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الـمـلـخص

تهدف هذه الدراسة إلى الكشف عن بعض الاضطرابات المبكرة في خلايا بيتا البنكرياسية، التي تُعدّ أحد العوامل الرئيسية في تطور داء السكري من النوع الأول، بالإضافة إلى تقييم التأثيرات البيولوجية لكل من الكركمين والنيكوتيناميد كمركبات يمكن أن يكون لها دور علاجي لهذا المرض. تم استخدام الخلايا NIT-1 المعالجة بالستربتوزوتوسين (STZ) كنموذج تجريبي لمحاكاة خلل الخلايا بيتا. وقد تبين أن معالجة الخلايا NIT-1والجزر البنكرياسية المعزولة من الفئران بالستربتوزوتوسين (STZ) أدى إلى موت كل هذه الخلايا، مصحوبة بزيادة في التعبير الجيني لكل منATF4 ، وNF-κB1، وCHOPعلى مستوىالخلايا NIT-1، وهي جينات مرتبطة بإجهاد الشبكة الإندوبلازمية، إلى جانب مؤشرات واضحة للإجهاد التأكسدي، بما في ذلك انخفاض مستوىالجلوتاثيون(GSH) ، وارتفاع مستوياتالمالوندياله يد (MDA) ، وتراكم أنواع مركبات الأكسجين التفعالية (ROS) ، وزيادة أنشطة أكسيدازالزانثين(XO) وسوبر أكسيد ديسميوتاز(SOD) .بعدها تمت" دراسة تأثيرات كل من الكركمين
والنيكوتيناميد الواقية لخلايا NIT-1 والجزر البنكرياسية. حافظ النيكوتيناميد على سلامة الخلايا المذكورة، في حين وبعد المعالجة بالكركمين، تمت ملاحظة سمية خلوية طفيفة في الخلايا NIT-1 من غير أن تكون لها دلالة إحصائية. وكتأثيرات إيجابية عزز كل من المركبين إفراز الإنسولين في الجزر البنكرياسية بشكل كبير. من جهة أخرى أدى الكركمين إلى انخفاضه في خلايا1-NIT، بينما زاد النيكوتيناميد من إفراز الأنسولين في هذه الخلايا. أما في ما يخص موت الخلايا فقد أسهم المركبان في إبقاء الخلايا على قيد الحياة رغم معالجتها بالستربتوزوتوسين (STZ) وقللا من تراكم ROS بشكل واضح.أظهر الكركمين بتركيز منخفض (30 ميكروغرام/مل) خصائص مضادة للأكسدة تمثلت في رفع مستويات GSH وخفض مستويات MDAوSOD، بينما أدى استخدامه بتركيز عال„ (60 ميكروغرام/مل) إلى الإجهاد التأكسدي. أما النيكوتيناميد، فقد أظهر نشاط"ا (في الزجاج) مضا"دا للأكسدة يتمثل في
تثبيط إنزيم أكسيدازالزانثين (XO) بطريقةتتعلقبالجرعة. من جهة أخرى، تمت دراسة تأثير المركبين على تعبير الجينات NF-κB1 و ATF4 وCHOP، إذ تبين أن الجرعات العالية (60 ميكروغرام/مل) لكلا المركبين ترفع من مستويات التعبير عن هذه المورثات.أما الدراسة البيولوجية الحاسوبية(bioinformatics) فقد أظهرت قدرة الكركمين على الارتباط بمجموعة من البروتينات المتدخلة في الالتهاب والإجهاد التأكسدي

والموت المبرمج للخلايا. كما تم تقييم تنظيم النشاط المناعي لكل من الكركمين والنيكوتيناميد على العدلات البشرية(neutrophils) ، حيث قلل الكركمين بشكل واضح من عمر هده الخلايا، ونشاطي الإيلاستاز والميلوبيروكسيداز بطريقة تتعلق بالجرعة، بينما لم يظهر النيكوتيناميد أي تأثير مناعي ملحوظ.أخير"ا، بينت التحليلات الحاسوبية (computational studies) الكفاءة التثبيطية للكركمين تجاه إنزيميHNEوMPOمن خلال تحليل التحام الجزيئات وحسابات نظرية الكثافة الوظيفية وتحليل الجهد الكهروستاتيكي، كما كشفت عن قوة ألفة ارتباطه بالمواقع الفعالة، إلى جانب خصائصه الإلكترونية والطاقوية المتميزة.

الكلمات المفتاحية: مرض السكري من النوع الأول، الخلايا بيتا البنكرياسية، الجزرالبنكرياسية، الخلايا المتعادلة، السمية الخلوية، إفرازالأنسولين، نشاط مضاد للأكسدة، إنزيم أكسيدازالزانثين، إنزيم الميلوبيروكسيديز، التعديل المناعي، إنزيم الايلاستاز،كركمين، نيكوتيناميد.

Abstract

This study aims to identify certain early pancreatic beta cell's dysregulations responsible for type 1 diabetes development and to evaluate certain biological effects of curcumin and nicotinamide that may be effective in the management of type 1 diabetes. Initially, β-cell disorders were studied using streptozotocin-treated NIT-1 cells as an experimental model. Streptozotocin significantly induced the death of both β-cells and mice-isolated pancreatic islets. Streptozotocine-treated NIT-1cells showed a set of disorders including an up-regulation of three genes expression related to ER-stress (ATF4, NF-κB1, and CHOP), and a state of oxidative stress which was observed as a decrease in GSH level and an increase in ROS accumulation, MDA level, and the activities of XO and SOD. Secondly, the potential beneficial effects of curcumin and nicotinamide on pancreatic beta-cells (NIT-1 cell line) and pancreatic islets (isolated from mice) were evaluated. Nicotinamide was non-toxic to both islets and NIT-1 cells, while curcumin showed a minor insignificant toxicity to NIT-1 cells .Curcumin and nicotinamide significantly enhanced insulin secretion in pancreatic islets. Curcumin significantly reduced insulin secretion in β-cells, whereas nicotinamide slightly increased this secretion. Both molecules significantly protected β-cells against the STZ-induced death and STZ-induced ROS accumulation. Curcumin at low dose (30µg/ml) exerted an antioxidant effect in β-cell as reflected by high level of GSH and low levels of MDA and SOD, while at high dose (60µg/ml), it induced oxidative stress. The antioxidant activity of nicotinamide was confirmed *in vitro* by evaluating its effect on xanthine oxydase activity. Nicotinamide showed remarquable and dose-dependent inhibition of XO activity. The possible side effects of these molecules on pancreatic β-cell was also verified by assessing their impact on the expression of NF-κB1, ATF4, and CHOP genes. Both molecules at high dose (60µg/ml) increased the expression of these genes. Bioinformatic study showed the interaction of curcumin with a set of proteins involved in apoptosis, oxidative stress, and inflammation.The immuno-modulatory activity of curcumin and nicotinamide on human neutrophil was also assessed. Curcumin significantly decreased neutrophil viability, elastase activity, and myeloperoxidase activity in a dose-dependent manner, while nicotinamide showed no immunomodulatory effects on these parameters. Curcumin's potential as a HNE and MPO inhibitor was confirmed through computational studies, including molecular docking analysis, DFT calculations, and MEP analysis, which revealed its high affinity with HNE and MPO enzyme active sites and provide informations on the electronic, energetic, and elecroteostatic characteristics of curcumin.

