protein and glycogen synthesis, lipolysis, and cell survival in muscle, adipose tissue, and liver. The MAPK pathway mainly controls cell growth, differentiation, and cytoskeletal organization **(Boucher et al., 2014; Lee et Pilch, 1994).** Both pathways are active in podocytes, and their dysfunction is linked to diabetes nephropathy-like phenotypes **(Hale et Coward, 2013; Welsh et al., 2010).**

In the PI3K/Akt pathway, PI3K converts PIP2 into PIP3, leading to activation of PDK1 and mTORC2, which fully activate Akt. Activated Akt regulates metabolism through mTORC1, inhibits gluconeogenesis via FoxO1, and promotes glycogen synthesis while reducing lipolysis and supporting cell survival **(Boucher et al., 2014).**

In the MAPK pathway, Grb2 and SOS1 activate Ras, which triggers a cascade through Raf, MEK, and ERK, ultimately regulating growth and differentiation **(Boucher et al., 2014).**

insulin signalling is essential for metabolic regulation and cell survival, and reduced Akt activity has been observed in type 2 diabetes models, contributing to podocyte damage **(Hale et Coward, 2013; Tejada et al., 2008).**

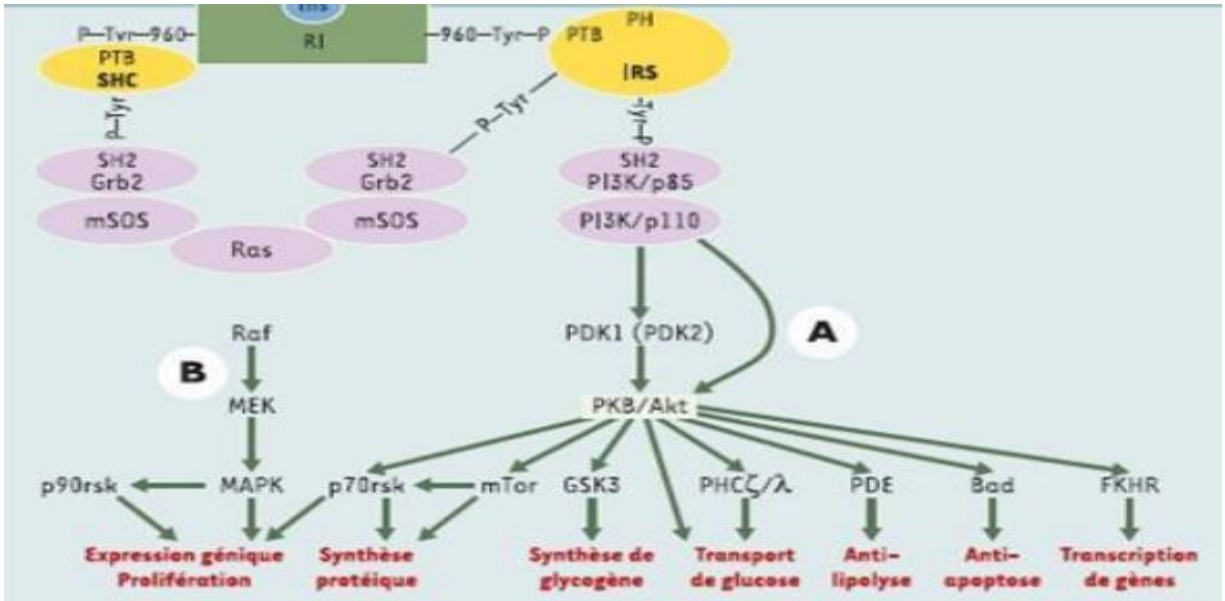


Figure 13: insulin signalling pathways (Le Roith, D. et Zick, Y.,2001)

I.3 insulin resistance:

After a meal, the body releases insulin to regulate blood sugar levels. However, in people who are overweight—especially those with excess abdominal fat—cells may become less responsive to insulin, a condition known as insulin resistance**.** In response, the body produces higher amounts of insulin to compensate, which can temporarily keep blood sugar levels normal. However, this leads to metabolic disturbances such as fat accumulation in the liver, imbalances in blood lipids, and increased blood pressure, ultimately raising the risk of cardiovascular

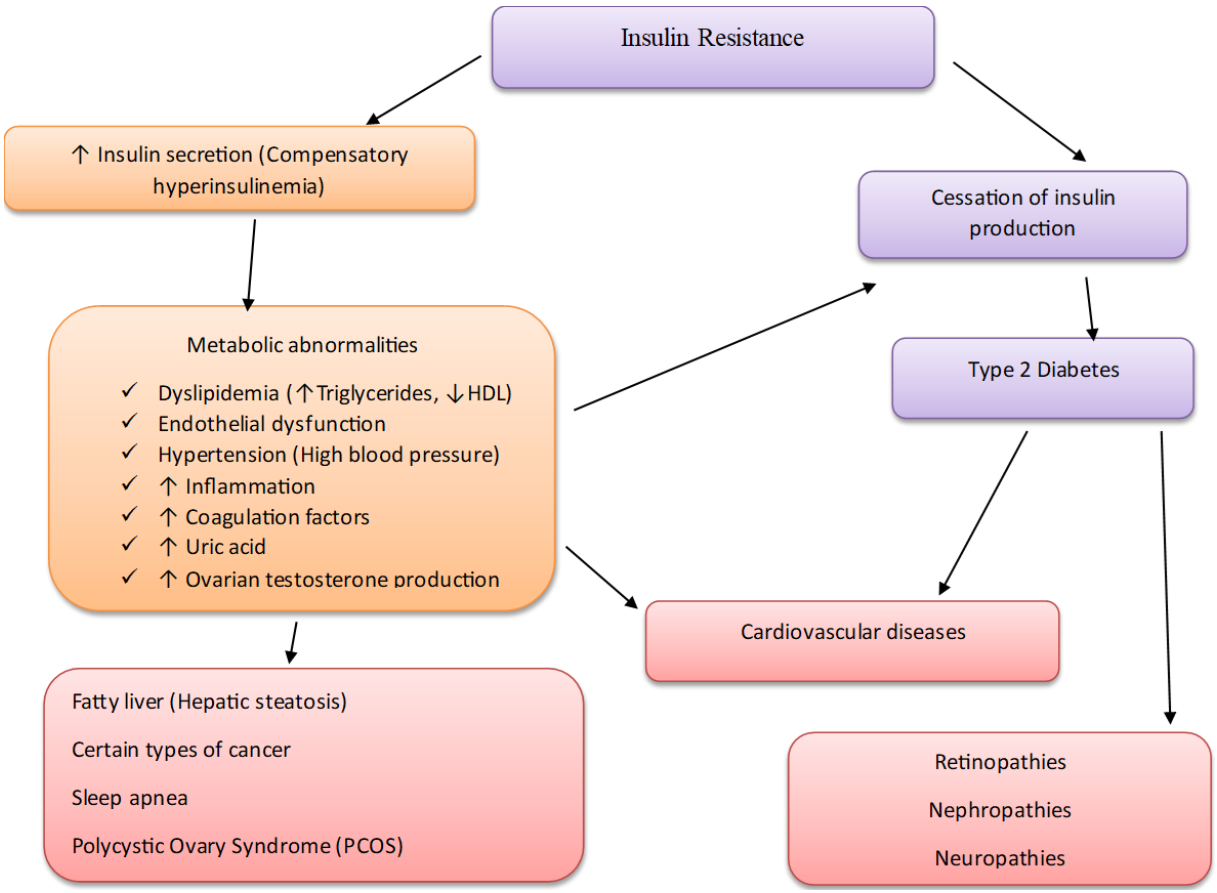


Figure14: pathophysiological mechanisms of insulin resistance and associated metabolic complications (https://observatoireprevention.org/2018/03/19/resistance-a-linsuline-consequence-dangereuse-de-lexces-de-poids/)

Insulin resistance is defined as a reduced response to insulin in targeted tissues (muscle, liver, and adipose tissue), leading to decreased glucose uptake and insufficient suppression of hepatic glucose production **(Monnier L et al.,2010).**

It involves complex mechanisms including inflammation, lipid accumulation, oxidative stress and mitochondrial dysfunction.

Adipose tissue actively contributes through adipokine secretion. In obesity, increased fat mass leads to higher levels of resistin, RBP4, and TNF-α, which impair insulin sensitivity **(Muoio DM, Newgard CB et al.,2008).**

Obesity also induces macrophage infiltration into adipose tissue, increasing pro-inflammatory cytokines (TNF-α, IL-6) and maintaining chronic inflammation **(Donath MY, Shoelson SE.,2011).**

Lipo-toxicity results from lipid overload and accumulation of DAG, ceramides, and triglycerides, partly due to increased malonyl-CoA, which inhibits fatty acid oxidation **(Muoio DM, Newgard CB et al.,2008).**

Endoplasmic reticulum stress and PKC activation disrupt insulin signalling by affecting insulin receptors and IRS1/IRS2 substrates **(Féry F, Paquot N et al.,2005).**

In skeletal muscle, insulin resistance is mainly linked to mitochondrial dysfunction, leading to incomplete fatty acid oxidation and increased ROS production, which inhibits GLUT4 translocation **(Muoio DM, Newgard CB et al.,2008).**

Under nor

mal conditions, insulin binding to its receptor induces IRS phosphorylation, activation of PI3K, and downstream signalling that promotes GLUT4 translocation, glycogen synthesis, and inhibition of gluconeogenesis and lipolysis **(Pinget M, Boullu-Sanchis S et al.,2002).**

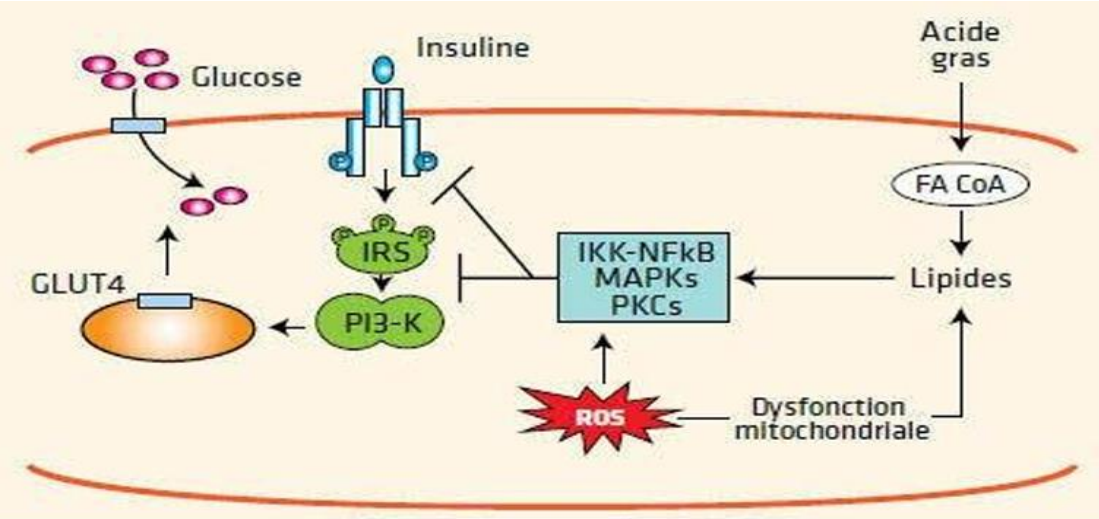


Figure15: Intracellular mechanisms of insulin resistance (Shoelson SE, Lee J, Goldfine AB,2006).

I.4 These processes are impaired in insulin resistance.

Various stimuli such as cytokines, free fatty acids, and AGEs activate inflammatory pathways including NF-κ B and JNK, promoting cytokine production and sustaining chronic inflammation **(Shoelson SE, Lee J, Goldfine AB ,2006).**

I.4.1 Impaired Insulin Secretion:

Glucolipotoxicity (hyper-glycemia and excess fatty acids) impairs β-cell function and induces cell death. Hyper-glycemia increases expression of the pro-apoptotic FAS receptor and IL-1α production, amplifying inflammation (**Donath MY et Shoelson S ,2011).**

Endoplasmic reticulum stress caused by increased insulin demand contributes to β-cell death **(Muoio DM et Newgard CB ,2008).**

I.4.2 Hyper-glycemia in insulin resistance:

Hyper-glycemia is one of the most recognized factors contributing to insulin resistance. Excess blood glucose, commonly observed in diabetes, can disrupt insulin signalling by over-activating inhibitory pathways that counteract insulin action. Large clinical studies have demonstrated that sustained glycemic control reduces the risk of diabetes-related cardiovascular diseases **(Conirol et al., 1995; Genuth et al., 2003).**

I.4.3 Negative regulation of insulin signalling:

Phosphatases: multiple negative regulators of the PI3K/Akt pathway exist, many of which act directly or indirectly as phosphatases targeting the insulin receptor, IRS proteins, or other components of the insulin signalling cascade. Protein phosphatases are classified into several groups based on their substrates, including lipid phosphatases, histidine phosphatases, serine/threonine phosphatases, tyrosine phosphatases, and dual-specificity phosphatases that targeted tyrosine, serine, and threonine residues **(E. Xu et al., 2014**). Since the insulin receptor is a tyrosine kinase, its deactivation mainly occurs through tyrosine phosphatases.

I.4.3.1 Protein tyrosine phosphatases (PTPs)

Protein tyrosine phosphatases remove phosphate groups from phosphorylated tyrosine residues and play a key role in regulating insulin signalling. They have been widely studied in the context of diabetes and insulin resistance **(E. Xu et al., 2014).** There are 37 classical PTPs located either at the membrane or in the cytoplasm. Their activity can be regulated through dimerization, expression levels, proteolysis, phosphorylation, oxidation, and simulation **(Soulsby et Bennandt, 2009).** Among them, PTP1B, SHP-1, and SHP-2 are the most studied in insulin signalling regulation, and are also expressed in podocytes**.**

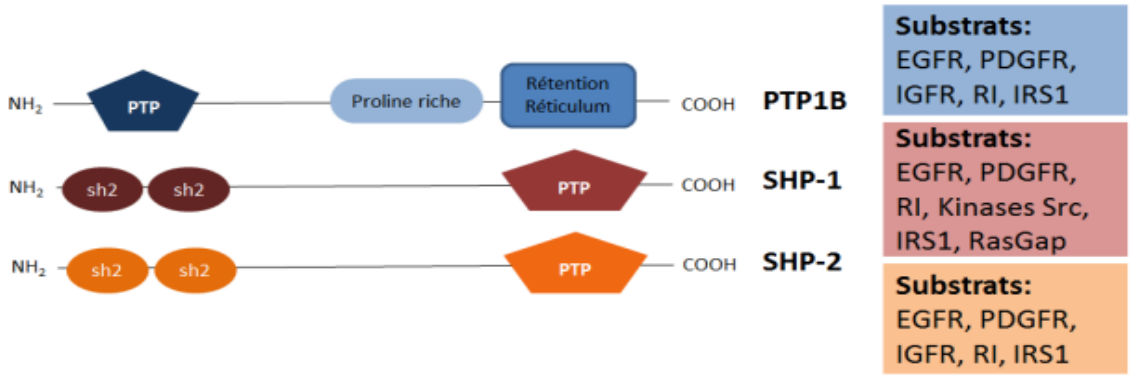


Figure16: Tyrosine phosphatase proteins involved in the insulin signalling pathway (Sherbrooke,2015)

I.4.3.2 PTP1B

PTP1B is a ubiquitously expressed cytoplasmic phosphatase encoded by the PTPN1 gene. It contains a PTP catalytic domain, a C-terminal endoplasmic reticulum rendition sequence, and a proline-rich region. Its substrates include receptor tyrosine kinases such as EGFR, PDGFR, IGF-1R, and the insulin receptor, as well as IRS1, making it a strong negative regulator of the PI3K/Akt pathway **(Soulsby et Bennandt, 2009).** PTP1B expression increases in obesity, and its hepatic deficiency enhances insulin sensitivity, highlighting its role in insulin resistance **(Owen et al., 2013).**

I.4.3.3 SHP-1 and SHP-2

SHP-1 and SHP-2 are encoded by PTPN6 and PTPN11, respectively. They contain two SH2 domains at the N-terminus and a C-terminal PTP domain. SHP-1 is mainly expressed in hematopoietic and epithelial cells, while SHP-2 is ubiquitously expressed. In their inactive state, they are autoinhibited through intramolecular interaction between SH2 and catalytic domains. Binding to phosphorylated substrates relieves this inhibition, enabling dephosphorylation activity.

Although highly homologous, SHP-1 and SHP-2 differ in function and substrate specificity. SHP-1 generally acts as a negative regulator of signalling pathways, whereas SHP-2 has context-dependent roles, sometimes promoting or inhibiting signalling.

SHP-2 targeted receptor tyrosine kinases such as EGFR, PDGFR, and the insulin receptor, and also regulates proteins including Src kinases, Ras-GAP, and IRS1. It can enhance ERK signalling. Mouse studies show that hepatic SHP-2 deletion reduces insulin sensitivity, while muscle-specific deletion worsens insulin resistance, indicating a context-dependent role in metabolism **(Matsuo et al., 2010; Princen et al., 2009).**

SHP-1 has a clearer inhibitory role in insulin signalling. Mutant mice with reduced SHP-1 activity (Motheaten viable) show improved glucose tolerance and increased insulin sensitivity, along with enhanced insulin signalling in liver and muscle .SHP-1 also regulates insulin receptor dephosphorylation and receptor recycling. Increased SHP-1 expression may impair insulin receptor recycling in the liver, while mutant mice show improved receptor recycling, supporting this mechanism **(Dubois et al., 2006**).

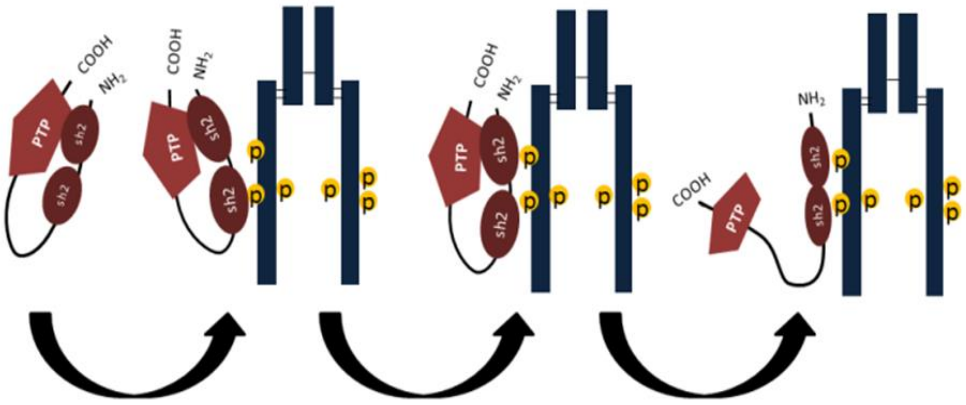


Figure 17: Activation of the SHP-1 protein (Farah Lizotte, Université de Sherbrooke, 2015).

I.5 Relationship between insulin resistance and vitamin D:

Vitamin D plays an important role in metabolic disorders related to insulin resistance, including type 2 diabetes, metabolic syndrome, NAFLD, and PCOS. Low vitamin D levels are inversely associated with HOMA-IR, while supplementation may improve insulin sensitivity and reduce insulin levels, particularly in obese individuals **(Monnier L, Thuan J-F et al., 2007; Forbes JM et Cooper ME, 2013).**

At the molecular level, vitamin D enhances insulin receptor expression in muscle, liver, and adipose tissues, regulates insulin signalling pathways, and improves glucose uptake. Vitamin D deficiency is also associated with inflammation, elevated pro-inflammatory cytokines, and obesity-related insulin resistance **(Forbes JM et Cooper ME, 2013; Shoelson SE, Lee J, Goldfine AB, 2006).**

Vitamin D acts through the vitamin D receptor (VDR), which regulates vitamin D-responsive genes and pancreatic β-cell activity. It promotes insulin secretion directly through VDR activation and indirectly via calcium-dependent pathways, while VDR impairment is linked to increased insulin resistance **(Joseph JJ et Golden SH, 2014).**

Furthermore, vitamin D exerts both genomic and non-genomic effects through signalling pathways such as PI3K, PLC, MAPK, and PKC, thereby influencing glucose metabolism and insulin action **(Lago RM et Nesto RW, 2009; Stratton IM, Adler AI, Neil HA, et al., 2000).**

II Vitamin D

II.1 Definition vitamin D

also known as calciferol or cholecalciferol, is a fat-soluble vitamin. Dietary fats facilitate its transport and absorption through the same mechanisms as lipids. It is then stored in relatively large amounts, depending on Dietary intake, mainly in adipose tissue and the liver. This storage capacity is advantageous as it allows the body to be regularly supplied with the amounts it needs **(Duchadeau J.C et al.,2001).**

Vitamin D was isolated and then synthesized in 1931 **(Duchadeau J.C et al.,2001).** It is an essential substance that behaves like a hormone and exerts multiple physiological effects **(Soccar,2007).**

II.2 Structure and Origin:

Under the term vitamin D, vitamin D3 is included. It has the same metabolism and exerts the same functions.