Keywords: Autoimmune type 1 diabetes, pancreatic β-cells, pancreatic islets, Neutrophils, Cytotoxicity, Insulin secretion, Antioxidant activity, Xanthine oxidase, Myeloperoxidase, Elastase, Immunomodulation, Curcumin, Nicotinamide.

Résumé

Cette étude vise à identifier certains dérèglements précoces de cellules bêta pancréatiques responsables du développement du diabète de type 1 ainsi qu'à évaluer certains effets biologiques de la curcumine et du nicotinamide qui peuvent être efficaces dans la gestion du diabète de type 1. Initialement, les troubles des cellules bêta ont été étudiés en utilisant des cellules NIT-1 traitées à la streptozotocine comme modèle expérimental. La streptozotocine a induit significativement la mort des cellules bêta et les îlots pancréatiques isolés de souris. Les cellules NIT-1 traitées par la streptozotocine ont montré un ensemble de troubles, y compris une augmentation de l'expression de trois gènes liés au stress du réticulum endoplasmique (ATF4, NF-κB1, et CHOP), et un état de stress oxydatif qui s'est manifesté par une diminution du niveau de GSH et une augmentation du niveau de MDA, et des activités de XO et de SOD. Deuxièmement, les effets bénéfiques potentiels de la curcumine et du nicotinamide sur les cellules bêta pancréatiques (la lignée cellulaire NIT-1) et les îlots pancréatiques (isolés des souris) ont été évalués. La nicotinamide n'était pas toxique pour les îlots et les cellules NIT-1, tandis que la curcumine présentait une toxicité mineure et insignifiante pour les cellules NIT-1. La curcumine et la nicotinamide ont augmenté significativement la sécrétion d'insuline dans les îlots pancréatiques. La curcumine a réduit significativement la sécrétion d'insuline dans les cellules bêta, alors que la nicotinamide augmente légèrement cette sécretion. Les deux molécules ont significativement protégé les cellules bêta contre la mort induite par la streptozotocine et contre l'accumulation des espèces réactives de l'oxygène induite par la streptozotocine. La curcumine à faible dose (30 µg/ml) a exercé un effet antioxydant dans les cellules bêta , ce qui s'est traduit par des niveaux élevés de GSH et de faibles niveaux de MDA et de SOD, tandis qu'à forte dose (60 µg/ml), elle a induit un stress oxydatif**.** L'activité antioxydante du nicotinamide a été confirmée *in vitro* en évaluant son effet sur l'activité de XO. La Nicotinamide a montré une inhibition remarquable et dose-dépendante de l'activité de XO**.** Les effets secondaires possibles de ces molécules sur les cellules bêta pancréatiques ont été également vérifiés en évaluant leur impact sur l'expression des gènes NF-κB1, ATF4 et CHOP. Les deux molécules à forte dose (60 µg/ml) ont augmenté l'expression de ces gènes. L'étude bioinfomatique a montré l'interaction de la curcumine avec un ensemble de protéines impliquées dans l'apoptose, le stress oxydatif et l'inflammation. L'activité immunomodulatrice de la curcumine et de la nicotinamide sur les neutrophiles humains a été également évaluée. La curcumine a significativement diminué la viabilité des neutrophiles, l'activité de l'élastase et l'activité de la myéloperoxydase de manière dose-dépendante, tandis que le nicotinamide n'a montré aucun effet immunomodulateur sur ces paramètres. Le potentiel de la curcumine en tant qu'inhibiteur de HNE et de MPO a été confirmé par des études computationelles, notamment une analyse d'amarrage moléculaire, des calculs de Théorie de la fonctionelle de la densité, et une analyse de potentiel électrostatique moléculaire , qui ont révélé sa forte affinité avec les sites actifs des enzymes HNE et MPO et ont fourni des informations sur les caractéristiques électroniques, énergétiques et électrostatiques de la curcumine.

Mots clés : Diabète auto-immun de Type 1, Cellules bêta pancreatiques, îlots pancréatiques, Neutrophiles, Cytotoxicité, Sécrétion d'insuline, Activité antioxydante , Xanthine oxydase, Myéloperoxydase, Élastase, Immunomodulation, Curcumine, Nicotinamide.

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4E-BP1: 4E-binding protein 1
4-HNE: 4-Hydroxynonenal
ACC: Acetyl-CoA carboxylase
ACLY: ATP citrate lyase
AIRE: Autoimmune regulator
AMPK: Adenosine monophosphate-activated protein kinase
AP-1: Activator protein 1
APC: Antigen-presenting cell
APRIL: A proliferation inducing ligand
Arg-1: Arginase 1
BAFF: B-cell activating factor
BM: Bone marrow
BMI: Body mass index
C5a: Complement component 5
Ca^{+2}: Calcuim ions
cAMP: Cyclic adenosine monophosphate
CAP: Catabolite activator protein
CAT: Catalase
Cbl: Casitas B-lineage Lymphoma proteins.
CTLA-4: Cytotoxic T- lymphocyte Antigen 4
Cu, Zn-SOD: Copper zinc superoxide dismutase
CXCL12: C-X-C motif chemokine ligand 12
CXCL8: C-X-C motif chemokine ligand 8
CXCR2: C-X-C motif chemokine receptor type 2
CXCR4: C-X-C motif chemokine receptor type 4
DMEM: Dulbecco's modified Eagle's medium
eNOS: Endothelial nitric oxide synthetase
ER: Endoplasmic reticulum
ERK1/2: Extracellular regulated kinase 1/2
ETC: Electron transport chain
FASN: Fatty acid synthetase
FBS: Fetal bovine serum.
G-6-Pase: Glucose-6-phosphatase
GAD 65: Glutamic Acid Decarboxylase 65
GADA: Glutamic Acid Decarboxylase antibodies
GIP: Gluco-dependent insulintropic polypeptide
GK: Glucokinase
GLP-1: Glucagon-like peptide 1
GLUT-1: Glucose transporter 1
GLUT-2: Glucose transporter 2
GLUT-4: Glucose transporter 4
GMPs: Granulocyte-monocytes precursors
GPAT: Glycerol-3-phosphate aceyltransferase
GPx: Glutathion peroxidase
GR: Glutathione reductase
GSH: Reduced glutathione
GSH-Px: Glutathione peroxidase
GSIS: Glucose-induced insulin secretion
GSK3: Glycogen synthase kinase 3