Vitamin D3 (cholecalciferol) is synthesized in the skin after solar irradiation from 7-dehydrocholesterol, or it can be obtained orally (through food or medications).

The main foods containing vitamin D3 are fatty fish, fish liver oils, and egg yolks.

Thus, the term “vitamin D” includes vitamin D3 in order to assess the vitamin D status in humans. Exogenous sources of vitamin D3 are limited. Foods that contain it are mainly fatty fish, fish liver oils, and egg yolks. It is also found in small amounts in milk, bread, and cereals, and in higher amounts when they are fortified.

A small amount of 25-hydroxyvitamin D (25OHD) can also be found naturally in foods; however, it is not taken into account when calculating exogenous vitamin D intake **(Rossy, N et al.,2010).**

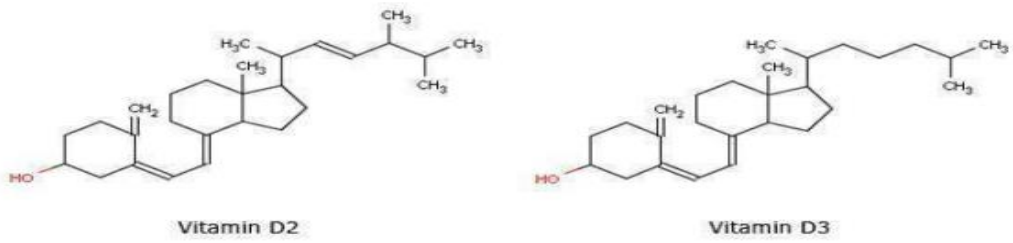


Figure18: structure chimique of vitamin D2, D3 (Duchadeau.2001)

II.3 Biosynthesis of vitamin D3:

This process begins in the skin, where UVB rays convert 7-dehydrocholesterol into pre-vitamin D3, which is then transformed into cholecalciferol (vitamin D3). Activation involves CYP enzymes in the liver and kidneys **(Brown AJ, Dusso AS, Slatopolsky E et al).**

The first step is hydroxylation in the liver (CYP2R1 mainly), producing 25(OH)D3, the storage form with a half-life of 2–3 weeks. It is transported by DBP to the kidneys **(Cheng JB, Levine MA, Bell NH ,2004; Prosser DE et Jones G,2004).**

The second step is renal hydroxylation by CYP27B1, forming 1,25(OH)2D3, the active form, with a short half-life (~4 hours). Minor production also occurs in other tissues but has little effect on circulating levels **(Nykjaer A, Dragun D, Wallther D et al,1999; Garabédian M,2000).**

Once formed, active vitamin D3 acts on targeted organs such as the intestine, bones, kidneys, and parathyroids, as well as other tissues like the epidermis **(Miller WL, Portale AA,2000; Schuessler M, Astecker N, Herzig G,2001).**

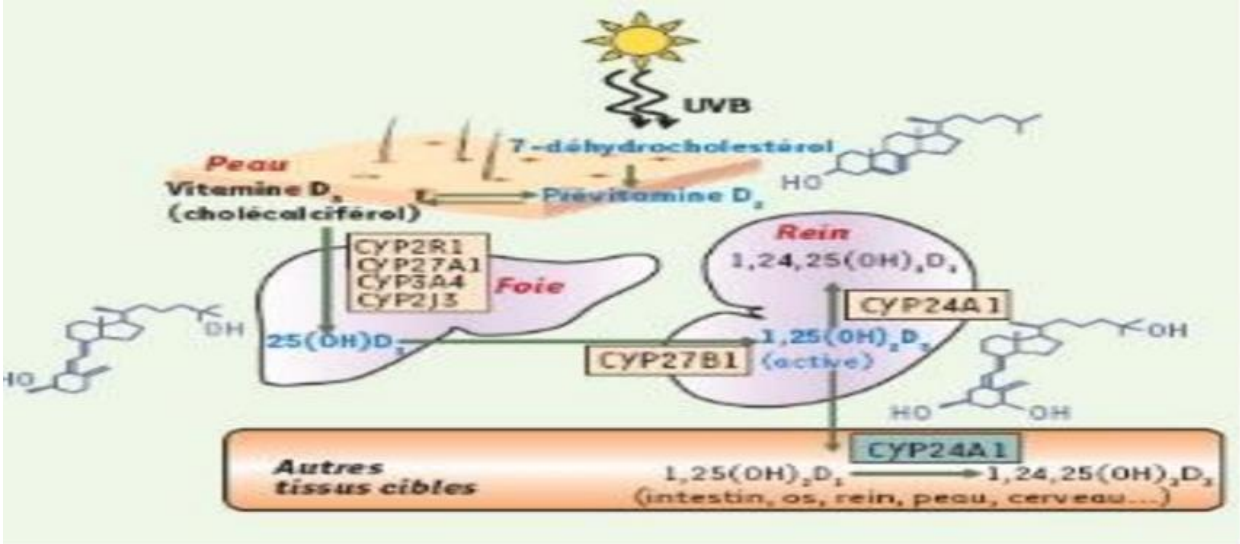


Figure19: Diagram of vitamin D metabolism. In the skin, the precursor of vitamin D (DELBEZE HADJER et al.,2021)

II.4 Regulation:

Vitamin D3 metabolism is mainly regulated by enzymes involved in its synthesis (CYP27A1, CYP27B1) and catabolism (CYP24A1). This regulation involves hormones, especially parathyroid hormone (PTH), which responds to changes in calcium homeostasis, as well as lipid-derived molecules acting via nuclear receptors. Circulating 25(OH)D3 is weakly regulated; its level increases with higher vitamin D synthesis or intake. In the liver, CYP27A1

is regulated at the transcriptional level by nuclear receptors such as PPARα, PPARγ, HNF4α, and SHP **(Post SM, Duez H, Gervois PP et al,2001; Delhoménie M,2011)**

In the kidney, CYP27B1 activity (responsible for producing active 1,25(OH)2D3) is tightly controlled. PTH stimulates its activity during hypo-calcemia, while hypercalcemia, hypophosphatemia, and high levels of 1,25(OH)2D3 inhibit PTH release. Calcium, phosphate, and 1,25(OH)2D3 can also directly influence enzyme activity **(Brown AJ, Dusso AS, Slatopolsky E,1999; Souidi M, Dubrac S, Parquand M, Lutton C,2003).**

PTH enhances CYP27B1 expression via CREB phosphorylation. Other factors such as IGF-I, insulin, calcitonin, and FGF23 also contribute to CYP27B1 regulation **(Brown AJ, Dusso AS, Slatopolsky E,1999; Miller WL, Portale AA,2000; Hewison M, Zehnder D, Bland R, Stewart PM,2000).**

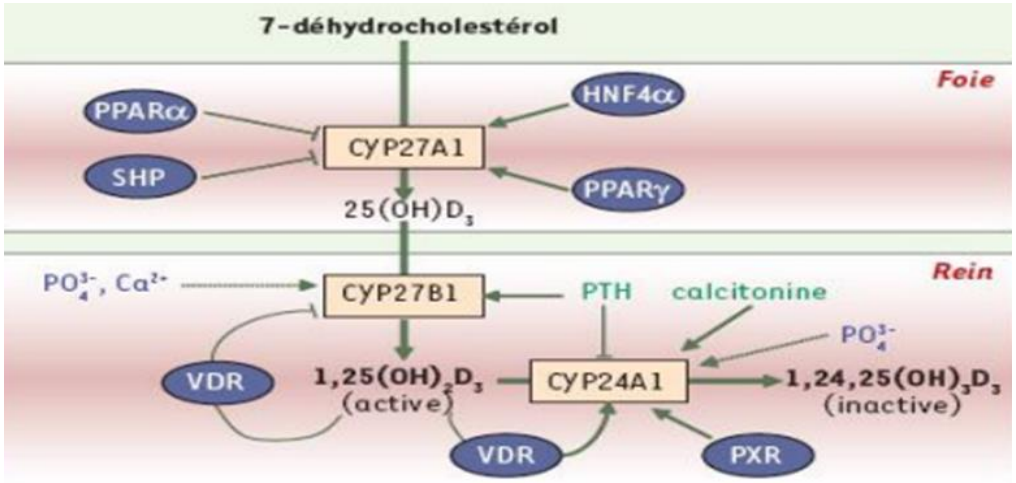


Figure 20: Regulation of vitamin D3 metabolism by hormones, regulation of its synthesis (DELBEZE HADJER et al.,2021)

The active metabolite of vitamin D is its hormonal form, 1α,25-dihydroxyvitamin D, and it exhibits both genomic and non-genomic effects **(Landrier, 2014).** It acts through the VDR to perform several essential functions, including intestinal absorption of calcium and phosphate, mobilization of calcium from bone, and renal calcium reabsorption. It also has non-calcemic and extra-Skeletal roles **(DeLuca, 2004).**

Vitamin D in its hormonal form functions via the VDR, a member of class II steroid hormone receptors, closely related to the retinoic acid receptor and the thyroid hormone receptor **(Jones et al., 1998).** The VDR consists of three domains: a DNA-binding domain (N-terminal) containing two zinc fingers that bind to VDREs, a ligand-binding domain (C-terminal), and a hinge region connecting these two domains **(Bikle, 2014)**. In addition, it generally forms a heterodimer with the retinoid X receptor (a vitamin A derivative) to create an open architecture**.**

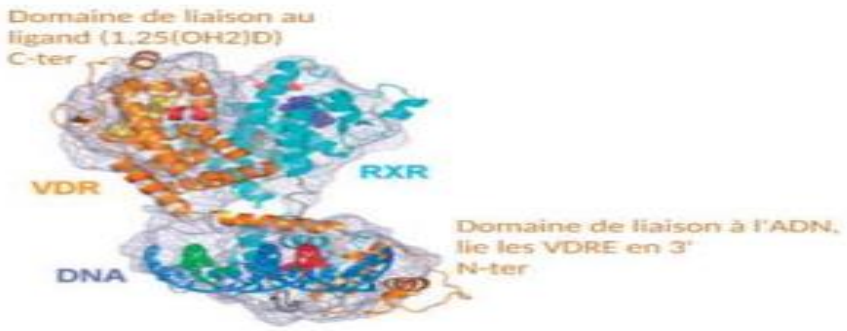


Figure 21: 3D model of the RXR/VDR/DNA complex obtained by cryo-electron microscopy (Orlov et al., 2012).

The receptor therefore acts through VDREs, which are repeated DNA sequences located in the promoter regions of genes regulated by vitamin D, with the 3' arm binding to the VDR. The binding between VDR and its ligand regulates the expression of many targeted genes **(DeLuca, 2004)**. Gene regulation mediated by VDR and vitamin D is a complex and multifactorial process involving the recruitment of multiple transcriptional cofactors (coactivators or corepressors) as well as changes in chromatin methylation and acetylation levels **(Landrier, 2014)**

The profile of VDR binding sites and the genes that are activated or repressed varies from one cell type to another and also depends on the exposure of the cell to $1,25(OH)_2D$. Moreover, these VDR binding sites can be located anywhere in the genome, often several thousand base pairs away from the gene being regulated, and are generally associated with binding sites for other transcription factors **(Bikle, 2014).**

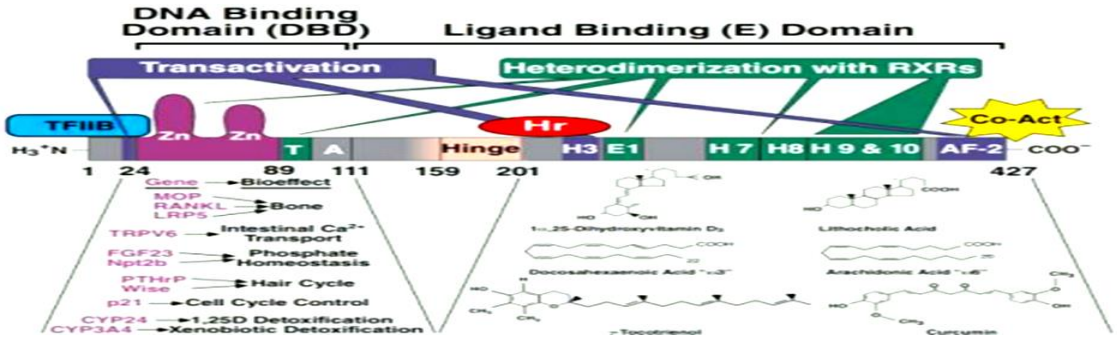


Figure 22: Functional domains of the human VDR showing DNA targeted, RXR interaction, and ligands including 1α,25-dihydroxyvitamin D (Haussler et al., 2008).

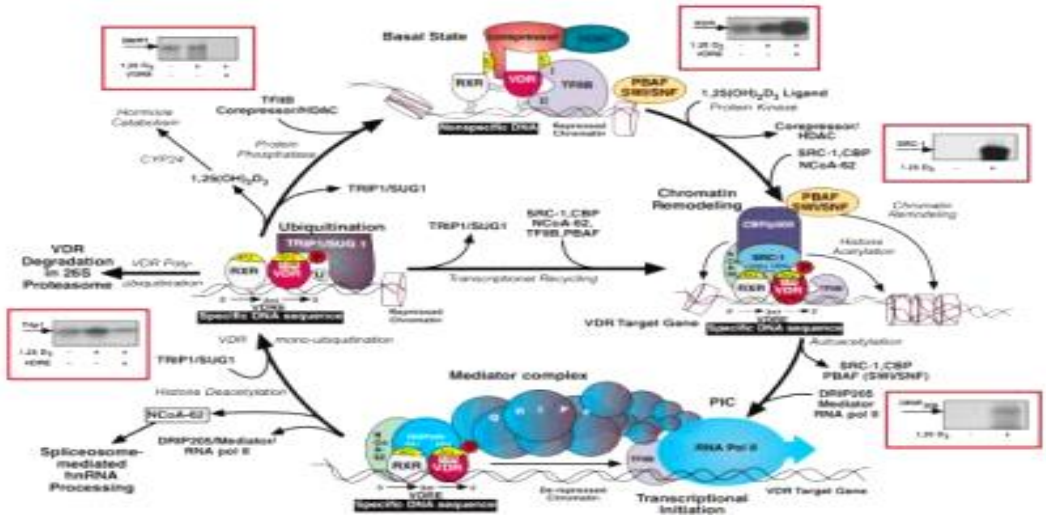


Figure 23: VDR transcriptional cycle model illustrating 1,25(OH)$_2$D$_3$–dependent gene activation and its role in calcium, phosphate, and bone homeostasis (Haussler et al., 2008).

This receptor is ubiquitously present in the human body, being expressed in most cell types and therefore in al tissues. As a result, almost all cells are targeted of calcitriol **(Landrier, 2014).** In particular, VDR is found not only in classical targeted cells such as enterocytes, osteoblasts, and distal renal tubular cells, but also in parathyroid gland cells, skin keratinocytes, promyelocytes, lymphocytes, colon cells, pituitary cells, and ovarian cells **(Jones et al., 1998).**

Vitamin D and its Metabolism also exhibit non-genomic effects, for example through a membrane receptor, protein disulfide isomerase family A member 3 (PDIA3), which contributes to rapid calcium uptake **(Landrier, 2014).**

Thus, vitamin D, via VDR, has numerous targeted across many tissues and therefore is associated with a wide range of potential physiological functions in the body.

II.5 Relationship of Vitamin D and Type 1 Diabetes mellitus (T1DM)

T1DM is an autoimmune disease caused by destruction of pancreatic β-cells, leading to insulin deficiency, with increasing incidence in genetically predisposed individuals **(Paschou, S.A et al.,2018; Soltesz, G et al.,2007; Huber, A et al.,2008).**

Vitamin D may act as a protective factor, especially early in life, by supporting immune maturation and reducing autoimmunity risk **(Mathieu, C et al.,2005; Norris, J.M.,2020).**

Vitamin D acts through VDR in immune cells and can be locally activated (CYP27B1), modulating immune responses by reducing pro-inflammatory cytokines and increasing anti-inflammatory ones **(Jiang, X. et al.,2019; Bikle, D.D et al.,2016; Dankers, W et al.,2016).** It promotes anti-inflammatory macrophage polarization (M2) and improves immune balance by suppressing effector T cells and enhancing Treg **(Casteels, K.M et al.,1998; Zella, J.B et**

al.,2003). Maternal vitamin D may lower autoimmune risk in offspring, with limited effects in adults **(Fronczak, C.M et al.,2003).**

II.6 Vitamin D and Type 2 Diabetes Mellitus (T2DM)

II.6.1 Vitamin D, T2DM and molecular Mechanisms

Type 2 Diabetes Mellitus (T2DM) is a major global public health problem, representing about 87–91% of all diabetes cases. It is a chronic metabolic disorder characterized by insufficient insulin secretion and persistent hyper-glycemia **(Muoio, D.M et al.,2008).** T2DM is associated with severe microvascular and macrovascular complications such as blindness, stroke, myocardial infarction, limb amputation, and renal failure, which significantly reduce quality of life and increase mortality risk **(Lotfy, M et al.,2017; Zheng, Y et al.,2018).** Pre-diabetes affects approximately 762 million people worldwide and is strongly associated with obesity **(Blaak, E.E et al.,2012).**

Vitamin D deficiency has been linked to both microvascular and macrovascular complications of T2DM. Low circulating vitamin D levels are frequently observed in obesity, prediabetes, and diabetes **(Kabadi, S.M et al.,2012).** The vitamin D receptor (VDR), expressed in insulin-sensitive tissues including pancreas, adipose tissue, and muscle, mediates many systemic effects of vitamin D, which also acts as an epigenetic regulator improving insulin sensitivity **(Gagnon, C et al.,2011).**

II.6.2 Studies and Research:

Meta-analyses show that vitamin D supplementation improves glycemic control, insulin sensitivity, and reduces HbA1c in T2DM patients (Mirhosseini, N and al.,2018; 60–64), likely through modulation of insulin secretion, inflammation, and PTH levels (58,59). It also enhances β-cell function, lipid metabolism, and insulin signalling via VDR activation and genomic effects **(Karkeni, E et al.,2018; Maestro, B et al.,2000).**

Vitamin D regulates calcium-dependent insulin secretion and improves skeletal muscle glucose uptake, enhancing overall insulin sensitivity (**Reusch, J.E et al.,1991; Ryan, Z.C et al.,2016).** Its deficiency is linked to metabolic dysfunctions and complications such as nephropathy, neuropathy, retinopathy, and foot ulcers, while supplementation may protect renal function and reduce proteinuria **(Earthman, C.P and al.,2012; Bajaj, S et al.,2014).**

Vitamin D deficiency is also associated with macrovascular complications, including endothelial dysfunction, arterial stiffness, and hypertension, partly via RAAS activation. Vitamin D suppresses RAAS, improves vascular function, and is inversely related to blood

pressure **(Al-Shoumer, K.A et al.,2015De Boer, I.H et al.,2009; Li, Y.C et al.,2002; Barbarawi, M et al.,2019).**

II.7 Experimental Techniques:

II.7.1 UV-Vis Spectroscopy:

UV-V is spectroscopy is a versatile and robust analytical technique used to investigate molecular structures and quantify substances based on their light absorption properties. This method operates within the ultraviolet and visible regions of the electromagnetic spectrum, specifically covering wavelengths from 100 to 800 nm. In the field of physical chemistry, this technique (often termed spectrometry) is essential for monitoring chemical reaction kinetics and determining equilibrium Constants in solution.