HBSS: Hank's Balanced Salt Solution
HLA: Human Leukocyte antigen
HSCs: Hematopoietic stem cells
HSL: Hormone-sensitive lipase
IAA: Insulin Autoantibodies
IFN-α: Interferon alpha
IFN-γ: Interferon gamma
IGF-2: Insulin-like growth fator 2
IL-1: Interleukin 1
IL-18: Interleukin 18
IL-1β: Interleukin 1beta
IL-2: Interleukin 2
IL2RA: α-chain of the IL-2 receptor
iNOS: Inductible nitric oxide synthetase
IRS: Insulin Receptor Substrate
ISGs: Insulin seretion granules
JNK: C-jun N-terminal kinase
LMPs: lymphomyeloids
LTB4: Leukotriene B4
MDA: Malondialdehyde
Mn-SOD: Manganese superoxide
MPO: Myeloperoxydase oxydase
mtDNA: mitochondrial DNA
mTEC: Medullary thymic epithelial cell
mTOR: Mammalian target of rapamycin
Munc13: Mammalian uncoordinated-13
Munc18: Mammalian uncoordinated-18
NE: Neutrophil elastase
NETs: Neutrophil extracellular traps
NF-κB: Nuclear factor- kappa B
NFS: N-ethylmaleimide sensitive factor
nNOS: Neuronal nitric oxide synthetase
NOS: Nitric oxide synthetase
NOX: NADPH oxidases
PC1/3: Proconvertase 1/3
PC2: Proconvertase 2
PDE-3: Phosphodiesterase -3
PDK: Pyruvate dehydrogenase kinase
PEPCK: phosphoenolpyruvate carboxynase
PGC-1α: Peroxisome proliferator-activated receptor $_{\gamma}$ coactivator 1α
PI3K: Phosphatidylinositol -3 kinase
PKA: Protein kinase A
PKB: Protein kinase B
PKC: Protein kinase C
PTPN22: Protein Tyrosine Phosphatase Non receptor 22
Rabs: Ras-associated binding proteins
RIMs: Rab3-interacting molecules
ROS: Reactive oxygen species
RRP: ready releasable pool
S6K: S6 Kinase 1
SAPK: Stress-activated protein kinases
SCD1: Stearoyl-CoA desaturase 1

SNAP-25: Synaptosome-associated protein 25
SREBP-1c: Sterol-regulatory Element-binding Protein-1c
SRP: Signal recognition particle
STZ: Streptozotocin
T1D: Type 1diabetes
TCR: T cell receptor
TLR: Toll-Like receptor
TNF-α: Tumor necrosis factor alpha
TPV: Total pancreatic volume
Tregs: Regulatory T cells
TSAs: Tissue-specific antigens
t-SNARE: target-Soluble N-ethylmaleimide-sensitive factor attachement protein receptor
UFA: Unsaturated fatty acid
v- SNARE: Vesicule- Soluble N-ethylmaleimide-sensitive factor attachement protein receptor
VAMP2: Vesicle-associated membrane protein 2
VCAM-1: Vascular cell adhesion molecule 1
VDR: Vitamin D receptor
VLA-4: Very late antigen-4
VNTR: Variable number of tandem repeats
ZnT8A: Zinc Transporter 8 antibodies
XO: Xanthine oxidase
ATF4: Activating transcription factor 4
CHOP: C/EBP Homologous Protein
NIT-1: Non-Obese Diabetic (NOD) Insulinoma Transgenic

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Introduction

Type 1 diabetes is a serious metabolic disease that occurs following the progressive autoimmune destruction of pancreatic beta cells, resulting in insufficient insulin production. Though symptoms can vary, type 1 diabetes typically features polydipsia, polyuria, polyphagia, fatigue, weight loss, and blurred vision (Singh et al., 2023).

T1D patients are susceptible to numerous severe acute and chronic complications. Acute complications comprise hypoglycemia and diabetic ketoacidosis. While, chronic complications comprise retinopathy, nephropathy, neuropathy, heart disease, peripheral arterial disease, cerebrovascular disease, and diabetic foot infections (Lucier & Weinstock, 2023).

Autoimmune type 1 diabetes represents 5 to 10% of all diabetes instances (American Diabetes Association Professional Practice Committee, 2024). This disease is associated with an increased rate of mortality. In 2021, around 8.4 million individuals suffer from T1D with an estimated number of newly diagnosed cases of 500,000. By 2040, the number of type1 diabetic cases is expected to reach 13.5-17.4 million (Gregory et al., 2022). In 2025, nearly 188, 200 people in Algeria were living with type 1diabetes, including approximately 56,000 children and adolescents (IDF Diabetes Atlas, 2025).

Currently, insulin replacement therapy is the first-line option for managing T1D. It is based on the delivery of exogenous insulin either by multiple daily injections or by insulin pumps. Nevertheless, this strategy has a number of drawbacks and undesirable consequences, such as a permanent reliance on exogenous insulin, hypoglycemia, insulin resistance, mild obesity, and mental health issues (Pathak et al., 2019; Subramanian et al., 2024). Therefore, the search for new approaches to manage type 1 diabetes has become an absolute necessity.

While immune system dysregulation is a primary driver of type 1 diabetes, evidence indicates a more complex etiology wherein beta cells themselves play a role in their destruction, implying factors beyond a purely autoimmune mechanism (Roep et al., 2021). Therefore, it seems that a thorough comprehension of the intracellular processes and the dysregulations of the pancreatic beta cells that precede the autoimmune reaction will enable us to identify new targets and effective strategies for the management of type 1 diabetes.

Furthermore, naturally occurring substances that are mostly found in herbs and animals are currently causing a lot of concern worldwide. This is due to their unique chemical diversity that offers many valuable biological properties useful for diseases prevention and treatment (Yuan et al., 2016).Curcumin and nicotinamide are bioactive compounds with

potent antioxidant, anti-inflammatory, and anti-apoptotic properties (Hewlings & Kalman, 2017; Permezel, 2011; Xie et al., 2017; Zhu et al., 2019). Current clinical trials on curcumin and nicotinamide for type 1 diabetes (T1D) are scarce and largely unsuccessful: curcumin studies remain preclinical or T2D-focused with no large-scale T1D human trials (Qin et al., 2014 ; Yaikwawong et al., 2024), while the ENDIT trial (2003-2010) showed no preventive benefit for nicotinamide in at-risk individuals despite high doses over 5 years (Gale et al., 2004). This significant evidence gap underscores a critical research niche for investigating their immunomodulatory and cytoprotective potential in T1D's autoimmune context, .Given this, we anticipate thatthese natural molecules will be beneficial in the development of medications to treat and prevent diabetes.

In this regard, our present work aims to explore certain pancreatic β-cell's early disorders that occur prior to the autoimmune response of type 1 diabetes, as well as assessing the valuable biological properties of two natural compounds, curcumin and nicotinamide that may be useful in the prevention and treatment of this disease.

Therefore, our present study was devoted to:

- ✓ Identify certain pancreatic β-cell's early dysregulations that occur before the onset of the autoimmune reaction of type 1 diabetes by studying the oxidative status as well as the expression of specific genes (NF-κB1, ATF4, and CHOP) related to ER stress-induced death using STZ-treated NIT-1 cells as an experimental model.
- ✓ Evaluate the *in vitro* effects of curcumin and nicotinamide on pancreatic β-cell using various models including the viability of mice-isolated pancreatic islets and β-cells (NIT-1 cells), insulin secretion in mice-isolated pancreatic islets and β-cells, protective effect against STZ-induced death and STZ-induced ROS accumulation, and measurement of oxidative stress markers in pancreatic β-cell, intending to target β-cell for type 1 diabetes management.
- ✓ Evaluate the effects of nicotinamide and curcumin on the expression of specific genes related to cellular death (NF-κB1, ATF4, and CHOP) in beta-cells to assess the profound cytotoxic effects of these molecules on beta-cells.
- ✓ Conduct an in silico study to investigate potential interactions between curcumin and genes involved in apoptosis, oxidative stress, inflammation, and pathways related to type 1 Diabetes.
- ✓ Evaluate the antioxidant activity of nicotinamide by studying its *in vitro* inhibitory effect on xanthine oxidase, with the aim of exploiting this biological property as a tool for managing type 1 diabetes.

- ✓ Evaluate the *in vitro* anti-inflammatory effects of curcumin and nicotinamide using several models including HNE and MPO activities modulation and human neutrophil lifespan modulation, and then applying these strategies for type 1 diabetes management.
- ✓ Perform Density Functional Theory (DFT) and molecular docking analysis to support the *in vitro* studies on the effectiveness of the examined molecules as inhibitors of HNE and MPO.

Literature review

1 The pancreas: structure and function

1.1 Pancreas

The human pancreas is an upper abdominal retroperitoneal organ that is situated behind the stomach (Gorai & Vashisth, 2022; Jouvet & Estall, 2017). It possesses an extended shape which is made up of a head part linked to the duodenum, a tail part linked to the spleen, and an intermediate body region (In't Veld & Smeets, 2014). Instead, there are no well-defined boundaries between these parts.This gland, with a weight ranging from 5 to 100g, is surrounded by a fibrous capsule from which it extend connective tissue septa, allowing the divisionof its parenchyma into several lobes and lobules (Dolenšek et al., 2015). On a microscopic scale, these lobules consistof two different structures: the exocrine pancreas, which accounts for approximately 98% of the pancreatic mass and is composed of duct and acinar cells that produce digestive enzymes and sodium bicarbonate, respectively; and the endocrine pancreaswhich maintains glucose homeostasis (In't Veld & Smeets, 2014).

In contrast to the human, mice have a less clearly defined pancreas that is instead diffusely and dendritically extended inside the mesentery of the proximal small intestine. The mice pancreas can be divided into three principal parts: duodenal, splenic, and gastric lobes that are mostly separated by adipose, connective, and lymphatic tissues (Dolenšek et al., 2015).

1.2 Human and mice endocrine pancreas

Pancreatic endocrine cells, occupying 1-4% of TPV, are typically arranged in islets of 50–200 microns in diameter. These latter contain from hundred to several thousands of endocrine cells that are irrigated by blood capillaries. The number of islets in the human adult pancreas varies from 1.000.000 to 15.000.000 (Dolenšek et al., 2015).

On the other hand, mice's pancreatic islets are predominantly found in the interlobular space and to a less degree within lobules compared to the human. A mouse pancreas can have from 1000 to 5000 islets that have a similar size to that of adult human (Dolenšek et al., 2015). Mouse islets can contains up to thousands of endocrine cells (Steiner et al., 2010).

The pancreatic islet is made up of five distinct cell types that release a range of hormones: glucagon-producing α-cells; amylin-, C-peptide and insulin-producing β-cells; pancreatic polypeptide (PP)-producing γ-cells; somatostatin producing δ-cells and ghrelin-producing ε-cells which make up only 1% of the total islet cells (Röder et al., 2016). Unlike

human, where these endocrine cells are randomly distributed within the islets, mice have islets with a beta-cell core surrounded by a mantle of the other endocrine cells (α,δ, γ, ε) (Steiner et al., 2010).

2 Insulin

2.1 Insulin gene

In human, the gene coding for the pre-pro-insulin precursor exists as a single copy on chromosome 11 close to the IGF-2 gene, while in mice two non-allelic copies, Insulin I and Insulin II, are founded on chromosome 9 and 19, respectively. Insulin I gene consists of only 2 exons, with the second exon containing the entire coding region. However, the structure of the Insulin II gene is similar to that of the human one. Human insulin gene has a length of 1355 pb. Its coding region comprises three exons: 1(42 pb), 2(187 pb), and 3(220 pb) which monitor the production of the signal peptide, the B chain of insulin and a portion of the connector peptide, and the A chain and the rest of the connector peptide, respectively. These three exons are separated by two introns that comprise 179 and 786 pb,respectively (Magnan & Ktorza, 2005).

2.2 Insuline structure

Mature insulin monomer is a globular protein with a molecular weight of approximately 6 kDa. It is formed of two small polypeptide chains, chain A and chain B which are linked together by two inter-chain disulfide bridges and an intra-chain disulfide bridge within chain A (Magnan & Ktorza, 2005). Chain A, comprising 21 residues, consists of two short α-helices (A1-A8 and A12-A19) linked by a turn (residues A9-A12) while the B chain of 30 residues is composed of a 8-residues fragment (residues B1 to B8) at the N-terminal part, which can take an elongated or helical structure. This latter isfollowed by an α-helix (B9- B19) extended by a β-turn and a β-strand (Huus et al., 2005; Ward & Lawrence, 2011). In the bloodstream, insulin is monomeric due to its very low concenrations.This monomerrepresents the physiologically active form of insulin that can bind to its specific receptor. At higher concentrations, insulin molecules form dimers, which are mainly stabilized by non-polar forces. However, under high levels of zinc ions, three dimers combine to form hexamers that are stabilized byZn^{2+}. These zinc ions specifically bind to the His10 residues found in the B chains of insulin. Along with the zinc ions, other residues (AlaA14, LeuA17, LeuB13, TyrB14, and GluB17) also play a role in stabilizing this hexameric structure. This later can have three distinct conformations, depending on the conformation of the B chain segment (residues Phe1 to Gly8).The hexamers

constitue the insulin storageform (Gorai & Vashisth, 2022; Huus et al., 2005; Li & Leblanc, 2014).