II.7.2 Fundamental Concepts of Light-Light:

functions as an electromagnetic wave composed of discrete energy packets known as photons. These waves oscillate to form peaks and valleys; the wavelength (λ) is defined as the linear distance between two consecutive peaks. The spectrum is categorized as follows:

- UV Radiation: 100 to 400 nm.
- Visible Light: 400 to 800 nm (Benbahmed et Outaleb, 2018).

II.7.3 The Beer-Lambert Law:

The Beer-Lambert Law serves as the fundamental principle for quantifying light absorption. It establishes a linear relationship between the absorbance of a medium and its intrinsic properties.

The relationship was expressed as:

- UV rays (from 100 to 400 nm).
- Visible light (from 400 to 800 nm). **(Benbahmed et Outaleb, 2018).**
- the Beer-Lambert Law is a fundamental principle that tells us how much light a substance absorbs.
- This relationship is written: Log10 I0/I (= f ε Cl).
- Or, as it is currently written: A: f ε Cl.

With:

A: absorbance.

ε: the extinction coefficient (mol-1.cm-1. L).

C: concentration (mol/L).

l: Thickness of the covalent.

I_0: intensity of the irradiation energy arriving at the sample (Incident light).

I: Intensity of the radiation that has passed through the sample (Transmitted light). (**Ibrahim et Ouazine, 2014).**

II.7.4 Principle of UV-Visible Spectroscopy

The electronic transitions observed in molecules via UV-V is spectroscopy involve some of the highest energy changes in chemical processes, ranging approximately from 160 to 665 kJ/mol. These energy levels are comparable to the forces maintaining atomic bonds within molecules. Consequently, while absorbed UV or visible light commonly induces the shifting of electrons between different energy levels, it can occasionally possess sufficient energy to undergo photodissociation and break chemical bonds **(Baudouin, 2012).**

II.8 The principal Biological Activities of Centaurium Erythraea:

II.8.1 Antioxidants

II.8.1.1 Definition

Antioxidants are substances that protect susceptible biomolecules from oxidative damage. They exert their effects through various mechanisms depending on the source of oxidation. For instance, they can neutralize free radicals, inhibit pro-oxidant enzymes, or chelate mandal ions that catalyse oxidative reactions. Overall, antioxidants serve as a defence system that delays or prevents oxidative damage **(HOUAS, 2022).**

II.8.1.2 Mechanisms of Action of Antioxidants:

Antioxidants exert their protective effects through multiple mechanisms. They can scavenge and neutralize reactive oxygen species, stabilize free radicals by donating electrons, reduce the reactivity of radicals and peroxides, and chelate mandal ions involved in the generation of oxidative stress, thereby limiting cellular damage **(WEYEPE LAH Fidèle Castro, 2021).**

II.8.1.3 Oxidative Stress

Oxidative stress is defined as a condition in which the natural balance between the production of reactive molecules (free radicals) and the body's antioxidant defence system is disturbed, leading to a predominance of free radicals. This imbalance may result either from a deficiency in antioxidant levels or from excessive production of free radicals **(Gupta, 2015).**

II.8.1.4 Free Radicals

Free radicals are chemical species (atoms, molecules, or ions) that possess at least one unpaired electron. This unstable electronic configuration makes them highly reactive, enabling them to donate or accept electrons from other molecules. As a result, they can function as both oxidizing and reducing agents in chemical reactions **(FERDJIOUI, 2020).**

II.8.1.5 Nature of Free Radicals

II.8.1.5.1 Reactive Oxygen Species (ROS):

Reactive Oxygen Species (ROS) constitute a group of highly reactive oxygen-containing molecules. This group includes oxygen-derived radicals such as the hydroxyl radical (OH•), superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and peroxyl radicals (RCOO•), among others **(FERDJIOUI, 2020).**

II.8.2 Anti-inflammatory activity:

II.8.2.1 Definition of Inflammation:

Inflammation is a protective response of the body against harmful stimuli such as injury, chemical exposure, or infection. Its main role is to eliminate the cause of damage and initiate tissue repair **(FERDJIOUI, 2020).**

II.8.2.2 Types of Inflammation:

II.8.2.2.1 Acute inflammation:

A rapid and short-term response characterized by vascular and exudative changes **(Kouachi, M. 2017).**

II.8.2.2.2 Chronic inflammation:

A prolonged inflammatory state that occurs when the initial stimulus persists in tissues, leading to long-term immune activation **(Kouachi, M. 2017).**

II.8.2.3 Mediators of Inflammation:

Inflammation involves the release of various mediators classified as pro-inflammatory or anti-inflammatory. These include cytokines (interferons, interleukins, TNF-α), chemokines (MCP-1), eicosanoids (prostaglandins and leukotrienes), and regulatory pathways such as NF-κ B, of which play key roles in inflammatory processes and disease development **(Azab, 2016).**

II.8.2.4 Anti-inflammatory Drugs:

II.8.2.4.1 Non-Steroidal Anti-Inflammatory:

NSAIDs are widely used drugs that reduce inflammation by inhibiting cyclooxygenase (COX) enzymes, which are responsible for the production of prostaglandins, key inflammatory mediators. COX enzymes exist in three forms: COX-1, which is constitutively expressed and involved in essential physiological functions; COX-2, which is induced during inflammation and represents the main targeted of NSAIDs for pain relief; and COX-3, mainly found in the brain with a still unclear role. Inhibition of COX-1 may lead to side effects such as gastric ulcers and renal complications due to metabolic imbalance and increased production of leukotrienes, which may also trigger allergic reactions. NSAIDs are classified according to their selectivity toward COX enzymes, ranging from non-selective inhibitors to selective COX-2 inhibitors **(FERDJIOUI, 2020).**

II.8.2.4.2 Steroidal Anti-Inflammatory:

Steroidal anti-inflammatory drugs are synthetic derivatives of cortisol that regulate stress response and energy metabolism. Their anti-inflammatory action involves suppressing pro-inflammatory gene expression, enhancing anti-inflammatory pathways, and inhibiting phospholipase A2, thereby reducing prostaglandin and leukotriene synthesis. They also decrease oedema, suppress immune cell activity, and reduce inflammatory mediator release. Their immunosuppressive effects mainly result from cytokine inhibition, which affects both cellular and humoral immunity. Despite their therapeutic benefits, these drugs may produce significant side effects due to their broad systemic actions **(Ferdjioui, 2020).**

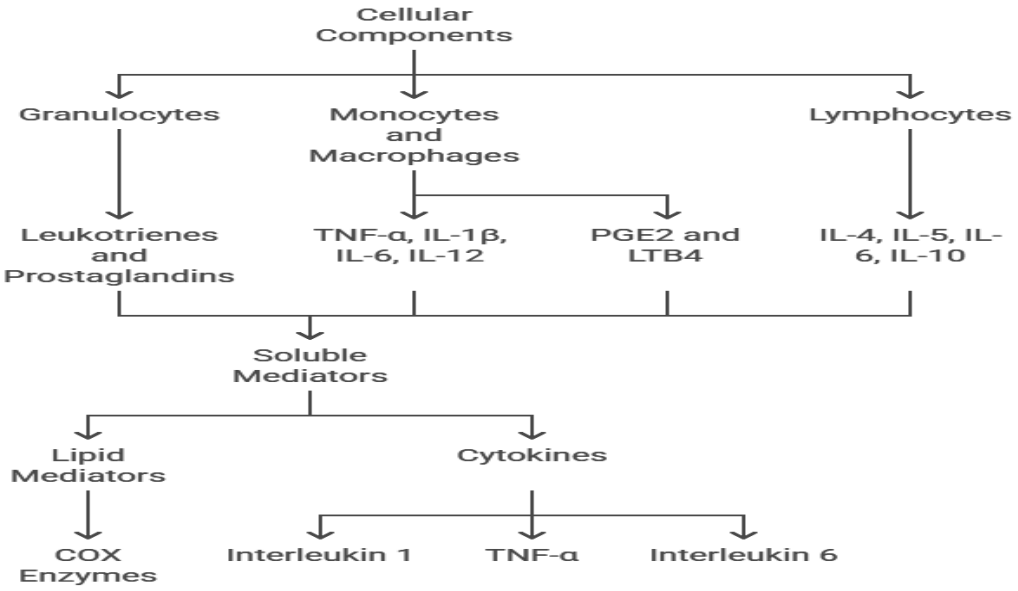


Figure 24: Different from Mediators of inflammation (Bahi, 2015).

II.8.3 Bovine Serum Albumin (BSA)

II.8.3.1 Definition:

Bovine Serum Albumin (BSA) is a globular protein derived from bovine blood serum and is one of the most abundant proteins in mammals. It has a molecular weight of approximately 66.43 K-Da (66430 g/mol) **(R. Feng, Y. Konishi et A. W. Bell et al.,1991)** and plays an essential role in maintaining colloidal osmotic pressure and transporting various molecules in the bloodstream. Due to its well-characterized structure and stability, BSA is widely used as a model protein in biochemical and pharmaceutical research.

II.8.3.2 Role of Bovine serum albumin (BSA):

Bovine serum albumin (BSA) plays an important role in maintaining colloidal osmotic pressure and ensuring proper distribution of body fluids between blood vessels and tissues. In addition, it is widely used as a model protein in various research fields such as molecular biology, medicine, food science, and environmental studies **(R. Feng, Y. Konishi et A. W. Bell et al.,1991).**

II.8.3.3 Structural and Physicochemical Properties of Bovine Serum Albumin (BSA):

Bovine Serum Albumin (BSA) exhibits a characteristic primary structure described as heart-shaped **(O. J. Bos, J. F. Labro, M. J. Fischer, J. Wilting et L. H. Janssen, et al.,1989).**

Its secondary structure is composed of approximately 50–68% α-helices and 16–18% β-she and **(J. F. Foster et al.,1977).** The tertiary structure of BSA has not yet been fully elucidated.

However, due to its high similarity with Human Serum Albumin (HSA), showing about 76% sequence homology—where hydrophobic amino acids are replaced by other hydrophobic residues and hydrophilic amino acids by residues with similar charge—researchers suggest that both proteins likely share a very similar three-dimensional structure.

Furthermore, recent studies have reported differences in certain lysine residues within the tertiary structure of BSA compared to HSA **(B. X. Huang, H.-Y. Kim et C. Dass et al.,2004).**

(BSA) exhibits a density of approximately 1.36 g/cm³ in neutral aqueous solution, which corresponds to the inverse of its partial specific volume. This density shows minimal dependence on pH, with reported values of 1.406 g/cm³ at pH 2 and 1.360 g/cm³ at pH 7 **(R. Jaenicke et M. A. Lauffer ,1969; K. Gekko, A. Kimoto et T. Kamiyama,2003).**

In addition, BSA is characterized by an isoelectric point ranging between 4.7 and 4.9 in aqueous solution at 25°C **(Sigma et al.,1997),** which reflects the pH at which the protein carries no net electrical charge.

II.8.3.4 Mechanisms of BSA–Ligand Interactions:

Protein–ligand interactions are stabilized through a combination of non-covalent forces, including hydrogen bonds, van der Waals contacts, electrostatic complementarity, and hydrophobic effects. Hydrogen bonds provide specificity and directional interactions, whereas van der Waals forces contribute to shape complementarity and close molecular packing. Electrostatic interactions often facilitate the initial recognition between charged groups, while hydrophobic effects enhance stability by promoting the clustering of non-polar regions away from the solvent.

Together, these interactions determine the affinity and selectivity of protein–ligand complexes, which is essential for understanding drug binding mechanisms and supporting rational drug design in pharmaceutical research. **(Madushanka et al., 2023; Grassmann et al., 2023)**

II.8.4 Gastric Ulcers:

II.8.4.1 Definition

A gastric ulcer is a lesion characterized by the destruction of the gastric mucosa and submucosa following damage to the protective layer by gastric juice, leading to a breach in the

stomach wall. The progression of gastric ulcers is classified into four stages **(Cohen, Pandit, Teissier, Merran, 2008):**

- **Stage 1:** Superficial lesions that do not reach the muscularis mucosae.
- **Stage 2:** The ulcer penetrates the muscularis mucosae and reaches the submucosa.
- **Stage 3:** The ulcer extends into the muscular layer (muscularis).
- **Stage 4:** The ulceration reaches the serosal layer after passing through the muscularis.

An illustration of the different layers of the gastric mucosa is provided in Appendix I.

II.8.4.2 The stomach:

is a large, hollow, muscular organ with a bean-like shape. Its upper part serves as a storage area for food, where the cardia and the body relax to accommodate incoming food. Meanwhile, the antrum contracts rhythmically to mix the food with gastric secretions, including acids and enzymes. Gastric mucosal cells also secrete important substances such as mucus, which plays a protective role against damage caused by acid and enzymes **(Ruiz, 2019).**

Figure 25: location of the stomach within the body (Roupioz, 2019).

II.8.4.2 Microscopic Anatomy

The stomach is histological composed of four layers arranged from the inside to the outside: mucosa, submucosa, muscular layer, and serosa. When the stomach is empty or contains little food, it remains in a contracted and reduced state. The mucosa appears folded and is lined with simple columnar epithelium covered by a protective alkaline mucus layer. This layer contains numerous invaginations that extend deeper into structures called gastric glands.

These glands are composed of different cell types responsible for the secretion of hydrochloric acid **(Rad, 2020).**

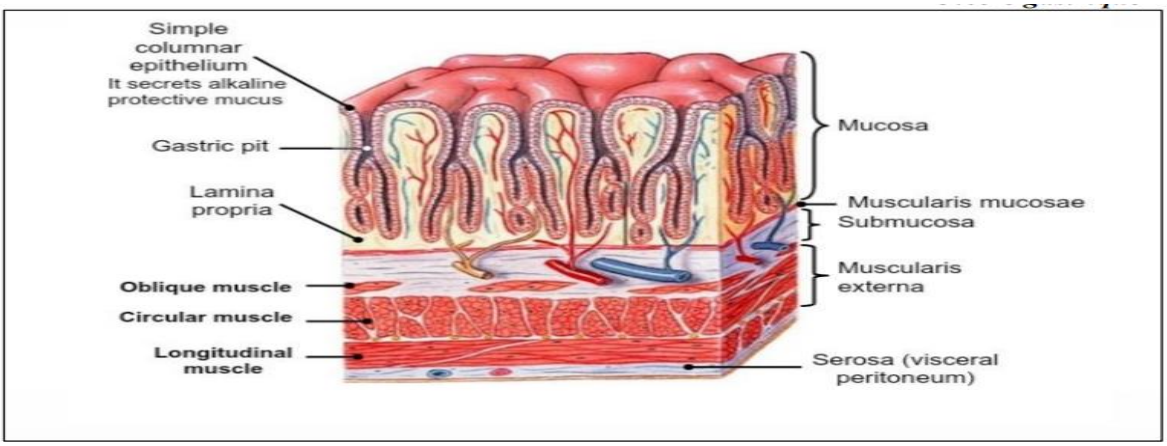


Figure 26: Histology of the stomach (Foster, 2016).

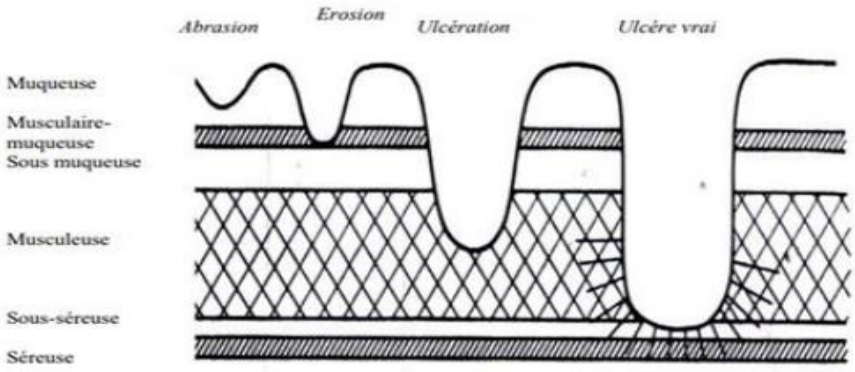


Figure 27: Histopathological aspects of gastric losses (Linguory, 1980)

II.8.4.3 Classification

Gastric ulcers are classified into different types **(Ottenjann, Elster, Cohen, 1980):**

- Circular ulcer: a round or oval-shaped loss of tissue that is deep and funnel-shaped.
- Linear ulcer: observed during the healing phase; it may appear horizontal or vertical.
- Multiple ulcers: can occur anywhere in the stomach and appear in linear patterns or groups.

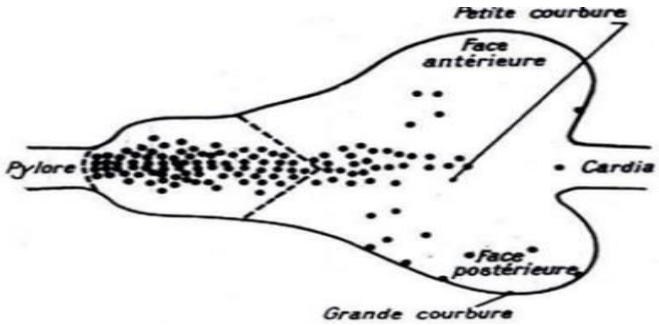


Figure 28: Relative frequency of the different locations of ulcers (Contre, 1980)

II.8.4.4 Aggressive and Defensive Factors in Peptic Ulcers:

Peptic ulcers develop due to an imbalance between aggressive factors, mainly acid-peptic secretions, and the protective mechanisms of the gastric mucosa, including mucus, epithelial integrity, and blood circulation. Duodenal ulcers are mainly caused by acid and pepsin, whereas gastric ulcers are primarily associated with weakened mucosal defence. Both genetic and environmental factors contribute to this imbalance **(Sorbonne, 2018).**

The gastric mucosa is protected by several mechanisms. The epithelial barrier, formed by mucus and bicarbonate-rich secretions, limits hydrogen ion penetration and protects against oxidative stress **(Kodjoh, 2014).** Adequate blood circulation also maintains mucosal integrity by removing harmful substances and supplying oxygen and nutrients, while reduced blood flow promotes oxidative damage and ulcer formation **(Ouledelhachemi, 2012).** In addition, prostaglandins reduce gastric acid secretion and enhance mucosal protection, particularly prostaglandins E1 and I1, which help prevent damage induced by NSAIDs, histamine, gastrin, and acetylcholine **(Rossant-Lumbroso, 2020).**

II.8.4.5 Pathophysiology:

Peptic ulcer disease is characterized by localized destruction of the gastric or duodenal mucosa, involving interruption of the mucosal and muscular layers, often associated with inflammation and fibrosis. The disease follows a chronic course with recurrent exacerbations and remission periods. Gastric ulcers commonly occur in the antrum and lesser curvature, whereas duodenal ulcers are mainly located in the duodenal bulb. Rare post-bulbar ulcers may indicate Zollinger–Ellison syndrome.

Normally, a balance exists between aggressive factors such as hydrochloric acid, pepsin, and gastrin, and protective factors including mucus, bicarbonate, mucosal blood flow, and cyto-protection. Peptic ulcer disease develops when this balance is disrupted due to increased aggressive factors or weakened mucosal defences, leading to tissue damage **(HAS, 2003; CDU-HGE, 2012; Raymand et al., 2010).**

II.8.5 Anti-glycation:

II.8.5.1 Definition:

When this sand of Maillard reactions occurs in vivo, it is referred to as non-enzymatic glycosylation or simply glycation, and it results in the formation of a heterogeneous group of compounds known as advanced glycation end-products (AGEs). Generally, the formation of these AGEs alters the chemical properties of protein residues (i.e., Lys and Arg), amino phospholipids (i.e., phosphatidylserine and phosphatidyl ethanolamine), or deoxyribonucleic

acids (mainly guanosine **(Jaramillo et al. 2017; Dutta et al. 2006))** on which they are formed. As a consequence, this may have significant effects on the intra- and/or intermolecular interaction patterns of biomacromolecules, potentially leading to macromolecular misfolding, aggregation, and, as has been recently recognized, the development of glycation-related diseases, particularly diabetes-related diseases.

II.8.5.2 Molecular mechanisms underlying protein glycation:

The extracellular glycation mechanism begins with the reaction between a protein's primary amino group (N-terminal α-amino group or ε-amino group of lysine residues) and the carbonyl group of glucose, forming a reversible Schiff base **(Cho et al.,2007).** This intermediate exists in equilibrium between an open-chain aldimine and a more stable glycosyl amine (Irvine and Campbell 1922), which then rapidly undergoes Amadori rearrangement to form a stable amine known as the Amadori product **(Cho et al, 2007; Yaylayan et Huyghues-Despointes,1994).** Schiff bases form quickly and reversibly, while Amadori products accumulate due to their greater stability **(Yaylayan et Huyghues-Despointes,1994).** Over time, Amadori products undergo further irreversible reactions via the Hodge pathway, leading to the formation of advanced glycation end-products (AGEs)

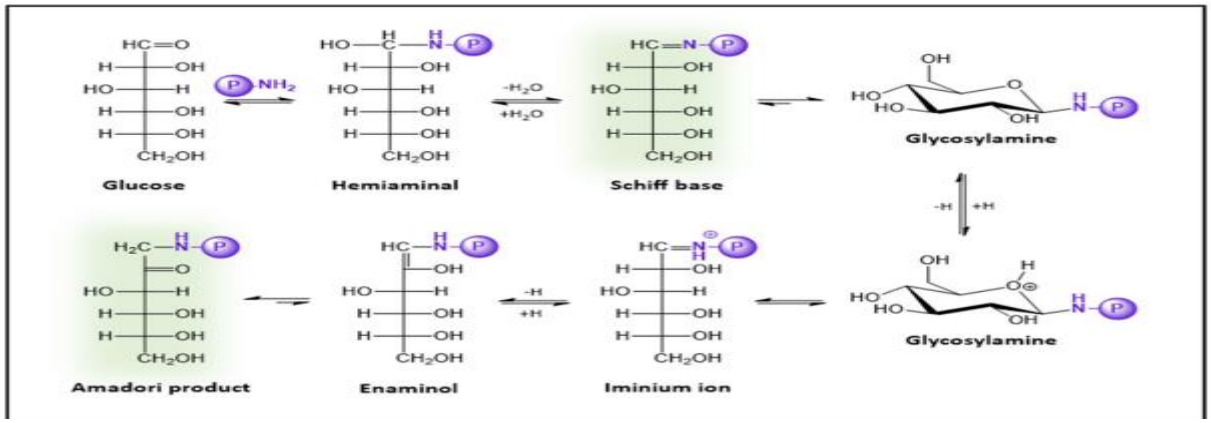


Figure 29: Formation of Schiff base and Amadori product during protein glycation (Ana Belén Uceda et al.,2024)

Glycation is enhanced by sugar fragmentation pathways (Wolff and Namiki), generating reactive carbonyl species (RCS) such as 3-DG, methylglyoxal (MG), and glyoxal (GO), which strongly react with lysine and arginine to form diverse AGEs, including hydroimidazolones

MG, mainly produced during glycolysis, is the most reactive glycating agent, forming multiple adducts (e.g., CEL, MOLD, THP, argpyrimidine, hydroimidazolones) with proteins **(Thornaley 2005; Ahmed et al. 1997; Brinkmann et al. 1998; Oya et al. 2000; Shipanova et al. 1997).**

Overall, glycation is influenced by factors such as pH, temperature, sugar type, and protein properties. RCS are the most harmful agents and, under carbonyl stress, play a major role in cellular damage and diabetes complications **(Johansen et al. 2006; Martins et van Boekel 2005).**

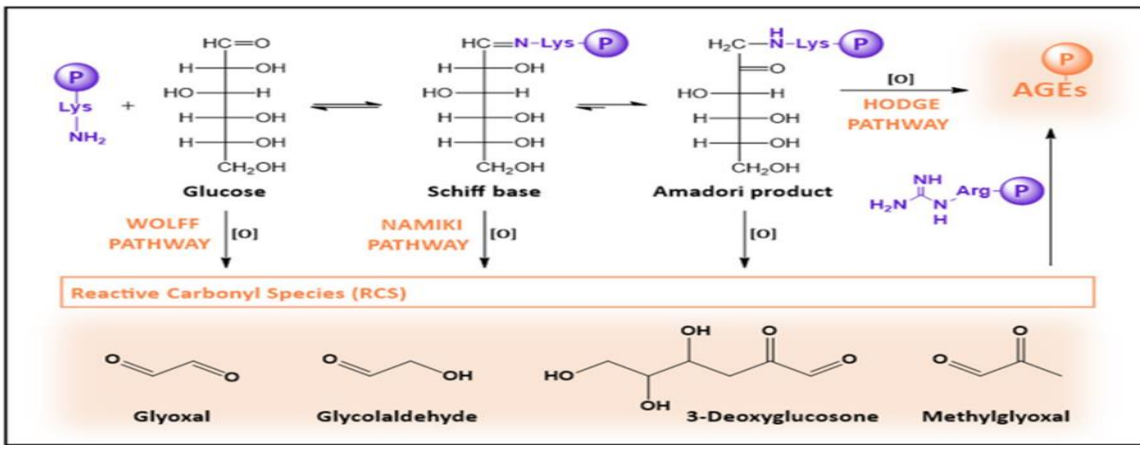


Figure 30: Main pathways involved in AGE formation from glucose and protein glycation intermediates (Ana Belén Uceda et al.,2024)

II.8.5.3 Effect of glycation on protein function:

Glycation and AGE formation impair protein function even without major structural changes by modifying lysine and arginine residues, altering charge, folding, aggregation, and protein interactions, and reducing enzymatic activity (kcat, KM) **(Adrover et al. 2014).**

Glycated serum albumin shows altered biological activity and increased cytotoxicity **(Anguizola et al. 2013; Baraka-Vidot et al. 2013; Byun et al. 2012**). Other proteins are also affected, such as cytochrome c (pro-apoptotic after glycation) and enzymes like paraoxonase, hemoglobin, and alkaline phosphatase, whose activities decrease **(Sharma et al. 2019; Hedrick et al. 2000; Bose et al. 2013; McCarthy et al. 1998).**

In some cases, glycation may enhance or modify activity depending on the protein (e.g., myoglobin, α-crystallin) **(Roy et al. 2010; Kumar et al. 2004).** AGEs also promote oxidative stress via ROS, contributing to cellular dysfunction **(Bansal et al. 2012; Nowotny et al. 2015).** Overall, effects depend on protein type, extent of modification, and cellular context.

II.8.5.4 Endogenous mechanisms of detoxification against glycation

The body has endogenous defence systems to limit glycation by detoxifying reactive intermediates and removing glycated biomolecules. The glyoxalase system (glyoxalase I and II) plays a key role by converting reactive di-carbonyls (methylglyoxal, glyoxal, 3-

deoxyglucosone) into less harmful metabolites, maintaining intracellular carbonyl balance **(Aragonès et al. 2021; Thornalley. 1990).**

Addit 4 ions pathways include DJ-1 protein, aldose-keto reductases, and aldehyde dehydrogenases, which detoxify reactive carbonyl species, though they may sometimes produce secondary toxic metabolites. Damaged or glycated proteins are removed via the ubiquitin–proteasome system and autophagy pathways, although their efficiency declines with glycation and aging.

Finally, enzymes such as fructose amine 3-kinase can reverse early glycation, helping repair proteins and limit AGE accumulation.

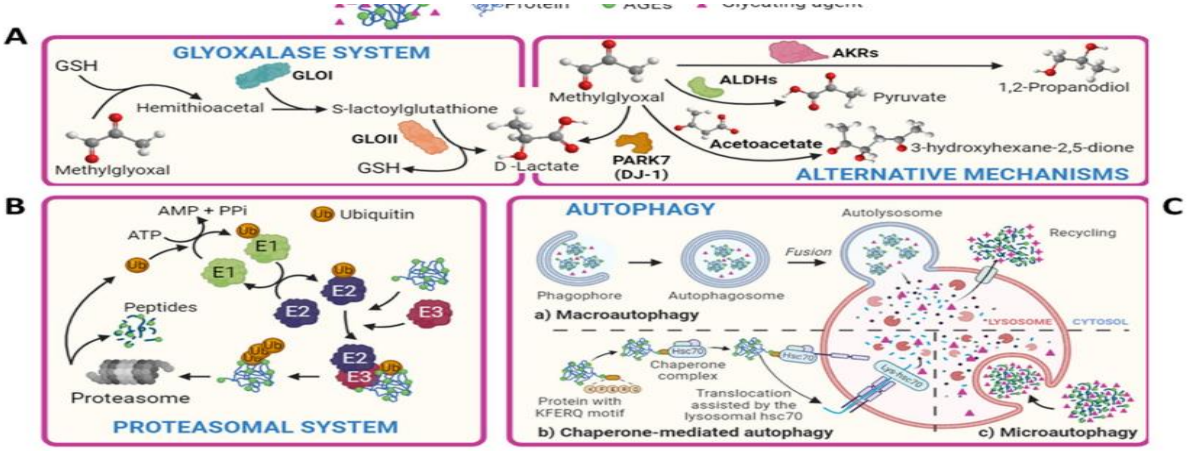


Figure 31: Endogenous Detoxification Mechanisms Against Glycation (Ana Belén Uceda et al.,2024)

II.8.5.5 Types and effectiveness of glycation inhibitors:

Pharmaceutical glycation inhibitors are used to reduce diabetic complications, particularly cardiovascular disorders, by compensating for insufficient natural defence mechanisms in diabetes **(Abbas et al., 2016; Yamagishi et al., 2008; Nenna et al., 2015).** These inhibitors may be synthetic or natural and act through various mechanisms, including inhibition of early glycation reactions, trapping reactive intermediates, antioxidant activity, metal chelation, and blocking AGE related pathways.

Major glycation inhibitors include compounds competing with carbohydrates, agents reacting with sugars or carbonyl intermediates, antioxidants, metal chelators, carbonyl scavengers, inhibitors of Amadori product progression, and AGE breakers such as ALT-711, which can reverse glycation damage and improve vascular function **(Wolffenbuttel et al., 1998; Coughlan et al., 2007).**

Aminoguanidine is considered an important glycation inhibitor due to its carbonyl-scavenging activity, which reduces AGE formation and delays diabetic complications such as neuropathy, nephropathy, and retinopathy (**Jud et Sourij, 2019; Peyroux et Sternberg, 2006; Rahbar et Figarola, 2002**). However, its clinical use remains limited because of inconsistent results, side effects, and reduced efficacy in advanced trials **(Freedman et al., 1999; Jud et Sourij, 2019; Nenna et al., 2015).**

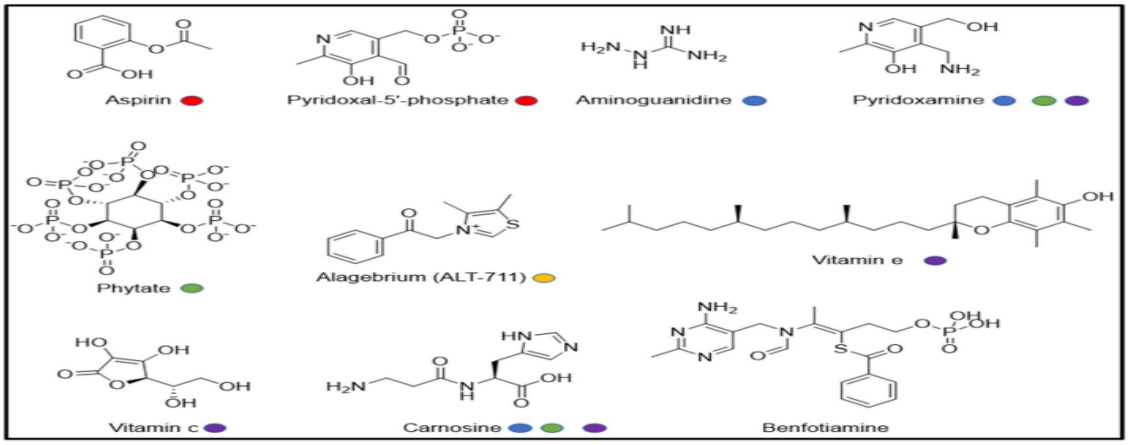


Figure 32: Classification of Glycation Inhibitors by Mechanism of Action (Ana Belén Uceda et al.,2024)

II.8.6 Antimicrobial activity:

The term “antimicrobial agent” refers to any substance capable of destroying microorganisms or inhibiting their growth, including antibacterial agents. Antimicrobial agents have been used for many decades in the treatment of infectious diseases and in the prevention of infections. Their mode of action against bacteria can be classified as either bacteriostatic, when the compound inhibits bacterial proliferation, or bactericidal, when it completely eliminates bacterial cells **(Cce, 2001).**

II.8.6.1 Antibiotics

An antibiotic is a natural, semi-synthetic, or synthetic substance that can kill or inhibit bacterial growth at low concentrations by targeting vital cellular processes **(Okusa, 2012).** More than 22,500 bioactive compounds have been obtained from microorganisms, mainly from actinomycetes (45%), fungi (38%), and other bacteria (17%). Around 5,000 antibiotics have been identified from bacterial and filamentous fungal cultures **(Gebreyohannes et al., 2013).**

II.8.6.2 Mode of action of antibiotics:

Antibiotics are designed to act on microbial pathogens at concentrations that are safe for the host while being sufficient to disrupt microbial metabolism or even lead to their destruction. To achieve this, they targeted essential cellular structures such as the bacterial cell wall, nucleoid, cytoplasmic membrane, and ribosomes. The activity of most antibiotics is based on one or more of the following mechanisms:

- Inhibition of bacterial cell wall synthesis.
- Disruption of cytoplasmic membrane permeability.
- Inhibition of protein synthesis.
- Inhibition of nucleic acid synthesis **(Minor a et nd Veron, 1989).**

II.8.6.3 Bacterial resistance to antibiotics:

Bacterial resistance to antibiotics is defined as the ability of a bacterial strain to grow in the presence of higher concentrations of antibiotics compared to other phylogenetically related strains. Some resistance mechanisms directly targeted the antibiotics, while others act on cellular processes involved in their transport. The most common mechanisms include reducing the intracellular concentration of antibiotics by decreasing membrane permeability and/or increasing efflux pump activity, enzymatic inactivation or modification of antibiotics, and alteration of their cellular targeted **(Levy and Marshal, 2004).**

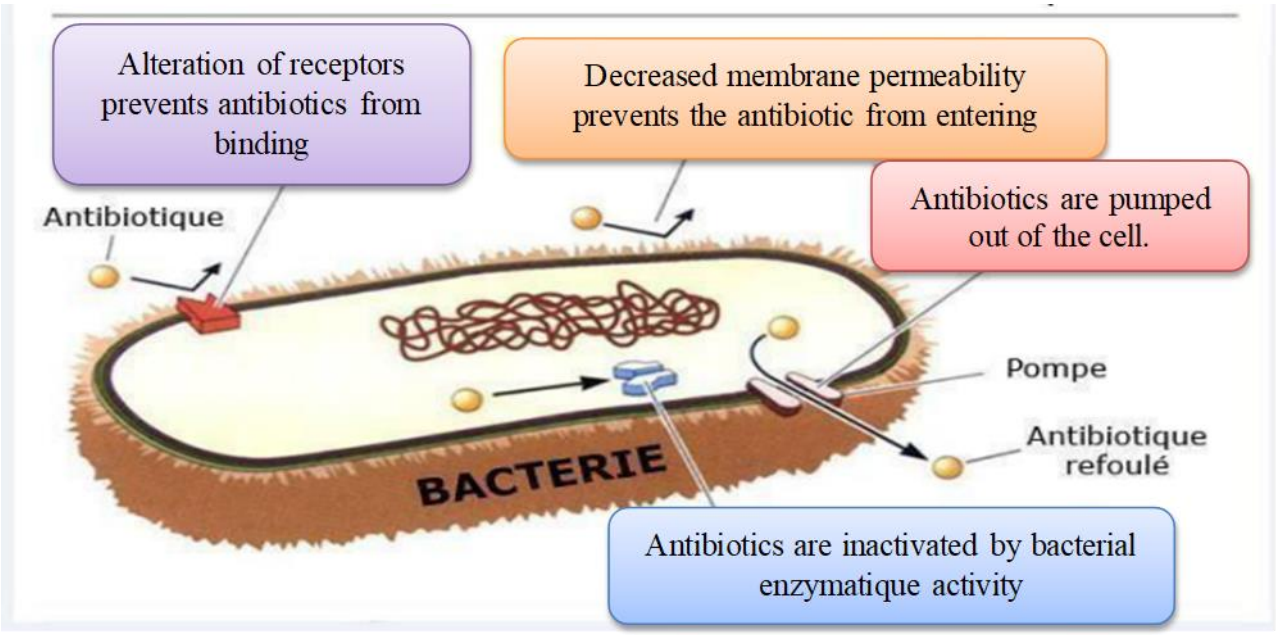


Figure 33: Bacterial resistance to antibiotics (Levy et Marshal, 2004).

II.8.6.4 Bacteria

Bacteria are unicellular prokaryotic microorganisms lacking a nuclear membrane, which distinguishes them from eukaryotes such as fungi, algae, and protozoa. They are divided into Bacteria and Archaea, with pathogenic species studied in bacteriology. Typically smaller than 1 µm, they can be observed under a light microscope after staining. Their shapes vary, including cocci, bacilli, vibrio, and spirochetes, while detailed structures are visible only by electron microscopy **(Ricci et al., 2005).**

II.8.6.5 Description of the studied bacteria

II.8.6.5.1 Escherichia coli

It is a Gram-negative bacillus, facultative anaerobe, non-halophilic, and non-spore-forming bacterium, usually motile. It belongs to the coliform group due to its ability to grow at relatively high temperatures (around 44.5°C). It includes numerous strains, some of which are pathogenic and can cause gastrointestinal and urinary tract infections as well as neonatal meningitis **(Nataro et Kaper, 1998; Boerlin et White, 2013).**

II.8.6.5.2 Staphylococcus aureus

It is a Gram-positive coccus, non-motile and non-spore-forming, with some strains producing a characteristic golden-yellow pigment. It can be found in different environments, including low-moisture foods. It is responsible for various infections such as meningitis, osteomyelitis, and diarrhoea, and some strains are antibiotic-resistant **(Hugas et al., 2002; Steven et al., 2004).**

II.8.6.5.3 Pseudomonas aeruginosa

It is a Gram-negative bacillus, motile due to a single polar flagellum, and non-spore-forming. It is one of the most important causes of hospital-acquired infections, particularly respiratory and urinary tract infections, and is among the leading bacterial pathogens in clinical settings **(Richard et Kiredjian, 1995).**

II.9 Molecular Docking:

Molecular docking is a pivotal computational technique used to predict how small molecules (ligands) bind to a specific blocked on a macromolecule (targeted). This process involves the virtual screening of various conformations, positions, and orientations of the ligand within the binding site. Only the configurations that demonstrate the most favourable interactions are randomized for further study. This approach is indispensable in biology,

pharmacy, and medicine, as the majority of therapeutic drugs function by binding to specific biological targeted **(EL Hadj Said, 2016; Lanez, 2016).**

II.9.1 Principles of Molecular Docking:

The molecular docking process is fundamental divided into two primary stages:

II.9.1.1 Sampling (Search Algorithm):

The ligand is positioned within the protein's active site, where the algorithm explores the "conformational space"—testing various shapes, locations, and orientations. Only the most promising arrangements with strong predicted interactions are selected.

II.9.1.2 Scoring:

These arrangements are evaluated and ranked using a mathematical

II.9.1.3 scoring function.