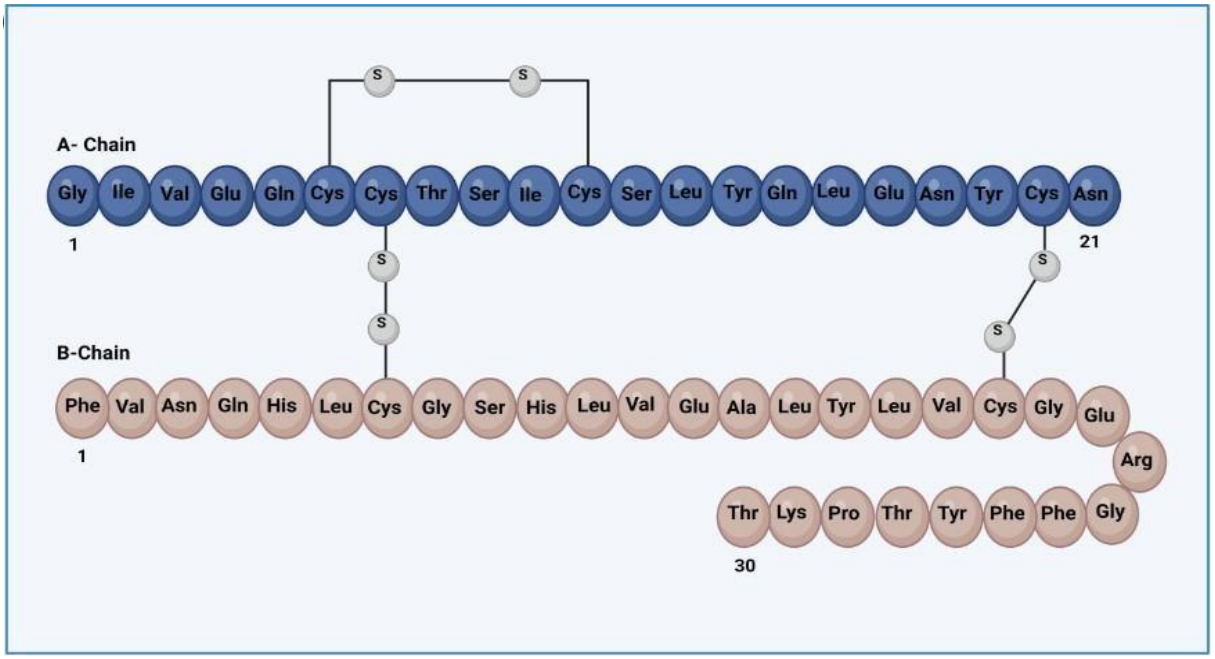

Figure 1. Primary structure of human insulin.

Source: reproduced from (Portha, 2007) . Created with Biorender.

2.3 Insulin biosynthesis and storage

Insulin biosynthesis begins in the nucleus where transcription of the gene coding for preproinsulin takes place. The latter is a single-stranded polypeptide with a molecular weight of 11.5kDa, and it comprises four segments: the signal peptide at the N-terminal end, insulin B-chain, C-peptide and insulin A-chain. The mRNA sequence will then be translocated to the cytoplasm and translated into preproinsulin. Subsequently, this precursor of 110 aminoacids will be recognized by the SRP which directs it towards the lumen of the rough endoplasmic reticulum. At this point, the 25-residue signal peptide will be immediately cleaved and degraded by specific peptidases thus generating proinsulin. Subsequently, the oxidizing environment of the endoplasmic reticulum promotes the formation of intracatenary and intercatenary disulfide bridges as well as the stabilization of proinsulin in 3D configuration catalyzed by protein-thiol reductase and chaprons, respectively. Proinsulin is then transported to the Golgi appartus where it packed into immature granules. Then, within these clathrine-coated granules, the prohormone will be processed into mature insulin. This is achieved by the cleavage of the C-peptide via two endopeptidases PC1/3 and PC2, which cleave at two dibasic sites (Arg 31, Arg 32 and Arg64 , Lys 65). Then, once the removal of the c-terminal

basic amino acids via the carboxypeptide E, mature insulin and a C-peptide are yeilded. Concurrently, these granules mature by losing their clathrine coating (Magnan & Ktorza, 2005; Uchizono, 2007).

The nascent insulin is finally stored in these granules as 2-Zn-hexameric complexes, thus promoting crystallization. The later is favored by the high zinc contenant, insulin accumulation within these granules as well as the acidic pH within these laters following basic aminoacids removal (Li, 2014; Magnan & Ktorza, 2005). A single pancreatic β-cell store roughly 10 000 insulin secretory granules (ISGs), each contains more than 250 000 insulin molecules. The number of ISGs within pancreatic β-cell is relatively maintained at an optimal constant level by balancing between biosynthesis, secretion and breakdown of insulin granules. In fact, the lost from ISGs store following exocytosisis instantly replenished by insulin biosynthesis activation. On the other hand, these β-granules have a half-life of 3 to 5 days; hence, those that do not undergo exocytosis during this period are targeted for subcellular degradation via crinophagy and/or autophagy (Magnan & Ktorza, 2005; Uchizono, 2007).

2.4 The insulin secretion

2.4.1 Insulin secretion stimulators

The release of insulin by the pancreatic beta cells is stimulated by a variety of agents. In fact, glucose is the only strong natural secretagogueable of inducing insulin release in absence of any other stimulating agents. This secretory reponse, known as GSIS, can be enhanced by agents called potentiators or amplifiers.These latter ones include some amino acids (leucine…), fatty acids, hormones (GLP-1, GIP, glucagon…), neurotransmitters (acetylcholine…), ketone bodies, fructose, and many pharmaceutical molecules. However, these agents are ineffective as secretagogue in low concentrations of glucose and can only exert their stimulatory effect on insulin secretion in the case of normo- or hyper-glycemia (Portha, 2007).

2.4.2 Glucose-induced insulin secretion pathway

Glucose enters the pancreatic beta cell via facilitate diffusion through the transporters GLUT-1 in humans and GLUT-2 in rodents (Leung, 2015; Newsholme et al., 2014). This glucose will then be phosphorylated by glucokinase before being metabolized by glycolysis and oxidative respiration to generate ATP. Consequently, the increase in the ATP/ADP ratio leads to the closure of ATP-dependant potassuim channels and, thus, a depolarization of the

plasma membrane and subsequent opening of the voltage-gated calcium channels. Then, a rise in cytosolic Ca^{+2} ions induces insulin vesicule exocytosis (Fu et al., 2013).