This system predicts the binding affinity and helps identify the most likely binding mode **(EL Hadj Said, 2016).**

Superior molecular docking algorithms are characterized by their ability to accurately predict experimental binding modes. Furthermore, an effective scoring system must distinguish the "native" or correct binding arrangement as the top-ranked pose among al generated possibilities **(Bouchagra, 2018).**

The resulting docking score provides a rough estimation of the Gibbs free energy change (ΔG) that occurs when a protein and ligand bind to form a stable complex, grounded in the laws of thermodynamics **(Arrault, 2007):**

$\Delta G = \Delta G \text{ complex} - \Delta G \text{ ligand} - \Delta G \text{ protein}$

In theory, the spontaneous binding between a protein and a ligand leading to a stable complex requires that the overall change in Gibbs free energy be negative (ΔG of complexation < 0) **(Lebbad, 2016).**

II.9.2 Steps of Molecular Docking:

Molecular docking generally involves three principal stages: the selection of the three-dimensional (3D) structures of both the targeted and the ligand, the preparation of these structures according to the chosen docking protocol, and final, the analysis and interpretation of the obtained results **(Morris, 2008).**

II.9.3 Tools of Molecular Docking:

The practical application of molecular docking relies on several essential components:

II.9.3.1 Protein: Macromolecules

A crucial prerequisite for performing molecular docking is the availability of the 3D structure of the targeted molecule, typically an enzyme **(Dar et Mir, 2017).** Such structural data are commonly accessible through specialized databases, particularly the Protein Data Bank (PDB).

II.9.3.2 Ligand

The acquisition of the ligand's 3D structure is equally important. These structures may be retrieved from public databases such as ZINC or PubChem, or alternatively generated using molecular modelling software like Chemosynthesis or Gaussian through geomancy optimization based on quantum chemical methods **(Jade et Gupta, 2016; El Hadji Said, 2016).**

II.10 Molecular Modelling

Prior to docking, ligand structures must undergo optimization using molecular modelling techniques to ensure accurate representation and reliable interaction predictions.

II.11 Docking Programs

Docking software constitutes a fundamental tool in biological, pharmaceutical, and medical research **(Amina et Khaoula, 2017).** Numerous programs—both commercial and open-source—are available, including Surfle x, GOLD, Flex X, DOCK, and Auto-Dock. These tools are particularly valuable for their ability to efficiently screen large libraries of compounds using advanced computational strategies such as Genetic Algorithms, Monte Carlo simulations, and Incremental Construction methods **(Dias et al., 2008; Forli et al., 2016).**

II.11.1 Auto-Dock Molecular Docking Software

Auto-Dock is a widely used docking program that relies on simulation of ligand trajectories to predict binding modes with high accuracy. The process begins with the ligand positioned outside the receptor's active site, followed by iterative exploration of the binding region through translations, rotations, and conformational adjustments. After each step, an energy function evaluates the interaction between the ligand and receptor, guiding subsequent movements. The simulation concludes upon identification of the most favourable binding conformation.

This approach effectively incorporates ligand flexibility and enables comprehensive exploration of the binding site **(LANEZ, 2019).**

II.11.2 Servers Used – Swiss-ADME

Swiss-ADME is a freely accessible web-based tool that utilizes computational models to predict key properties of drug-like molecules in a rapid and reliable manner. These include physicochemical characteristics, pharmacokinetic parameters (ADME), drug-likeness, and medicinal chemistry suitability. Its intuitive interface allows users to input multiple compounds and easily interpret the predictions, thereby supporting various stages of the drug discovery process **(Daina and al., 2017).**

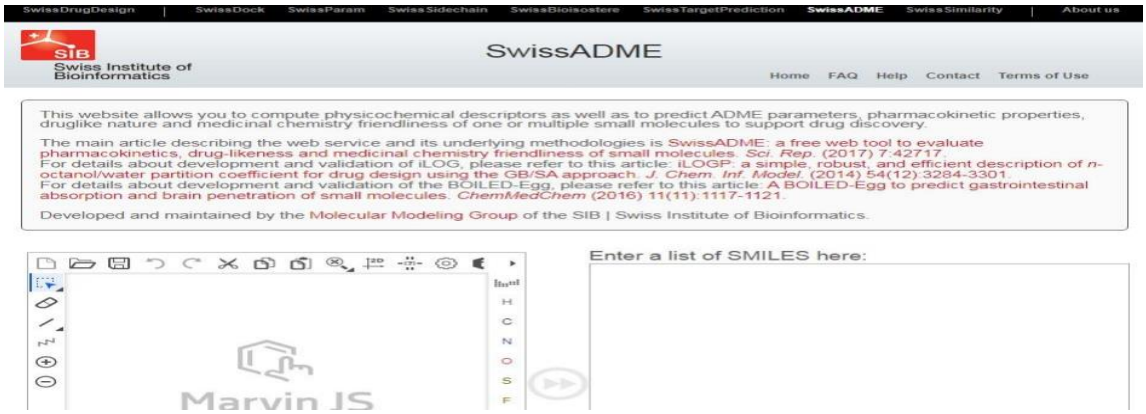


Figure 34: Home page of the Swiss-ADME server (Daina and al., 2017)

II.11.3 The PubChem Database:

The PubChem database, established in 2004, has become a major public resource for chemical information, serving students, researchers, and scientists worldwide. It provides access to millions of users each month through its online platform. PubChem contains data on a wide range of chemical substances, including small molecules as well as larger biomolecules such as nucleotides, peptides, lipids, carbohydrates, and chemicaly modified macromolecules. It compiles extensive information related to chemical identifiers, molecular structures, and various properties, in addition to biological activities, physicochemical characteristics, safandy data, health-related information, toxicity, and patent records **(Trad, 2020).**

Figure35 : Home page of the Pub-chem database server (Trad, 2020)

II.11.4 Pro-Tox

Pro-Tox-II is a free online tool (accessible at http://tox.charite.de/protox_II without needing to log in) that helps predict how toxic a chemical might be. It's designed for toxicologists, regulators, computer and medicinal chemists, and anyone else interested. You just enter the 2D structure of a chemical, and Pro-Tox-II will give you a possible toxicity profile based on 33 different models, along with how confident it is in each prediction. It also shows a radar chart summarizing the overall toxicity and compares your chemical to the three most similar compounds with known immediate (acute) toxicity **(Banerjee et al., 2018).**

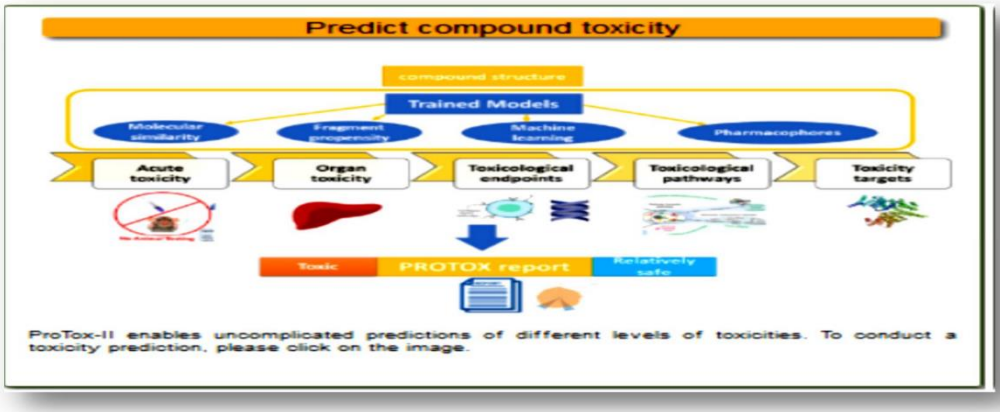


Figure 36: Home page of the pro-Tox server (Banerjee et al., 2018).

II.11.5 Auto-Dock-Tools (ADT)

Auto-Dock-Tools (ADT) is a free graphical user interface developed by the creators of Auto-Dock to simplify the preparation, execution, and analysis of molecular docking

simulations as well as Auto-Grid calculations. In addition to its primary role in docking workflows, ADT provides several functionalities for molecular modelling, including the calculation of molecular surfaces, visualization of secondary structures, and analysis of hydrogen bonding interactions, among other features **(Ahmedou, 2021).**

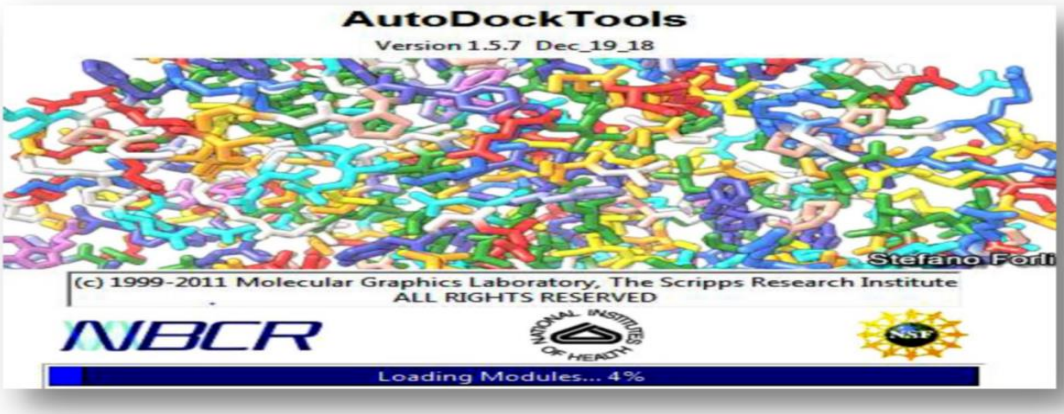


Figure 37: Auto-Dock-Tools server home page (Ahmedou, 2021).

II.11.6 Discovery Studio

Discovery Studio is a comprehensive software platform designed for the simulation and modelling of both small and large molecular systems. It is widely used in various fields of research for applications such as molecular simulations, ligand design, pharmacophore modelling, structure-based drug design, quantitative structure–activity relationship (QSAR) analysis, and ADM and prediction **(Ahmedou, 2021).**

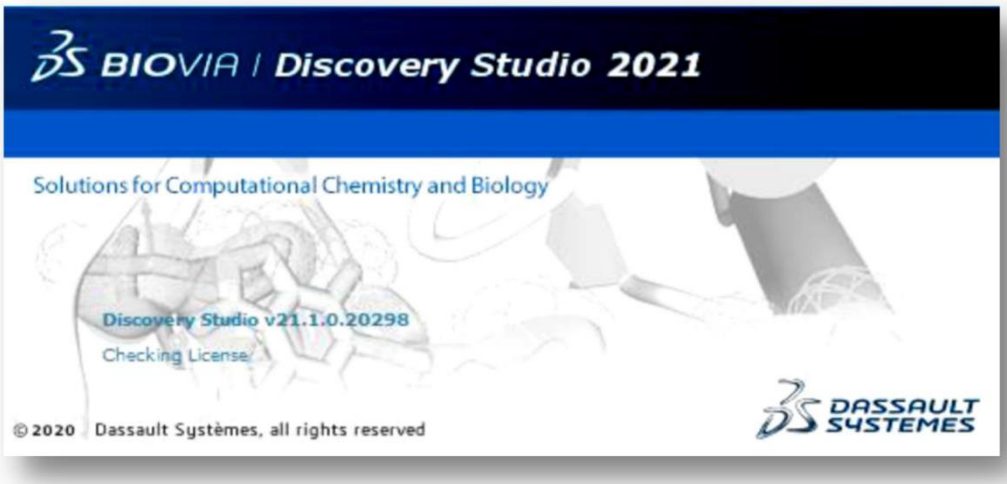


Figure 38: Discovery Studio server home page (Ahmedou, 2021)

II.11.7 The Protein Data Bank (PDB)

The Protein Data Bank (PDB) is the principal global repository for freely available three-dimensional structures of biological macromolecules. Structural data are contributed by researchers from around the world, often as a requirement for publication in leading scientific journals. The PDB serves a broad and diverse international community, including biologists from various fields such as structural biology and pharmacology, bioinformaticians, software developers, educators, students, science communicators, and the general public. As a result, it

attracts a large number of users, with approximately 140,000 unique visitors each month from nearly 140 countries **(Dorey, 2012).**

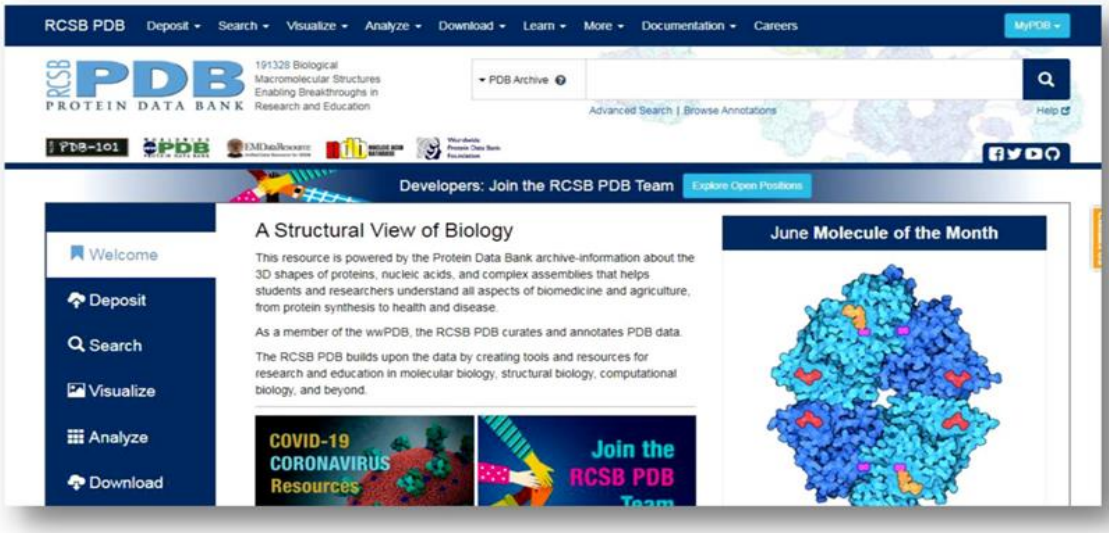


Figure 39: Protein Data Bank (PDB) server home page (Dorey, 2012).

II.11.8 Py MOL

Py MOL (Pleomorphic Analysis Methodology) is a free and open-source molecular visualization software developed by Warren Lyford DeLano. It is widely used for generating high-quality three-dimensional representations of both small and large molecules, making it an essential tool in scientific research and education, particularly in the field of structural biology. Its open-source nature further promotes its accessibility and widespread use within academic and research communities.

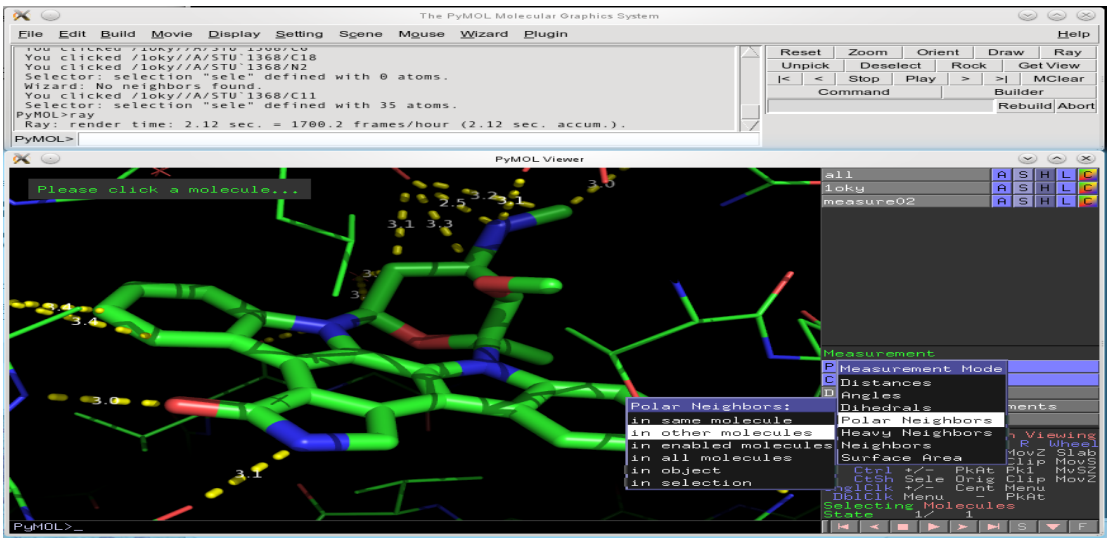


Figure 40: Py MOL software interface

II.11.9 PLIP (Protein–Ligand Interaction Profiler)

PLIP (Protein–Ligand Interaction Profiler) is an online tool designed to analyse and visualize interactions between proteins and small molecules in three dimensions. It enables the identification of ligand binding sites on proteins, predicts potential interactions, and provides detailed characterization of the specific atoms involved as well as the types of molecular forces governing these interactions **(Salentin, 2015).**

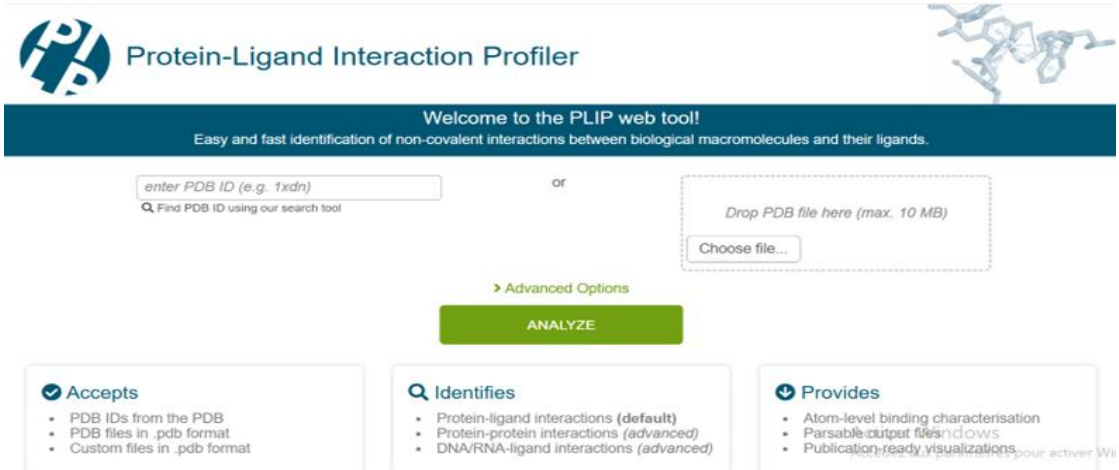


Figure 41: PLIP home page (Salentin, 2015).

General Conclusion

The present study demonstrated that *Centaurium erythraea* extract and vitamin D possess important biological properties that may contribute to reducing mechanisms associated with insulin resistance and oxidative stress. The obtained findings revealed that the plant extract exhibits considerable antioxidant, anti-inflammatory, antibacterial, and protein-protective activities, including the prevention of protein oxidation and glycation. These effects may be attributed to its richness in bioactive phytochemicals such as polyphenols, flavonoids, secoiridoids, and other phenolic compounds.

In addition, the results indicated that vitamin D plays a significant role in improving metabolic balance and regulating insulin signaling pathways through its antioxidant and anti-inflammatory effects. The interaction studies performed using both in vitro and in silico approaches supported the existence of important interactions between vitamin D and α-amylase, suggesting its possible involvement in the regulation of carbohydrate metabolism and the reduction of metabolic disturbances related to insulin resistance.

Furthermore, the combination of vitamin D with *Centaurium erythraea* extract exhibited enhanced biological activities compared with the individual compounds, particularly in combating oxidative stress, inhibiting inflammation, and protecting proteins from oxidative and glycation damage, indicating a potential synergistic effect between both agents. These findings highlight the importance of combining natural bioactive compounds with essential nutrients as a promising strategy for the prevention and management of complications associated with Type 2 Diabetes Mellitus.

Overall, this work provides valuable scientific evidence supporting the therapeutic potential of both *Centaurium erythraea* and vitamin D. It also opens promising perspectives for further in vivo and clinical investigations to confirm their therapeutic efficacy, clarify their molecular mechanisms of action, and evaluate their safety for future biomedical and pharmaceutical applications.

References Bibliography

References

- Abbas G, Al-Harrasi AS, Hussain H, Hussain J, Rashid R, Choudhary MI (2016) Antiglycation therapy: discovery of promising antiglycation agents for the management of diabetic complications. Pharm Biol 54(2):198–206. https://doi.org/10.3109/13880209. 2015.1028080
- Adrover M, Mariño L, Sanchis P, Pauwels K et al (2014) Mechanistic insights in glycation-induced protein aggregation. Biomacromol 15(9):3449–3462. https://doi.org/10.1021/bm501077j
- Agelet, A., Vall`es, J., 2003. Studies on pharmaceutical ethnobotany in the region of Pallars (Pyrenees, Catalonia, Iberian Peninsula). Part II. New or very rare uses of previously known medicinal plants. J. Ethnopharmacol. 84, 211–227. https://doi.org/10.1016/S0378-8741(02)00319-7.
- Ahmed MU, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW (1997) Nε -(carboxyethyl)lysine, a product of chemical modification of proteins by methylglyoxal, increases with age in human lens proteins. Biochem J 324(2):565–570. https://doi.org/10.1042/bj324056 5
- Anguizola J, Matsuda R, Barnaby OS, Hoy KS, Wa C, DeBolt E, Koke M, Hage DS (2013) Review: glycation of human serum albumin. Clin Chim Acta 425:64–76. https://doi.org/10.1016/j.cca.2013. 07.013
- Aragonès G, Rowan S, Francisco SG, Whitcomb EA, Yang W et al (2021) The glyoxalase system in age-related diseases: nutritional intervention as anti-ageing strategy. Cells 10:1852. https://doi.org/10.3390/cells1008185 2
- Arapitsas P. Hydrolyzable tannin analysis in food. Food Chemistry 135, 1708-1717, (2012)
- Arbouche Lezoul Z. (2007). Les effets du traitement substitutif post ménopausique chez la diabétique de type 2, sur le métabolisme des lipoprotéines et le métabolisme glucidique ,Science Médicales., Université d'Alger., p 11.
- Azab, A., Nassar, A., & Azab, A. N. (2016). Anti-Inflammatory Activity of Natural Products. *Molecules*, *21*(10), 1321. https://doi.org/10.3390/molecules21101321
- B. X. Huang, H.-Y. Kim and C. Dass, "Probing Three-Dimensional Structure of Bovine Serum Albumin by Chemical Cross-Linking and Mass Spectrometry", Journal of American Society for Mass Spectrometry, 15, (2004) 1237 – 1247
- Baba Aissa, F. 1991. Les plantes médicinales en Algérie. Bouchène et Ad.Diwan.
- Baba Aissa, F., Encyclopedie des plantes utiles: Flore d"Algérie et du Maghreb Rouiba: Librairie moderne; p: 243 - 244., (1999).

- Baba Aissa, F.2000. Encyclopédie des plantes utiles Flore d'Algérie et du Maghreb. EDAS Librairie moderne Rouiba. 368 p.
- Bahi, A. (2014-2015). L'Effet protecteur des antioxydants naturels dans l'intoxication du mercure chez le rat Albinos Wistar (Aspects biochimiques, immunologiques et histologiques) (Thèse de doctorat, Université des Frères Mentouri-Constantine) p.7-10-11-12-13-16-17
- Bajaj S, Singh RP, Dwivedi NC, Singh K, Gupta A, Mathur M. (2014). Vitamin D levels and microvascular complications in type 2 diabetes. Indian Journal of Endocrinology and Metabolism, 18(4), 537–541.
- Bansal S, Siddarth M, Chawla D, Banerjee BD, Madhu SV, Tripathi AK (2012) Advanced glycation end products enhance reactive oxygen and nitrogen species generation in neutrophils in vitro. Mol Cell Biochem 361(1–2):289–296. https://doi.org/10.1007/s11010-011-1114- 9
- Baraka-Vidot J, Guerin-Dubourg A, Dubois F, Payet B, Bourdon E, Rondeau P (2013) New insights into deleterious impacts of in vivo glycation on albumin antioxidant activities. Biochim Biophys Acta 1830(6):3532–3541. https://doi.org/10.1016/j.bbagen.2013.01.019
- Barbarawi M, Kheiri B, Zayed Y, et al. (2019). Vitamin D supplementation and cardiovascular disease risks in more than 83,000 individuals in 21 randomized clinical trials. JAMA Cardiology, 4(8), 765–776.
- Berkan, T., L. Ustunes, F. Lermioglu, and A. Ozer. 1991. Antiinflammatory, analgesic, and antipyretic effects of an aqueous extract of *Erythraea* centaurium. Planta Med. 57:34–37.
- Berthod A, Billardello B, Geoffroy S.Polyphenols in countercurrent chromatography. An example of large scale separation 1. Analusis. EDP Sciences, Wiley-VCH. 27: 750-757. (1999).
- Bikle DD. (2016). Extraskeletal actions of vitamin D. Annals of the New York Academy of Sciences, 1376(1), 29–52.
- Bikle. (2014). Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. Bone, 23(1), 1–7. https://doi.org/10.1016/j.chembiol.2013.12.016.Vitamin
- Blaak EE. (2012). Current metabolic perspective on malnutrition in obesity and type 2 diabetes. Current Opinion in Clinical Nutrition & Metabolic Care, 15(6), 553–558.

- Bose T, Bhattacherjee A, Banerjee S, Chakraborti AS (2013) Methylglyoxal-induced modifications of hemoglobin: structural and functional characteristics. Arch Biochem Biophys 529(2):99–104. https://doi.org/10.1016/j.abb.2012.12.001
- Boucher, J., Kleinridders, A. et Kahn, C. R. (2014). Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harbor perspectives in biology, 6(1), a009191
- Bouillon R, Carmeliet G, Verlinden L, et al. (2008). Vitamin D and human health: lessons from vitamin D receptor null mice. Endocrine Reviews, 29(6), 726–776.
- Bouyahya, A., Abrini, J., EtTouys, A., Bakri, Y., Dakka, N., 2017a.Indigenous knowledge of the use of medicinal plants in the North-West of Morocco and their biological activities. European Journal of Integrative Medicine13, 9-25. https://doi.org/10.1016/j.eujim.2017.06.004.
- Brinkmann F, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW (1998) Role of the Maillard reaction in aging of tissue proteins: advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. J Biol Chem 273:18714–18719. https://doi.org/10.1074/jbc.273.30.18714
- Brinkmann F, Brinkmann Frye E, Degenhardt TP, Thorpe SR, Baynes JW (1998) Role of the Maillard reaction in aging of tissue proteins: advanced glycation end product-dependent increase in imidazolium cross-links in human lens proteins. J Biol Chem 273:18714–18719. https://doi.org/10.1074/jbc.273.30.18714
- Brown AJ, Dusso AS, Slatopolsky E. Vitamin D. Am J Physiol 1999; 277: F157–75. [Google Scholar]
- Brown AJ, Dusso AS, Slatopolsky E. Vitamin D. Am J Physiol 1999; 277: F157–75. [Google Scholar]
- Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales, 3 ème Ed Tec & Doc, 1120 p, (1999).
- Buysschaert M.et al. (2006). Diabétologie clinique. Belgique: 3ème éddition, De Beock.Carey VJ, Walters EE, Colditz GA, et al. (1997). Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. Am J Epidemio l9-614 : 145 1997
- Buysschaert M.et al. (2006). Diabétologie clinique. Belgique: 3ème éddition, De Beock.
- Byun K, Bayarsaikhan E, Kim D, Kim CY et al (2012) Induction of neuronal death by microglial AGE-albumin: implications for Alzheimer's disease. Plos One 7(5): e37917. https://doi.org/10. 1371/journal.pone.0037917

- Carey VJ, Walters EE, Colditz GA, et al. (1997). Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. The Nurses' Health Study. Am J Epidemiol 9-614 :145; 1997
- Casteels KM, Mathieu C, Waer M, et al. (1998). Prevention of autoimmune diabetes in NOD mice by early intervention with vitamin D. Diabetologia, 41(3), 307–314.
- Cell Biol 2008; 9: 193–205.
- Cheng JB, Levine MA, Bell NH, et al. Genetic evidence that the human CYP2R1 is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 2004; 101: 7711–5. [Google Scholar]
- Cho SJ, Roman G, Yeboah F, Konishi Y (2007) The road to advanced glycation end products: a mechanistic perspective. Curr Med Chem 14:1653–1671. https://doi.org/10.2174/0929867077 80830989
- Chou O, Ali A, Subbiah V, Barrow CJ, Dunshea FR,Suleria HAR. LC-ESI-QTOFMS/MS characterization of phenolics in herbal tea infusion and their antioxidant potential. Fermentation. 2021, 7, 73 - 97. https://doi.org/10.3390/fermentation7020073.
- Chou O, Ali A, Subbiah V, Barrow CJ, Dunshea FR,Suleria HAR. LC-ESI-QTOFMS/MS characterization of phenolics in herbal tea infusion and their antioxidant potential. Fermentation. 2021, 7, 73 - 97. https://doi.org/10.3390/fermentation7020073.
- Conirol, T. D., Trial, C., Control, D. et Control, T. D. (1995). The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. Annals of internal medicine, 122(8), 561-8.
- Coughlan MT, Forbes JM, Cooper ME (2007) Role of the AGE crosslink breaker, alagebrium, as a renoprotective agent in Fazili NA, Naeem A (2013) In vitro hyperglycemic condition facilitated the aggregation of lysozyme via the passage through a molten globule state. Cell Biochem Biophys 66:265–275. https://doi.org/10.1007/s12013-012-9479- 2
- Crozier A., Jaganath I.B., Clifford M.N. Dietary phenolics: Chemistry, bioavailability and effects on health. Natural Product Reports 26, 1001-1043 (2009).
- Cunha, A.P.; Silva, A.P.; Roque, O. Plantas e Produtos Vegetais em Fitoterapia; Fundação Calouste Gulbenkian: Lisbon, Portugal, 2003; pp. 216–334. ISBN978-972-31-1435-5.
- Curr Opin Endocrinol Diabetes Obes April 2014 2014; 21: 109–20.

- Dankers W, Colin EM, van Hamburg JP, Lubberts E. (2016). Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. Frontiers in Immunology, 7, 697.
- De Boer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS. (2009). 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Kidney Diseases, 50(1), 69–77.
- DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. The American Journal of Clinical Nutrition, 80(6 Suppl), 1689–1696 . https://doi.org/10.1093/ajcn/80.6.1689s
- Di Carlo G., Mascolo N., lzzo A.A., et Capasso F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life. Sci. 65 (4): 337-53, (1999)
- Di Novella, R., Di Novella, N., De Martino, L., Mancini, E., De Feo, V., 2013. Traditional plant use in the national park of cilento and Vallo di Diano, Campania, southern, Italy. J. Ethnopharmacol. 145, 328–342. https://doi.org/10.1016/j.jep.2012.10.06 5.
- Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev 157 Immunol 2011; 11: 98–107.
- Douira, J. B., 2002; LES PLANTES MEDICINALES DANS LA FORET DE L'ACHACHE (PLATEAU CENTRAL, MAROC). Acta Botanica Malacitana , p. 136.
- Du, Y. et Wei, T. (2014). Inputs and outputs of insulin receptor. Protein & Cell, 5(3), 203-213.
- Duchadeau J.C vitaminothérapie chez les voilés, thèse de doctorat Toulouse 2001
- Dutta U, Cohenford MA, Guha M et al (2006) In vitro nonenzymatic glycation of DNA nucleobases: an evaluation of advanced glycation end products under alkaline pH. Anal Bioanal Chem 386:1633–1640. https://doi.org/10.1007/s00216-006-0753-2
- El Menyiy, N., Guaouguaou, F.-E., El Baaboua, A., El Omari, N., Taha, D., Salhi, N., … Bouyahya, A. Phytochemical properties,biological activities and medicinal use of *Centaurium erythraea* Rafn. Journal of Ethnopharmacology, 276,114171. doi:10.1016/j.jep.2021.114171 10.1016/j.jep.2021.114171, (2021).
- El-Hilaly, J., Hmammouchi, M., Lyoussi, B., 2003. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). J. Ethnopharmacol. 86, 149–158. https://doi.org/10.1016/S0378-8741(03)00012-6.

- Farooq M, A, l. Shaukat A. Ali H. Hafz T. Samaira M. Shumaila M. (2021). Biosynthesis and industrial applications of α amylase: a review. Archives of Microbiology Vol. 203. pp. 1281–1292. https://doi.org/10.1007/s00203-020-02128- y
- FERDJIOUI S,(2020). Activités biologiques de deux plantes médicinales Mentha rotundifolia L. et Lamium amplexicaule L.Doctorat en Sciences,Université Ferhat Abbas Sétif 1.
- Féry F, Paquot N. [Etiopathogenesis and pathophysiology of type 2 diabetes]. Rev Médicale Liège 2005; 60: 361–8.
- Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiol Rev 2013; 93: 137–88.
- Foster, E (2016). Histology of the stomach.
- Freedman BI, Wuerth JP, Cartwright K, Bain RP, Dippe S, Hershon K, Mooradian AD, Spinowitz BS (1999) Design and baseline characteristics for the aminoguanidine clinical trial in overt type 2 diabetic nephropathy (ACTION II). Control Clin Trials 20(5):493–510. https://doi.org/10.1016/s0197-2456(99)00024-0
- Gagnon C, Lu ZX, Magliano DJ, et al. (2011). Low serum 25-hydroxyvitamin D is associated with increased risk of the development of the metabolic syndrome. Diabetes Care, 35(5), 1133–1138.
- Garabédian M. La 1,25dihyroxyvitamine D et son récepteur. Rev Rhum 2000; 67 (suppl 2): 39– 41. [Google Scholar]
- Gaspar, N., Godinho, J., Vasconcelos, T., Caldas, D., Mendes, P., Barros, O., 2002. Ethnobotany in the center of Portugal (Santar´em), in: Rauter, A.P., Palma, F.B., Justino, J., Araújo, M.E., dos Santos, S.P. (Eds.), Natural Products in the New Millennium: Prospects and Industrial Application, Proceedings of the Phytochemical Society of Europe. Springer Netherlands, Dordrecht, pp. 271–284. https://doi.org/10.1007/978-94-015-9876-7_29
- Genuth, S., Eastman, R., Kahn, R., Klein, R., Lachin, J., Lebovitz, H., Nathan, D., et al. (2003). Implications of the United-kingdom prospective diabetes study. Diabetes care, 26 Suppl 1, S28-32.
- Gjoshe Stefkov, B. M.-K., 2014; Chemical characterization of Centaurium erythrea L. andits effects on carbohydrate and lipid metabolism in experimental diabetes. Journal ofEthnopharmacology ,74.
- Grimaldi A, (2009). Traité de diabétologie. 2e éd. Paris, France: Médecine Sciences

- Grünwald, J., T. Brendler, C. Jaenicke, and T. Fleming T (eds.). 2000. PDR for Herbal Medicines, 4th ed. Medical Economics Company, Montvale, NJ. pp. 174– 175.
- Hale, L. J. et Coward, R. J. M. (2013). The insulin receptor and the kidney. Current opinion in nephrology and hypertension, 22(1), 100-6.
- Haloui, M., L. Louedec, J. B. Michel, and B. Lyoussi. 2000. Experimental diuretic effects of Rosmarinus officinalis and Centaurium *Erythraea*. J. Ethnopharmacol. 71:465–472.
- Hamza, N., B. Berke, C. Cheze, R. Le Garrec, R. Lassalle, et al. 2011. Treatment of high fat diet induced type 2 diabetes in C57BL/6J mice by two medicinal plants used in traditional treatment of diabetes in the east of Algeria. J. Ethnopharmacol. 133:931–933.
- Hänsel, R., and O. Sticher (eds.). 2010. Pharmakognosie—Phytopharmazie, 9th ed. Springer Verlag, Heidelberg. 189 pp.
- Hedrick CC, Thorpe SR, Fu MX, Harper CM, Yoo J, Kim SM, Wong H, Peters AL (2000) Glycation impairs high-density lipoprotein function. Diabetologia 43(3):312–320. https://doi.org/10. 1007/s00125005004
- Holick MF. (2007). Vitamin D deficiency. New England Journal of Medicine, 357(3), 266–281.
- https://observatoireprevention.org/2018/03/19/resistance-a-linsuline-consequence-dangereuse-de-lexces-de-poids/
- International Diabètes Federation. (2006). The Diabetes Atlas. Third Edition
- J. F. Foster, (V. M. Rosenoer, M. Oratz, and M. A. Rothschild, Editors) “Albumin Structure, Function and Uses”, Oxford; New York: Pergamon Press, (1977) 53 - 84
- Jaramillo R, Shuck SC, Chan YS et al (2017) DNA advanced glycation end products (DNA-AGEs) are elevated in urine and tissue in an animal model of type 2 diabetes. Chem Res Toxicol 30:689–698. https://doi.org/10.1021/acs.chemrestox.6b00414
- J-C. Souberbielle, D. Prié, M. Courbebaisse, G. Friedlandera, P. Houillier, G. Maruani, et al. Actualité sur les effets de la vitamine D et l’évaluation du statut vitaminique D. Annales d’Endocrinologie. déc 2008; 69(6) :501-10.
- Jiang X, O'Reilly PF, Aschard H, et al. (2019). Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nature Communications, 9, 260.
- Johansen MB, Kiemer L, Brunak S (2006) Analysis and prediction of mammalian protein glycation. Glycobiology 16:844–853. https://doi.org/10.1093/glycob/cwl00 9

- Jones, Strugnell, & DeLuca, H. F. (1998). Current understanding of the molecular actions of vitamin D. Physiological Reviews, 78(4), 1193–1231. https://doi.org/10.1152/physrev.1998.78.4.1193
- Joseph JJ, Golden SH. Type 2 diabetes and cardiovascular disease: what next?].
- Jud P, Sourij H (2019) Therapeutic options to reduce advanced glycation end products in patients with diabetes mellitus: a review. Diabetes Res Clin Pract 148:54–63. https://doi.org/10.1016/j. diabres.2018.11.016
- Jujjavarapu S.E et Dhagat. (2018). Evolutionary Trends in Industrial Production of a-amylase. Recent Patents on Biotechnology 12, 1-15.
- K. Gekko, A. Kimoto and T. Kamiyama, "Effects of Disulfide Bonds on Compactness of Protein Molecules Revealed by Volume, Compressibility, and Expansibility Changes during Reduction", Biochemistry, 42, 46 (2003) 13746 - 13753 [97] Sigma, Product Information, CAS number 9048-46-8, (1997)
- Kabouche, A. (2016). Investigation phytochimique et biologique de l'espèce *Astragalus armatus* ssp. numidicus (Coss. et Dur.) Maire. et Synthèse catalytique de nouveaux dérivés julolidines 14, https://dx.doi.org/10.1016/j.procbio.2016.11. 0
- Kashani A.H, Hoseini E.S, Nikzad H, Aarabi M.H. Pharmacological properties of medicinal herbs by focus on secondary metabolites. Life Sci. 9 (1):509-520, (2012). [39]-Mohr, N., Budzi, K. H et El-Tawil, B. A. H. 1982. Phytochemistry, 7(9), 1838.
- Kodjoh, N (2014). Clinique Universitaire d'Hépato-gastroentérologie Centre National Hospitalier et Universitaire, Cotonou, Bénin: Ulcère gastrique et duodénal.
- Krul-Poel YHM, Ter Wee MM, Lips P, Simsek S. (2017). Management of endocrine disease: The effect of vitamin D supplementation on glycaemic control in patients with type 2 diabetes mellitus. European Journal of Endocrinology, 176(1), R1–R14.
- Kumarasamy, Y., Nahar, L., Sarker, S.D. 2003. Bioactivity of gentiopicroside from the arial parts of Centaurium Erythreae. Fitoterapia, tome 74(1-2) 151-4
- Kumarasamy, Y., Nahar, L., Sarker, S.D. 2003. Bioactivity of gentiopicroside from the arial parts of Centaurium Erythreae. Fitoterapia, tome 74(1-2) 151-4
- Kutchan T.M. et Zenk M.H. -, Enzymology and molecular biology of benzophenanthridine alkaloid biosynthesis. journal of Plant Research, 3, 165-173 (1993).
- Landrier, J. F. (2014). Vitamin D: Sources, metabolism and mechanisms of action. Cahiers de Nutrition et de Dietetique, 49(6), 245–251. https://doi.org/10.1016/j.cnd.2014.07.008

- Le Roith, D. and Zick, Y. Recent advances in our understanding of insulin action and insulin resistance.2001, Diabetes Care 24, 588-97.
- Lee, J. et Pilch, P. F. (1994). The insulin receptor: structure, function, and signaling. The American journal of physiology, 266(2 Pt 1), C319-34.
- Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. (2002). 1,25-Dihydroxyvitamin D (3) is a negative endocrine regulator of the renin-angiotensin system. Journal of Clinical Investigation, 110(2), 229–238.
- Lotfy M, Adeghate J, Kalasz H, Singh J, Adeghate E. (2017). Chronic complications of diabetes mellitus: A mini review. Current Diabetes Reviews, 13(1), 3–10.
- Maestro B, Campión J, Dávila N, Calle C. (2002). Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression. Endocrine Journal, 49(1), 77–83.
- Maestro B, Dávila N, Carranza MC, Calle C. (2000). Identification of a vitamin D response element in the human insulin receptor gene promoter. Journal of Steroid Biochemistry and Molecular Biology, 72(5), 223–230.
- Mandal S.M., Chakraborty D. and Dey S. Phenolic acids act as signaling molecules in plant- microbe symbioses. Plant Signaling and Behavior, 5(4); 359-368, (2010).
- Martins SIFS, van Boekel MAJS (2005) Kinetics of the glucose/glycine Maillard reaction pathways: influences of pH and reactant initial concentrations. Food Chem 92:437–448. https://doi.org/10.1016/j.foodchem.2004.08.01 3
- Matsuo, K., Delibegovic, M., Matsuo, I., Nagata, N., Liu, S., Bettaieb, A., Xi, Y., et al. (2010). Altered glucose homeostasis in mice with liver-specific deletion of Src homology phosphatase. Journal of Biological Chemistry, 285(51), 39750-39758.
- mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol
- Mecier C. (1985). Les enzymes amylolitiques. In Mauranche A et Costes C. (Ed): Hydrolases et polymérases. Ed. Gauthier Villars. Pp, 109-142.
- Menkovi´c, N., ˇSavikin, K., Tasi´c, S., Zduni´c, G., Steˇsevi´c, D., Milosavljevi´c, S., Vincek, D., 2011. Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije Mountains (Montenegro). J. Ethnopharmacol. 133, 97–107. https://doi.org/10.1016/j.jep.2010.09.008.
- Miller WL, Portale AA. Vitamin D 1 alpha hydroxylase. Trends Endocrinol Metab 2000; 11: 315–9. [Google Scholar]
- Mirhosseini N, Vatanparast H, Kimball SM. (2018). The effects of vitamin D supplementation on glycemic control in type 2 diabetes patients: a systematic review and meta-analysis. Journal of Clinical Endocrinology & Metabolism, 103(4), 1–12.