The Ca^{+2}-dependent release of insulin secretory granules (ISGs) occurs in a series of steps: docking with the plasma membrane, maturation (priming) and fusion. This process relies on the formation of complex known as the SNARE complex, which triggers the release of ISG granules.This complex involves the interaction of t-SNARE proteins on the plasma membrane (SNAP-25 and syntaxin 1A) with the v- SNARE protein VAMP2 on the ISG membrane. Other proteins like Munc18, Munc13, Rabs, RIMs, Synaptotagmins, NFS and the regulatory protein Go are also involved (Omar-Hmeadi & Idevall-Hagren, 2021).Upon the increase in calcium concentration, the regulatory protein Go that prevent exocytosis in the absence of stimulation, becomes inactive. Simultaneously, the protein synaptotagmin becomes active.This activation enables synaptotagmin to bind with its partner NFS, leading to the fusion of lipid bilayers, and the subsequent release of insulin (Magnan & Ktorza, 2005).

2.4.3 Kinetics of insulin secretion

Insulin secretion upon glucose stimulation is a biphasic process .The first phase correspond to the immediate exocytosis of the readily releasable pool (RRP) that referred to as a population of mature insulin granules already docked and primed to the plasma membrane. This phase has high amplitude but short duration, it returns to the baseline within 5 to 10 minutes following the depletion of RRP. This later represent only 1% of the total insulin-containing granules. While, the second phase consists of the release ofboth stored insulin-granules, which aredeeper inside the beta cell as well as newly synthesized granules. Exocytosis ofthese granules involves their mobilization, docking with the cell membrane as well as their priming. This second phase is slower and has a lower amplitude but maintain insulin secretion for sustained period lasts from 30 to 60 min (Jouvet & Estall, 2017; Leung, 2010; Newsholme et al., 2014).

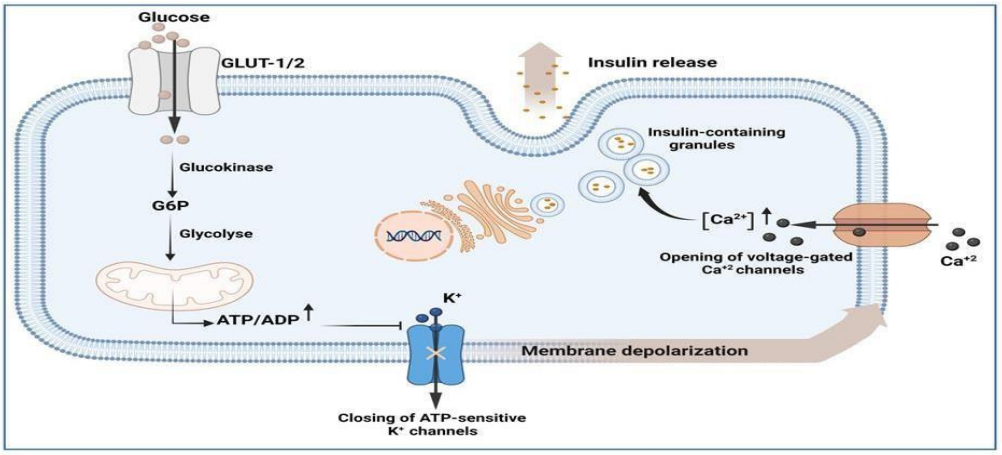

Figure 2. Insulin secretion pathway (Created with Biorender (self creation).

2.5 Major metabolic Effects of insulin

Insulin exerts a wide range of anabolic actions in metabolic tissues including: liver, skeletal muscle, and adipose tissue. It stimulates the mobilization and storage of carbohydrates, proteins and lipids in these tissues and prevents the breakdown of this reserve (Wu & Garvey, 2010a). Indeed, insulin effects are initiated following its interactions to its specific receptor on target tissues. This transmembrane receptor is a heterotetramer (2α2β) and belongs to the family of tyrosine kinase receptors. Its binding to its ligand, insulin, induce its autophosphorylation and consequently the recruitement of insulin receptor substrates (IRSs) which activate two signaling pathways, the PI3K/Akt pathway and the MAPK pathway. However, the metabolic effects of insulin are primarly mediated by the PI3K/Akt pathway (Newsholme et al., 2014; Rahman et al., 2021).

2.5.1 Regulation of liver metabolism

Unlike its effects on myocytes and adipocytes, insulin does not stimulate glucose uptake by hepatocytes. Glucose enters these latter through the GLUT-2 transporter in response to hyperglycemia rather than hyperinsulinemia (Adeva-Andany et al., 2016).

The primary effect of insulin on the liver is related to the promotion of glycogenesis and glycolysis and the suppression of glycogenolysis and glyconeogenesis. Mechanistically, insulin can affect the liver's metabolic functions in two ways: directly and indirectly. Directly, the interaction of insulin with its receptor activates the PI3K pathway and subsequently the AKT protein. The latter protein induce the inhibition of GSK3 and consequently the activation of glycogene synthetase, thus promoting the conversion of glucose into glycogen. On the other hand, AKT protein inhibits the transcription of genes encoding PEPCK and G-6-Pase, two key enzymes for gluconeogenesis and glucose output, respectively. However, the

insulin effect is mainly indirect. It involves the inhibition of glucagon secretion, the impact of insulin on hypothalamus, and the deprivation of gluconeogenesis substrate through the inhibition of lipolysis (Rahman et al., 2021; Wu & Garvey, 2010).Concerning its effect on lipid metabolism, insulin induces liver De novo lipogenesiswhile inhibiting fatty acid oxidation. Insulin promotes fatty acid biosynthesis and triglycerides accumulation primarly through stimulation of SREBP-1c expression and its translocationto the nucleus. SREBP-1c is a transcription factor that promotes the expression of lipogenic genes (FASN, GPAT, ACLY, ACC, SCD1, GK). Simultaneously, insulin shuts off fatty acid oxidation via stimulation of PGC-1α phosphorylation. These latter effects are also mediated through the PI(3)K pathway (White, 2017; Rui, 2014).

2.5.2 Regulation of Skeletal Muscle and adipose tissue metabolism

The effect of insulin on skeletal muscles and adipose tissues consists mainly in the promotion of glucose uptake via facilitate diffusion transporter, GLUT-4. Under basal conditions, this transporter is intracellulary sequestered in vesicles. Upon stimulation by insulin and subsequent activation of the PI3K/PDK and the CAP/Cbl/TC10 pathways, the vesicles carrying GLUT-4 will be translocated to the plasma membrane. This is a reversible process; GLUT-4 will be resequestred in the intracellular compartement as the blood insulin level drops (Santoro et al., 2021; Wu & Garvey, 2010). Skeletal muscles account for the majority of insulin-induced glucose uptake (Wu & Garvey, 2010). This glucose will be stored as glycogen. Insulin also prevent glycogenolysis in this tissue. In adipocytes, insulin promotes the conversion of glucose into fatty acids (de novo lipogenesis) by stimulating SREBP-1c expression. Additionally, insulin suppresses lipolysis via inhibition of HSL and perilipin following a decrease in cAMP and subsequent inactivation of PKA. This drop in cAMP is due to the insulin-mediated phosphorylation and activation of PDE-3 through the AKT/PKB pathway (Wu & Garvey, 2010). Beyond its effects on glucose and lipids metabolism, insulin promotes protein synthesis in both muscle and liver by activating two main regulators of protein translation: S6K and 4E-BP1 via PI3K/AKT pathway (Khalilov & Abdullayeva, 2023; Wu & Garvey, 2010).

3 Type 1 diabetes

3.1 Definition

Type 1 diabetes, formely known as "insulindependant diabetes" or " juvenile-onset

diabetes", is an autoimmune disease characterized by the destruction of beta cells of langerhans islets (American Diabetes Association, 2015).The reduction of beta cell mass leads to a deficiency of insulin secretion hence a development of hyperglycemia (Katsarou et al., 2017). The disease occurs in genetically susceptible subjects following exposure to environmental factorsand involves both humoral and cellular islet autoimmunity (Paschou et al., 2018). The presence of anti-pancreatic islet antibody in patients with type 1 diabetes is a reliable marker of the autoimmune process (American Diabetes Association, 2015).

3.2 Stages of T1D pathogenesis

Stage1: It is charachterized by the appearance of two or more islet autoantibodies that reflect beta-cell autoimmunity. Four main islet autoantibodies have been detected: IAA, GADA, IA-2A, and ZnT8A. However, beta cell function and normoglycemia are still preserved without any T1D symptoms. The risk of progression to clinical disease increases with an increased number of islet autoantibodies. These immune biomarkers can be detected weeks to years prior to the clinical stage.

Stage2: It is characterized by a decline in functionnal beta-cell mass, which is manifested by dysglycemia without any concomitant clinical symptoms.

Stage3: It is charachterized by a progressive loss of beta-cell mass, resulting in insulin deficiency and subsequent hyperglycemia. The latter is associated to polydipsia, polyphagia, and polyuria, three main clinical signs of overt type 1 diabetes (Lindbladh et al., 2020).

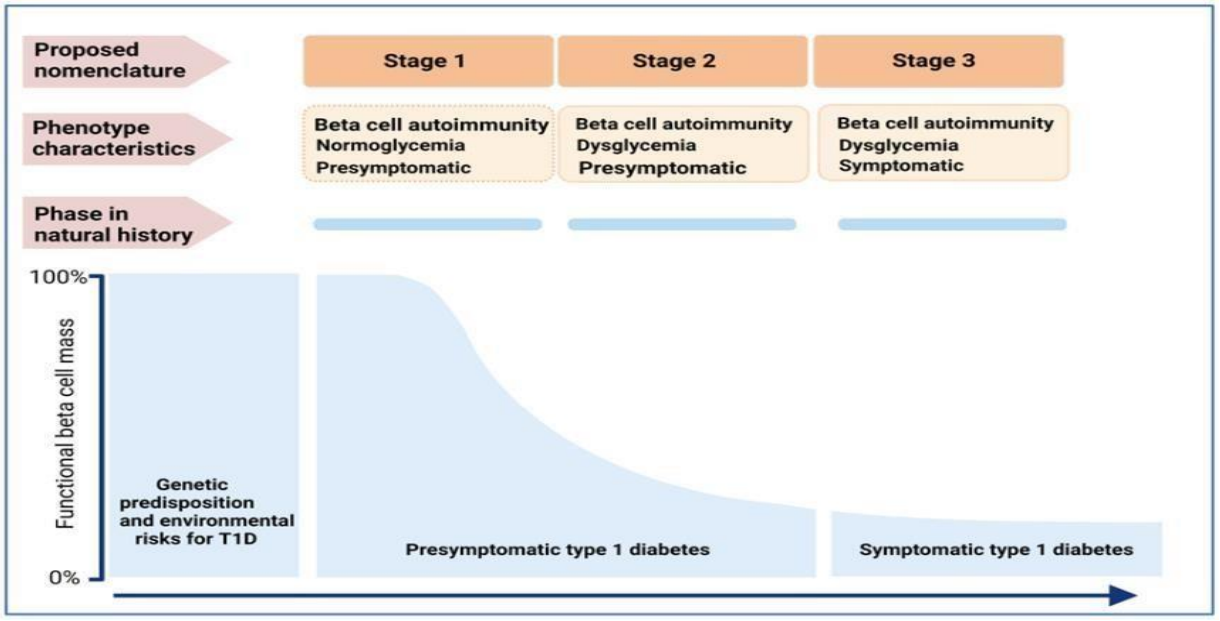

Figure 3. Schematic illustration of T1DM stages. Source: reproduced from Lindbladh et al., 2020 (Created with Biorender).

3.2. Causes of failure of immunological self-tolerance to pancreatic beta-cells

3.2.1 Failure of central self-tolerance

Under the control of the transcription factor AIRE, thymic epithelial cells express and present several tissue-specific antigens of different organs, including those of pancreatic beta cells. This permits the process of negative selection, resulting in the elimination of self-reactive T cells prior to their maturation into fully immunocompetent cells. However, this central tolerance mechanism may be broken in case of type 1 diabetes; certain T lymphocytes escape negative selection and reach the periphery, and corresponding tissue-specific antigens (TSA) become autoantigens (Brezar et al., 2011; Clark et al., 2021).

3.2.2 Failure of peripheral self-tolerance

The so-called central tolerance is aprocess that does not entirely remove autoreactive T cells. Indeed, a number of self-forbidden T lymphocytes avoid thymic negative selection and find their way into lymph nodes and peripheral tissues. However, there are several mechanisms that neutralize and suppress auto-reactivity in the periphery. These mechanisms are mainly: anergy, ignorance, deletion, and Treg-mediated immunoregulation. While, failure of peripheral tolerance mechanisms to counteract autoreactive T cells targeting pancreatic β-cells might lead to type1 diabetes development (Burrack et al., 2017). A number of gene such as PTPN22, IL-2RA and CTL-4, which impact T cell activation, function , and regulation, have been reported to predispose to less efficient immune peripheral control in type 1 diabetes (Pugliese, 2014).

3.2.3 Beta cell stress and neoantigens formation

Loss of peripheral tolerance in case of type 1 diabetes may also attributed to organelle stress includingendoplasmic reticulumstress which lead to the formation of neoantigens that are recognized as foreign by the immune system. It is widely known that β-cell normal secretory functionin response to chronic hyperglycemia may develop to a pathological state generating ER stress that is exerbated by a panel of environmental factors including virus, exposure to chemicals, exposure to ROS, and inflammation (Marré et al., 2015). ER stress, in turn, induces the leakage of Ca^{+2} from the ER lumen to the cytosol, allowing: on theone hand, the raising of cytosolic Ca^{+2}level, which promotes the activation of Ca^{+2}-dependant PTM enzymes and consequently PTM-modifications of β-cell proteins (Marré & Piganelli, 2017); on the other hand, ER calcuim depletion leads to improper protein folding, asER-resident

enzymes responsibles for protein folding such as chaperones and protein disulfide isomerase possess calcuim-dependant activity.Both PTM-modified and abberant folding β-cell proteins constitue neoantigens that trigger autoimmunity against pancreatic β-cells.Several native β-cell proteins including preproinsulin, proinsulin, GAD 65, and chromogranin A, have changed into neo-antigens through abberant post-translational modifications (Clark & Urano, 2016).