- Monnier L, Thuan J-F. [Type 1 diabetes of the child and the adult. Type 2 diabetes of the adult. Complications of diabetes]. Rev Prat 2007; 57: 653–64
- Monnier L, Thuan J-F. [Type 1 diabetes of the child and the adult. Type 2 diabetes of the adult. Complications of diabetes]. Rev Prat 2007; 57: 653–64.
- Monnier L. Diabétologie. Issy-les-M oulineaux: Elsevier M asson, 2010
- Muoio DM, Newgard CB. Mechanisms of disease: molecular and metabolic
- Nenna A, Nappi F et al (2015) Pharmacologic approaches against advanced glycation end products (AGEs) in diabetic cardiovascular disease. Res Cardiovasc Med 4(2): e26949. https://doi.org/10.5812/cardiovascmed.4(2)2015.2694 9
- Nowotny K, Jung T, Höhn A, Weber D, Grune T (2015) Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules 5(1):194–222. https://doi.org/10.3390/biom501019 4
- Nykjaer A, Dragun D, Walther D, et al. endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3 Cell 1999; 96: 507–15. [Google Scholar]
- O. J. Bos, J. F. Labro, M. J. Fischer, J. Wilting and L. H. Janssen, “The Molecular Mechanism of the Neutral-to-Base Transition of Human Serum Albumin. Acid/base Titration and Proton Nuclear Magnetic Resonance Studies on a Large Peptic and a Large Tryptic Fragment of Albumin”, Journal of Biological Chemistry, 264, 2 (1989) 953 – 959
- Odile Kusnik. J., Alain W. (2008). Philippe R., Hubert A. Diabète traité en France en 2007: Un taux de prévalence proche de 4% et des diparités geographiques croissantes BEA. Bulletin epidemiologique hebdomadaire ; N° 43.
- Olempska-Beer Z. (2004). Alpha-amylase from bacillus licheniformis Containing a genetically engineered alpha-Amylase gene from b. licheniformis (thermostable); chemical and technical assessment (cta)
- Oliveira L.D.L.D., Carvalho M.V.D., Melo L. Health promoting and sensory properties of phenolic compounds in food. Revista Ceres 61,764-779 (2014).
- Oroian M., Escriche I. Antioxidants: Characterization, natural sources, extraction and analysis. Food Research International 74, 10-36, (2015).

- Oroian M., Escriche I. Antioxidants: Characterization, natural sources, extraction and analysis. Food Research International 74, 10-36, (2015).

- Ouledelhachemi, S (2012). Ulcère gastroduodénal : prise en charge thérapeutique et accompagnement à l'officine. Thèse. Faculté: Pharmacie, Rabat: Université Mohammed V .16.
- Owen, C., Lees, E. K., Grant, L., Zimmer, D. J., Mody, N., Bence, K. K. et Delibegović, M. (2013). Inducible liver-specific knockdown of protein tyrosine phosphatase 1B improves glucose and lipid homeostasis in adult mice. Diabetologia, 56(10), 2286-2296.
- Oya T, Hattori N, Mizuno Y et al (2000) Methylglyoxal modification of protein: chemical and immunochemical characterization of methylglyoxal-arginine adducts. J Biol Chem 274:18492–18502. https://doi.org/10.1074/jbc.274.26.18492
- Panchal C.J (1990). Yeats strain selection. Marcel Dekker (ed) USA, p: 189.
- Penna G, Adorini L. (2000). 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells. Journal of Immunology, 164(5), 2405–2411.
- Peyroux J, Sternberg M (2006) Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathol Biol (paris) 54(7):405–419. https://doi.org/10.1016/j.patbio.2006. 07.006
- Pinget M, Boullu-Sanchis S. [Physiological basis of insulin secretion abnormalities]. Diabetes Metab 2002; 28: 4S21–32.
- Pittas AG, Lau J, Hu FB, Dawson-Hughes B. (2007). The role of vitamin D and calcium in type 2 diabetes. Journal of Clinical Endocrinology & Metabolism, 92(6), 2017–2029.
- Post SM, Duez H, Gervois PP, et al. Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-alpha-mediated downregulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase expression. ArteriosclerThrombVascBiol 2001; 21: 1840–5. [Google Scholar]
- Princen, F., Bard, E., Sheikh, F., Zhang, S. S., Wang, J., Zago, W. M., Wu, D., et al. (2009). Deletion of Shp2 tyrosine phosphatase in muscle leads to dilated cardiomyopathy, insulin resistance, and premature death. Molecular and cellular biology, 29(2), 378-88.
- Princen, F., Bard, E., Sheikh, F., Zhang, S. S., Wang, J., Zago, W. M., Wu, D., et al. (2009). Deletion of Shp2 tyrosine phosphatase in muscle leads to dilated cardiomyopathy, insulin resistance, and premature death. Molecular and cellular biology, 29(2), 378-88.
- Prosser DE, Jones G. Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci 2004; 29: 664–73. [Google Scholar]

- Psotovà J., Lasovsky J. and Vicar J. Metal-chelating properties, electrochemical behavior, scavenging and cytoprotective activities of six natural phenolics. Biomedical Papers, 147(2); 147-153, (2003).
- R. Feng, Y. Konishi and A. W. Bell, "High Accuracy Molecular Weight Determination and Variation Characterization of Proteins up to 80 ku by Ionspray Mass Spectrometry" Journal of the American Society for Mass Spectrometry, 2, 5 (1991) 387-401
- R. Jaenicke and M. A. Lauffer, Determination of Hydration and Partial Specific Volume of Proteins with the Spring Balance", Biochemistry, 8, 7 (1969) 3077 - 3082
- Rad, A (2020). Article: Microscopic anatomy. [66]
- Rafiq S, Jeppesen PB. (2021). Body mass index, vitamin D, and type 2 diabetes: A systematic review. Nutrients, 13(4), 1182.
- Rahbar S, Figarola JL (2002) Inhibitors and breakers of advanced glycation endproducts (AGEs): a review. Curr Med Chem Endocr Metab Agents 2:135–161. https://doi.org/10.2174/1568013023 358889
- Rossant-Lumbroso, J (2020). Revue médicale: Les causes de l'ulcère duodénal.
- Rossy, N, Epidémie de carence en vitamine D, supplémentassions en vitamine D3 et taux sanguin optimal de 25 (OH) D 2010
- Roupioz,A(2019). Article scientifique : Viscère
- Roy A, Sil R, Chakraborti AS (2010) Non-enzymatic glycation induces structural modifications of myoglobin. Mol Cell Biochem 338:105–114. https://doi.org/10.1007/s11010-009-0343-7
- Ruiz,A.R Jr (2019). Le Manuel MSD. Article: l'estomac.
- Ryan ZC, Craig TA, Folmes CD, et al. (2016). 1α,25-Dihydroxyvitamin D3 regulates mitochondrial oxygen consumption and dynamics in human skeletal muscle cells. Journal of Biological Chemistry, 291(3), 1514–1528.
- Sadasivam S, Thayumanavan B. Molecular host plant resistance to pests. Books in soils, plants and the environment. CRC Press, 221 p, (2003).
- Saltiel AR, Olefsky JM. Insulin resistance and the pathogenesis of non-insulin-dependent diabetes mellitus. Diabetes. 1990
- Saric T., Rogosic J., Zupan I., Beck R., Bosnic S., Sikic Z., et al. Anthelmintic effect of three tannin-rich Mediterranean shrubs in naturally infected sheep. Small Ruminant Research 123, 179-182, (2015)
- Schomburg D and Salzmann M (1991). Enzyme Hand book 4. Classe 3: Hydrolases Springer-Verlag (ed). Berlin Heidelberg. Germany. p: 1-12

- Schuessler M, Astecker N, Herzig G, et al. Skin is an autonomous organ in synthesis, two-step activation and degradation of vitamin D3: CYP27 in epidermis completes the set of essential vitamin D3-hydroxylases. Steroids 2001; 66: 399–408. [Google Scholar]
- Shahat A.A., Marzouk M.S. Chapter 13; Tannins and related compounds from medicinal plants of Africa. Medicinal Plant Research in Africa. Elsevier, (2013)
- Sharma GS, Warepam M, Bhattacharya R, Singh LR (2019) Covalent modification by glyoxals converts cytochrome c into its apoptotically competent state. Sci Rep 9(1):4781. https://doi.org/10.1038/s41598-019-41282- 2
- Shehab D, Al-Jarallah K, Mojiminiyi OA, Abdella NA. (2012). Does vitamin D deficiency play a role in peripheral neuropathy in type 2 diabetes? Diabetic Medicine, 29(1), 43–49.
- Shipanova IN, Glomb MA, Nagaraj RH (1997) Protein modification by methylglyoxal: chemical nature and synthetic mechanism of a major fluorescent adduct. Arch Biochem Biophys 344:29–36. https://doi.org/10.1006/abbi.1997.0195
- Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006; 116: 1793–801.
- Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest .801–1793 :116 ;2006
- Sorbonne (2018). Expliquer schématiquement la conception physiopathologique, et lesfacteurs favorisant l'apparition d'un ulcère gastrique ou duodénal.
- Souberbielle JC, Prié D, Courbebaisse M, et al. Actualité sur les effets de la vitamine D 2009
- Souccar, T vitamine D, le nouveau scandale sanitaire 2007
- Souidi M, Dubrac S, Parquet M, Lutton C. Hepatic and extrahepatic sterol 27-hydroxylase: roles in cholesterol and bile acid metabolism and associated diseases. Gastroenterol Clin Biol 2003; 27: 100–
- Soulsby, M. et Bennett, A. M. (2009). Physiological signaling specificity by protein tyrosine phosphatases. Physiology (Bethesda, Md.), 24(5), 281-9.
- Stalikas, C.D., Extraction, separation, and detection methods for phenolic acids and flavonoïds. Journal of Separation Science 30, 3268-3295 (2007).
- Stoiko Liliya, D. I., 2017 (juin); POLYSACCHARIDES IN *CENTAURIUM ERYTHRAEA* RAFN. International Journal of Research in Ayurveda and Pharmacy.

- Subash C, B, G. Anbu, P. Arshad M, K, M.d, Lakshmipriya T. Voon, C, H ,Hashim U. Chinni S, V. (2017) Biotechnological Processes in Microbial Amylase Production. BioMed Research International, p 9,https://doi.org/10.1155/2017/1272193
- Süntar I. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochemistry reviews. 2020:19(5):1199-1209.
- Tahraoui A, Z. H., 2010; Acute and sub-chronic toxicity of a lyophilised aqueous. Journal of Ethnopharmacology, 48.
- Tapas AR, Sakarkar DM, Kakde RB..Flavonoids as Nutraceuticals: A Review. Trop. J. Pharma. Res 7 (3):1089-1099, (2008).
- Tejada, T., Catanuto, P., Ijaz, a, Santos, J. V, Xia, X., Sanchez, P., Sanabria, N., et al. (2008). Failure to phosphorylate AKT in podocytes from mice with early diabetic nephropathy promotes cell death. Kidney international, 73(12), 1385-93.
- Thornalley PJ (2005) Dicarbonyl intermediates in the Maillard reaction. Ann NY Acad Sci 1043:111–117. https://doi.org/10.1196/annals.1333.01 4
- Ticli, B. L'herbier de santé. 1°édition, Paris, édition VECCHI SAO, 01.206 p (1997)
- Trifunović-Momčilov, M., Zdravković-Korać, S., Ćirić, A., Soković, M., Gođevac, D., & Ninković, S. (2017). Secondary metabolite profile of transgenic centaury (*Centaurium erythraea* Rafn.) plants, potential producers of anticancer compounds. In S. Jha (Ed.), Transgenesis and secondary metabolism (pp. 227–246). Springer. https://doi.org/10.1007/978-981-10-3473-5_ 11
- Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients, 2; 1231-1246, (2010).
- Welsh, G. I., Hale, L. J., Eremina, V., Jeansson, M., Maezawa, Y., Lennon, R., Pons, D. A., et al. (2010). Insulin signaling to the glomerular podocyte is critical for normal kidney function. Cell Metabolism, 12(4), 329-340.
- Whitcomb D.C., Lowe M. E. (2007). Human pancreatic digestive enzymes. Dig Dit Sci 52, 1-17
- Wolffenbuttel BH, Boulanger CM, Crijns FR et al (1998) Breakers of advanced glycation end products restore large artery properties in experimental diabetes. Proc Natl Acad Sci U S A 95(8):4630– 4634. https://doi.org/10.1073/pnas.95.8.4630
- World Health Organization. WHO monographs on selected medicinal plants. Geneva: WHO; 2010
- Yamagishi S, Nakamura K, Matsui T, Ueda S, Noda Y, Imaizumi T (2008) Inhibitors of advanced glycation end products (AGEs): potential utility for the treatment of

cardiovascular disease. Cardiovasc Ther 26(1):50–58. https://doi.org/10.1111/j.1527-3466. 2007.00038.x

- Yaylayan VA, Huyghues-Despointes A (1994) Chemistry of Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. Crit Rev Food Sci Nutr 34:321– 369. https://doi.org/10.1080/10408399409527667
- Zakharova I, et al. (2019). Vitamin D insufficiency in overweight and obese children and adolescents. Pediatric Endocrinology Reviews, 17(1), 61–69.
- Zella JB, McCary LC, DeLuca HF. (2003). Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. Archives of Biochemistry and Biophysics, 417(1), 77–80.
- Zhang Q, Xiao H. (2016). Microbial-amylase: A biomolecular overview School of Chemical Engineering and Technology, Tianjin University.Tianjin. China.
- Zhang X, et al. (2015). Vitamin D inhibits pro-inflammatory cytokines and promotes regulatory T cells. Immunology Letters, 164(2), 128–135.
- Zhao J, et al. (2014). Vitamin D improves diabetic nephropathy by reducing proteinuria. Experimental and Therapeutic Medicine, 8(3), 805–809.
- Zheng Y, Ley SH, Hu FB. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews Endocrinology, 14, 88–98.
- Zubtsova I.V., Sklyar V.G. Morphological fea-tures of plants and size structure of centaurium erythrea coenopopulations on floodplain meadows of the Krolevets-Glukhiv geobotanical district.

الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي
جامعة الشهيد حمه لخضر بالوادي

مركز تطوير المقاولاتية لجامعة الوادي

الكلية/ المعهد: الكلية علوم الطبيعة والحياة
القسم. بيولوجيا
المستوى والتخصص: 2 ماستر بيوكيمياء

مذكرة التخرج لنيل شهادة جامعية – مشروع مؤسسة اقتصادية

عنوان المشروع:

تطوير مكمل غذائي طبيعي يحتوي على فيتامين د ومستخلص نبات القنطريون الصغير لدعم التحكم في مقاومة الإنسولين وتثبيط إنزيم الفا اميلاز

مقدمة في اطار الحصول على شهادة مؤسسة اقتصادية (مصغرة) في اطار القرار الوزاري

1275 المعدل والمتمم بالقرار الوزاري 008 المؤرخ في 23 فيفري 2025

Biobalance

السنة الجامعية: 2025 / 2026

الفهرس

أولا: فريق العمل والإشراف

1-فريق العمل

فريق المشروع	التخصص	الكلية/ المعهد	الخبرة/ الكفاءة في مجال المشروع
الطالب: هارون نور الاسلام	بيوكيمياء تطبيقية	الكلية علوم الطبيعة والحياة	/
الطالب: ولابي سندس	بيوكيمياء تطبيقية	الكلية علوم الطبيعة والحياة	/
الطالب:			
الطالب:			
الطالب:			
الطالب:			

2-فريق الإشراف

المشرف	التخصص	الكلية
المشرف الرئيسي :عدائكة عائشة	كيمياء صيدلانية	الكلية علوم الطبيعة والحياة
المشرف المساعد:مروة جار الله	كيمياء	الكلية علوم الطبيعة والحياة

ثانيا: تعريف المشروع: يكون النشاط وفقا للمدونات التي تحدد قائمة الأنشطة لكل هيئة تسجيل (المركز الوطني للسجل التجاري، الفلاحة، الصناعة التقليدية والحرف)؛

تحديد النشاط	قطاع النشاط	رمز النشاط(وفق مدونة الأنشطة للمركز الوطني للسجل التجاري cnrc)
إنتاج وتطوير مكمل غذائي طبيعي مستخلص من مصدر نباتي ومدعم بقيتامين د للمساهمة في تحسين مقاومة	الصناعات الغذائية الصحية والمكملات الغذائية	يحدد حسب مدونة CNRC الخاصة بالمكملات الغذائية والصناعات الغذائية الصحية.

الانسولين والحد من الاظربات الايضية المرتبطة بداء السكري		

❖ في حالة نشاط مقنن، أو نشاط يحتاج إلى اعتمادات او تراخيص خاصة، يجب ذكرها وذكر الجهة المانحة لها :

في حالة هذا النشاط، وباعتباره يتعلق بإنتاج وتسويق مكمل غذائي طبيعي، فإنه يخضع لإجراءات تنظيمية تتعلق بالنشاطات الغذائية والصحية، وتشمل ورقابية

-التسجيل التجاري لدى المركز الوطني للسجل التجاري

احترام شروط النظافة والسلامة ومطابقة المنتجات الغذائية تحت رقابة وزارة التجارة الداخلية وضبط السوق

-الوطنية

-إجراء التحاليل ومراقبة الجودة عبر مخابر معتمدة

الحصول على التراخيص أو الاعتمادات الصحية المطلوبة في حالة التصنيع أو التسويق الواسع للمكملات -

-الغذائية، بالتنسيق مع وزارة الصحة والهيئات المختصة حسب التنظيم المعمول به

-الالتزام بالقوانين الخاصة بوسم المكملات الغذائية وعدم تضمين ادعاءات علاجية مباشر

ثالثا: وصف فكرة المشروع:

ينغي ان يكون صاحب / أصحاب المشروع على دراية بكل تفاصيل المشروع وقادرين على تقديم وصف ملخص ودقيق عنه ، من خلال الإجابة بدقة على العناصر التالية:

ما هي منتجاتي أو خدماتي: (حدد بدقة منتجاتك. خدماتك):

يتمثل المشروع في تطوير مكمل غذائي مبتكر يحتوي على فيتامين د ومستخلص نبات القنطريون الصغير الغني بالمركبات الفلافونويدية و الفينولية، بهدف

-المساهمة في تقليل ارتفاع سكر الدم بعد الوجبات

-دعم تحسين حساسية الإنسولين

-المساهمة في الحد من مقاومة الإنسولين

-دعم مرضى السكري من النوع الثاني بطريقة طبيعية

من سيشتري منتجاتي/ خدماتي (حدد زبائنك بدقة) : الفئات المستهدفة:

-مرضى السكري من النوع الثاني الذين يعانون من اضطربات في تنظيم سكر الدم ومقاومة الانسولين

-الأشخاص الذين يعانون من مقاومة الإنسولين

-الأشخاص المعرضون للإصابة بالسكري

-الاشخاص الذين يعانون من السمنة اوزيادة الوزن المرتبطة باضطرابات ايض الغلكوز

-الصيدليات ومحلات المنتجات الصحية.

أين سيقام مشروعي وكيف تصل منتجاتي / خدماتي للزبائن :(بين مكان إقامة مشروعك وطرق التواصل مع زبائنك)

يقام المشروع داخل ورشة او مخبر مصغر متخصص في تحضير وتغليف المكملات الغذائية يكون في وسط المدينة الوادي قريب من الصيدلايات والشبه الصيدليات، مع الاعتماد على

-التسويق الإلكتروني

-البيع عبر شبه الصيدليات

التوزيع على محلات المنتجات الطبيعية

المشاركة في المعارض الصحية.

كيف سيشتغل مشروعي (بين من سيقوم بتشغيل المشروع ومن سيساهم في ذلك**)**: سيقوم بتشغيل المشروع انا وزميلتي وايضا سنقوم با الإشراف الكامل على جميع مراحل الدراسة، بما في ذلك جمع المادة النباتية، وتحضير العينات، وتنفيذ مراحل الاستخلاص والتجارب المخبرية، ثم تحليل النتائج وتوثيقها بشكل منهجي. ويتكفل الأستاذ المؤطر بالإشراف العلمي من خلال توجيه العمل البحثي، والمصادقة على المنهجية المعتمدة، ومراجعة مختلف مراحل الإنجاز لضمان دقتها العلمية. كما يوفر المخبر الجامعي الوسائل والتجهيزات الضرورية لإجراء التحاليل والاختبارات البيوكيميائية، مع إمكانية مساهمة التقنيين أو طلبة البحث في دعم الجانب التطبيقي وتشغيل الأجهزة المخبرية عند الحاجة.

مبررات اختيارفكرة مشروعي (اشرح لماذا تم اختيار هذه الفكرة بالذات):

:تم اختيار هذه الفكرة بسبب

-الانتشار المتزايد لداء السكري ومقاومة الإنسولين

-الحاجة الى بدائل علاجية طبيعية

-الاهتمام العلمي بالمركبات النباتية النشطة حيويا ودورها في تحسين الحساسية للانسولين

إمكانية التطبيق محليا بتكلفة منخفضة وفعالية محتملة

-أهمية فيتامين D في دعم حساسية الإنسولين وتنظيم الاستقلاب.

رابعا: دراسة السوق

1. **طبيعة وشدة المنافسة:** من هم المنافسون المباشرون وغير المباشرون؟

المنافسون	طبيعة المنافسة		شدة المنافسة		
	مباشرة	غير مباشرة	قوية	متوسطة	ضعيفة

BIOMAX Algérie شركة جزائرية متخصصة في المكملات الغذائية لديها منتجات مؤتبطة بحساسية الانسولين	نعم		نعم		
Thera Sante مختبر جزائري يعمل في المكملات الغذائية		نعم		نعم	
Nutraxin Algérie شركة تعمل في المكملات الغذائية والعناية بالصحة العامة		نعم			نعم

أذكر استراتيجيات مؤسستك في التعامل مع منافسيها:

-تعتمد المؤسسة على تقديم مكمل غذائي طبيعي مبتكر

-الاستناد على نتائج علمية ومخبرية

- اعتماد اسعار مناسبة مقارنة ببعض المنتجات المتشابهة

-التركيز على الجودة والسلامة في التصنيع

-التسويق الرقمي والتوعية الصحية للتعريف بالمنتج

2. **حجم السوق المستهدف والمحتمل:**

- **حجم السوق المستهدف:** يمثل مجموعة الافراد أو المؤسسات والتي تقدم لها او تعرض عليها – منتجاتك/خدامتك. (يطلب تحديد الشريحة المستهدفة، تعدادها، مكان تواجدها، .....).
- الشريحة المستهدفة: الرجال والنساء (25-55 سنة) بالولاية، المصابون بمقاومة الإنسولين، تكيس المبايض، ومرحلة ما قبل السكري.
- تعداد السوق: الشريحة المحتملة بالولاية تُقدر بحوالي 60,000 إلى 80,000 شخص (حسب نسب اضطرابات الأيض في الجزائر). الهدف الأول استقطاب 1% منهم (حوالي 800 زبون).
- مكان التواجد: السوق الجزائري
- نقاط الوصول:
- واقعياً: عيادات أطباء السكري والتغذية بالوادي، والصيدليات الكبرى.
- رقمياً: مجموعات الفيس بوك المحلية النشطة في الولاية.
- القدرة الشرائية: الطبقة المتوسطة والميسورة المهتمة بالصحة البديلة والمكملات..

- **حجم السوق المحتمل**: السوق المحتمل هو مجموعة الافراد او المؤسسات التي تطلب أو يحتمل ان تطلب منتجاتك او خدماتك لإشباع حاجا تهم ورغباتهم. (من يشتري منتجاتنا/ خدماتنا؟ من وما الذي يحفزه على ذلك؟ أين يتواجدون؟ كم أعدادهم؟).
- من يشتري؟ مصابو مقاومة الإنسولين، تكيس المبايض، والوزن الزائد (عمر 25-55 سنة).
- ما المحفز؟ التخلص من السمنة، تنظيم الهرمونات، وتجنب الإصابة بالسكري والأدوية المزمنة.
- أين يتواجدون؟ بلدية الوادي والبلديات الكبرى (عيادات التغذية، الصيدليات، ومجموعات فيسبوك المحلية).
- كم أعدادهم؟ حوالي 60,000 إلى 80,000 شخص محتمل في الولاية**تحليل SWOT:** نقاط القوة والضعف والفرص والتهديدات لمشروعي:

- **نقاط القوة:** كل العناصر (داخل المؤسسة) التي تشكل مصدر قوة لمشروعي (قد تكون: مهارات خاصة، موارد بشرية، موارد مادية، .....)
- **نقاط الضعف**: كل العناصر (داخل المؤسسة) التي تشكل نقطة ضعف أو تقف عائقا امام مشروعي حاليا أو مستقبليا
- **الفرص**: كل العناصر الخارجية التي لها أثر إيجابي على تنفيذ مشروعي أو تطوره في المستقبل
- **التهديدات**: كل العناصر الخارجية التي لها أثر سلبي على تنفيذ مشروعي أو تطوره في المستقبل

جدول تحليل SWOT

SWOT	عوامل تساعد في تحقيق الأهداف	عوامل تعطل تحقيق الأهداف
عوامل داخلية	نقاط القوة Strengths 1 مكمل غذائي طبيعي مبتكر 2 تخصص دقيق في حل مشكلة (مقاومة الإنسولين) 3 احتواء المنتج على فيتامين د ومستخلص نبات القنطريون الصغير	نقاط الضعف Weaknesses 1 علامة تجارية جديدة تحتاج لبناء الثقة. 2 ميزانية تسويقية محدودة في البداية 3 الاعتماد على الموردين لشحن المنتج لولاية الوادي.

	الفرص Opportunities	التهديدات Threats
عوامل خارجية	1زيادة وعي سكان الوادي بالحميات والصحة 2 إمكانية الشراكة مع عيادات التغذية وأطباء الولاية. 3 توجه المستهلكين نحو البدائل الطبيعية والمحلية.	1 منافسة المكملات المستوردة بالصيدليات وبدائل العشابين. 2 استعجال الزبائن للنتائج(مقارنةبالأدوية). 3 صعوبة اكتساب ثقة المستهلك

3. نموذج العمل التجاري BMC لمشروعي:

العملاء	العلاقة مع العملاء	القيمة المقترحة	الأنشطة الرئيسية	الشركاء
الاشخاص الذين يعانون من مقاومة الانسولين والمهتمون بالمكملات الغذائية	تقديم استشارات ومتابعة الزبائن عبر الهاتف -مستمرة مع والفيسبوك بناء الثقة عبر تقديم محتوى طبي-توعوي تقديم عروض وتخفيضات الدائمين-للمشتركين	مكمل طبيعي، آمن، لعلاج مقاومة -ومخصص المبايض الإنسولين وتكيس بديل فعال للأدوية وبدون أعراض -الكيميائية جانبية سعر تنافسي يناسب .المحلي مقارنة بالمستورد -السوق	تأمين وتعبئة المكملات الط .بيعية بجودة عالية التسويق الرقمي وتوعية ا لزبائن بمقاومة الإنسولين إدارة المبيعات والتوصيل لبلديات الولاية	الرئيسيون طباء السكري بالواد-والتغذية الصيدليات -الكبرى بالولاية
	قنوات توزيع رقمياً: البيع المباشر عبر صفحات الفيسبوك وإنستغرام والواتساب واقعياً: الصيدليات الكبرى، عيادات أطباء السكري والتغذية بالوادي		الموارد الرئيسية المواد الاولية التجهيزات المجبرية الموارد البشرية المتخصصة	

	التوصيل: شركات الشحن السريع لتوصيل الطلبات لبلديات .الولاية			**شركات التوصيل السريع**

الإيرادات	**التكاليف**
مبيعات المكملات مباشرة للزبائن البيع بالجملة للصيدليات ومحلات الأعشاب	شراء السلع ومواد التغليف إعلانات فيسبوك الممولة مصاريف الشحن والتوصيل

خامسا: الدراسة التقنية

1. **احتياجات المشروع من الموارد البشرية:** حدد بدقة احتياجات مشروعك من الموارد البشرية

طبيعة المستخدمين	العدد	الكفاءات / الشهادات
المسير	1	تسيير وإدارة
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق، رؤساء الورشات...إلخ.)	1	بيوكيمياء
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	1	تحضير وتحليل
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	1	تسويق رقمي

2. **اختيارموقع إقامة المشروع : أين سيكون موقع المشروع ومبررات اختياره ؟** (قدم المبررات اللازمة لاختيار موقع المشروع)

موقع إقامة المشروع:

- **الموقع المقترح** :السوق الجزائري

مبررات اختيار الموقع:

- **القرب من شبكة التوزيع والأطباء** :التواجد في عاصمة الولاية يسهل الوصول السريع لعيادات أطباء السكري، التغذية، والصيدليات الكبرى التي تمثل قنوات التوزيع الرئيسية للمنتج.
- **سهولة الدعم اللوجستي والشحن** :عاصمة الولاية هي مركز انطلاق شركات التوصيل السريع **(** مثل **ZR express**،**Yalidine**، وغيرها مما يضمن شحن الطلبيات لبلديات الوادي (مثل قمار، الرقيبة، والمغير) والولايات الأخرى بسرعة وبتكلفة أقل.
- **توفر اليد العاملة والمواد** :سهولة استقطاب الموارد البشرية المؤهلة (خريجي جامعة حمه لخضر بالوادي في تخصصات البيولوجيا والكيمياء) للعمل في المخبر.
- **انخفاض تكاليف المقرات** :اختيار موقع في ضواحي المدينة أو المناطق النشطة المجاورة يضمن توفير مساحة كافية للتخزين والإنتاج وبأسعار إيجار معقولة مقارنة بالمحلات التجارية وسط المدينة.

سادسا: تقدير تكاليف و إيرادات المشروع

1 - تقدير تكاليف المشروع

1.1 تقدير المبلغ الإجمالي للاستثمار: (تكلفة المشروع)

البيان	المبلغ (دج)
آلات ومعدات الإنتاج	1,800,000 دج
تهيئة المحل	650,000 دج
الأصول البيولوجية (الحيوانات- الأشجار...)	لا ينطبق على)دج 0
العتاد المتنقل	0 دج
العتاد المكتبي	150,000 دج
عتاد الإعلام الآلي	300,000 دج
عتاد السمعي البصري	60,000 دج
عتاد الاتصالات	50,000 دج
تركيب التجهيزات	120,000 دج
المصاريف العامة المتعلقة بالإنشاء	90,000 دج
رأس المال العامل (المادة الأولية)	500,000 دج
المبلغ الإجمالي للاستثمار(تكلفة المشروع)	3,720,000 دج

2.1 تقدير مشتريات المشروع من المواد الأولية

قائمة المواد الاولية		السنة 1	السنة 2	السنة 3
المادة الاولية 1: مستخلص نبات القنطريون الصغير	الكمية المشترات	60kg	80kg	100kg
	سعر الوحدة	20,000DA	20,000DA	20,000DA
	تكلفة شراء المادة الاولية 1	120.000دج	160,000دج	200.000دج
المادة الاولية 2: فيتامين د والمواد الرابطة والكبسولات	الكمية المشترات	10kg	12kg	15kg
	سعر الوحدة	5,000DA	5.000DA	5.000DA
	مبلغ المادة الاولية 2	50,000دج	50.000دج	50000دج
المادة الاولية 3: العبوات البلاستيكية المعتمة والملصقات	الكمية المشترات	1.000u	1.300u	1,600u
	سعر الوحدة	80DA	80DA	80DA

	تكلفة شراء المادة الاولية	80DA	104.000DA	136.000DA
إجمالي مبالغ المشتريات من المواد الاولية		250,000دج	324,000دج	418.500دج

3.1 – تقدير تكاليف اليد العاملة

تسمية المنصب	عدد العمال			قيمة الاجر		
	السنة 1	السنة 2	السنة 3	السنة 1	السنة 2	السنة 3
المسير / أو المسيرون	2	2	2	840000 دج	960000 دج	1020000 دج
الإطارات (مهندسين، رؤساء المصالح، المكلفين بالتسويق، رؤساء الورشات...إلخ.)	2	3	4	720000 دج	1080000 دج	1440000 دج
عمال التحكم (المكلفين بالمخزن، السكريتاريا...إلخ.)	2	3	5	600.000 دج	1080000 دج	1800000 دج
عمال التنفيذ (السائق، الحارس، عامل النظافة...إلخ.)	3	4	4	960000دج	1320000 دج	1320000دج

- ينبغي ان لا يقل الاجر الشهري الصافي عن الحد الأدنى المضمون

4.1 تقدير التكاليف الخارجية للمشروع

البيان	السنة 01	السنة 02	السنة 03
مصاريف الكهرباء /الغاز /الماء	600000 دج	840000 دج	960000 دج
تكاليف الصيانة والتصليح	360000 دج	420000 دج	480000 دج
تكاليف الايجار	360000 دج	360000 دج	420000 دج
مصاريف تامين العتاد والمعدات	30000 دج	40000 دج	40000 دج
مصاريف التسويق (الدعاية والإشهار)	240000 دج	300000 دج	300000 دج
تكاليف التكوين والتدريب	120000 دج	240000 دج	360000 دج
مصاريف عامة (أعباء خارجية) أخرى	30,000 دج	45,000 دج	60,000 دج
المجموع	1.740.000دج	2.245.000 دج	2.620.000 دج

1.5 تقدير تكاليف اهتلاك الاستثمارات

البيان	مدة الاهتلاك (بالسنوات)	قسط الاهتلاك السنوي
آلات ومعدات الإنتاج	5سنوات	90,000 دج
العتاد المتنقل	0	0 (غير موجود) دج
العتاد المكتبي	5سنوات	200.000 دج
عتاد الإعلام الآلي	3سنوات	200.000 دج
عتاد السمعي البصري	0	0 (غير موجود) دج
عتاد الاتصالات	3سنوات	20.000 دج
.....		
المبلغ الإجمالي		1.010,000. دج

- يطبق الاهتلاك الخطي (تحدد مدة الاهتلاك وفقا لقرار وزارة المالية المؤرخ في 14 أفريل 2025 المعدل والمتمم للقرار الصادر بتاريخ 25 فيفري 2024 الذي يحدد مدة اهتلاك التثبيتات المطبقة لتحديد النتيجة الجبائية – الجريدة الرسمية عدد 29 لسنة 2025)

3-تقدير إيرادات المشروع

1.3 تقدير حجم المبيعات السنوية

البيان		السنة 1	السنة 2	السنة 3
المنتج / الخدمة 1: مبيعات مباشرة للمستهلكين (عبوات علاجية)	الكمية المباعة	3600	4000	4500
	سعر الوحدة	2500	2200	2200
	قيمة مبيعات المنتج 1	900.000 دج	880.000 دج	990.000 دج
المنتج / الخدمة 2: التوزيع بالجملة (الصيدليات ومحلات التغذية الصحية)	الكمية المباعة	500	625	750
	سعر الوحدة	2200	2100	2000
	قيمة مبيعات المنتج 2	1,400,000 دج	1,750,000 دج	2,250,000 دج
المنتج / الخدمة 3: الباقات العلاجية المتكاملة (شاملة المكمل والاستشارة)	الكمية المباعة	150	200	250
	سعر الوحدة	6,000	6,000	6,500
	قيمة مبيعات المنتج 3	900,000 دج	1,200,000 دج	1,625,000 دج
إجمالي المبيعات		2.900.000 دج	3.392.500 دج	4.115.000 دج

2.3 حساب النتيجة السنوية

البيان	السنة 01	السنة 02	السنة 03
رقم الأعمال (المبيعات) ... (1)	3.200.000	3.200.000	4.865.000
المشتريات المستهلكة ... (2)	250.000	250.000	418.500
الأعباء الخارجية ... (3)	1.000.000	1.250.000	1.450.000
القيمة المضافة ... (4) = (1)-(2)-(3)	1.950.000	2.256.000	2.996.500
الأجور والأعباء الاجتماعية ... (5)	900.000	1.100.000	1.300.000
إجمالي فائض الاستغلال ... (6) = (4)-(5)	1.050.000	1.156.000	1.696.500
الإهتلاكات والمؤونات.... (7)	200.000	200.000	200.000
ناتج الاستغلال ... (8) = (6) - (7)	850.000	956.000	1.496.500
الضرائب على الشركات/ IFU .... (9)	120.000	150.000	220.000
صافي الدخل ..... (10) = (8) - (9)	730.000	806.000	1.276.500
تطوررقم الأعمال	-	**19.7**	**27.2**

خاتمة:

القيمة المضافة المتوقعة للمشروع على الاقتصاد المحلي والوطني.....

من المتوقع أن يساهم هذا المشروع في تعزيز الاقتصاد الوطني من خلال تقليل الاعتماد على استيراد المكملات الغذائية الجاهزة، وبالتالي دعم الإنتاج المحلي وتقليل خروج العملة الصعبة. كما يساهم في خلق فرص عمل جديدة، خاصة في مجالات التصنيع، المراقبة المخبرية، والتسويق. وعلى المستوى المحلي، يمكن للمشروع أن يدعم تطوير الصناعات الصغيرة والمتوسطة في المنطقة، ويحفّز الاستثمار في قطاع الصناعات الصيدلانية والطبيعية. إضافة إلى ذلك، فإن اعتماد المشروع على مواد نباتية محلية قد يشجع على استغلال الموارد الطبيعية بشكل مستدام ورفع قيمتها الاقتصادية