3.3 Factors contributing to type 1 diabetes

3.3.1 Genetic background

3.3.1.1 HLA

The strongest genetic susceptibility for type 1 diabetes resides in HLA class II locus (Saberzadeh-Ardestani et al., 2018). The product of this gene shapes immunological central tolerance as it mediates the presentation of self-peptides by mTEC during T cell development and maturation.The HLA II mechanism mediating resistance or susceptibility to T1D depends on the affinity between HLA II moleculesand self-epitopes. The HLA II protective variant form strong interaction with self-peptidesleading to efficient presentation of these latters and deletion of autoreactive T cell. The risk variants are linked to unstable HLAII –peptide complexes and suboptimal presntation of self-peptides thus promoting the survive of autoreactive T cells and autoimmunity (Pugliese, 2010). The HLA class II risk alleles include DR3-DQ2 (DRB103:01-DQB102:01) and DR4-DQ8 (DRB104:01-DQB103:02)—with their heterozygous combination conferring the highest odds ratio, while DR15-DQ6 (DRB115:01-DQB106:02) provides strong dominant protection, reducing risk by over 90% (Noble et al., 2024; Pociot & Lernmark, 2023).

3.3.1.2 INS

Insulin gene is located on chromosome 11p15.5. This gene lies a polymophic variable number tandem repeat (VNTR) that is highly associated with type 1 diabetes. Two main T1D-related VNTR alleles exist: a predisposing allele, class I, and a protective allele, class III. The mechanism by which these alleles confer protection or susceptibilty to T1D is linked to the level of insulin expression by mTEC. The protective allele is related to high expression of thymic insulin. While, the risk allele is associated with suboptimal insulin expression in the thymus leading to an impaired negative selection and the esscape of insulin-specific autoreactive T cells to the periphery (Brezar et al., 2011; Burrack et al., 2017). In addition to the allelic variation of the VNTR region, epigenetic regulation can also influences the rate of thymic insulin expression. Epigenetic regulation may inhibit this latter process particulary for T1D-protective alleles (Pugliese, 2017).

3.3.1.3 PTPN22

The PTPN22 gene encodes lymphoid-specific tyrosine phosphatase (LYP) on chromosome 1p13.3-p13.1. In addition to its broad expression by Natural Killer cells and neutrophils, LYP enzyme is also found in immature and mature B and T lymphocytes (Burn et al., 2011). PTPN22 predisposing variants are linked to elevated phosphatase activity which impair thymic negative selection and thus immunological self-tolerance. This effect is mediated through downregulation of TCR signaling allowing the survival of autoreactive T cells specific for pancreatic beta cells. Thymic development of CD4+CD25+ regulatory T cells may also be blocked by a rise in PTPN22 activity (Clark et al., 2017; Tisch & Wang, 2008).

The single-nucleotide polymorphism PTPN22 C1858T, also known rs2476601, has been revealed to be significantly associated with T1D in several studies performed in different populations (El Fotoh et al., 2019; Lee & Song, 2013), whereas, a study performed by Campos et al. (2020) has not shown this relationship. rs1310182 (Sharma et al., 2021) and 1123 G>C (Jaya et al., 2018) polymorphisms at PTPN22 has also exihibited its correlation with T1D.

3.3.1.4 CTLA-4

The CTLA-4 gene is encoded on chromosome 2q33 (Jović et al., 2020). Its correspondant protein is exclusively expressed on activated T-lymphocytes, where the CTLA-4 molecule interacts to its ligands CD80 or CD86 expressed on the surface of antigen-presenting cells leading to IL-2 synthesis reduction and consequently to the inhibition of activated T-lymphocytes expansion. Thus, CTLA-4 prevents autoimmune reactions by its inhibitory effect on T-cell activation (Jović et al., 2020; Rudd, 2008). Through this anergy pathway, CTL-4 susceptibility alleles are expected to act by providing a weaker inhibitory signal that promotes an increase of autoreactive clones (Brezar et al., 2011).

In 1996, CTLA4 gene has been revealed by Nisticò and his colleagues as a third loci related to T1D (Nisticò et al., 1996).The association between T1D and CTLA4 49 A–G polymorphism located at exon 1 is well established in great number of populations (Mosaad et al., 2012; Sharma et al., 2021).This contrasts with the findings of other studies that have not shown any significant link of this polymorphism and the studied disease (Çelmeli et al., 2013; Jaya et al., 2018). Besides, many other polymorphisms in the same gene have exibited its relation with susceptibility to T1D, among them CT60 single-nucleotide polymorphism (Sharma et al., 2021), the CTLA4 1661(Baniasadi et al., 2006), the CTLA4159 (Osei-Hyiaman et al., 2001), CTLA4 -318 C/T (Anjos et al., 2004), rs231727 and rs231775 (C.

Sharma et al., 2021). While, other works were inable to proved this association concerning CTLA4 -318 C/T (Baniasadi et al., 2006; Caputo et al., 2007), CTLA4-1722 (Baniasadi et al., 2006; Bouqbis et al., 2003), CTLA4 -1661 (Anjos et al., 2004), CTLA4-CT60 (Baniasadi et al., 2006), and CTLA4-159 (Caputo et al., 2007)polymorphisms.

3.3.1.5 IL2RA/CD25

The IL2RA/CD25 gene which encodes the α-chain IL-2 receptor is resided on chromosome 10p15 (Jović et al., 2020). This molecules is extremly expressed on Tregs in constitive manner, up-regulated on activated effector T-cells, and some innate immune cells (國安, 2001). IL-2/IL-2 receptor (IL-2R) signaling is crucial for the maintenance of immune self-tolerance by mediating $CD4^+CD25^+$ regulatory T-cells development and activity, thus controlling T-cell immune responses and preventing autoimmunity (Bayer et al., 2013; Malek & Bayer, 2004).

A report of Vella et al. (2005) has revealed for the first time that variants of IL2RA gene is firmly associated with T1D. According to the studies performed on different populations, the ss52580101 (rs41295061) is the most locus in the IL2RA gene associated with T1D risk (Maier et al., 2009; Tang et al., 2015). However, in contrast to the above mentioned studies, other authors have not found any link of this polymorphism with the disease (Kawasaki et al., 2009; Ranjouri et al., 2016). Further polymorphisms in this gene such as: rs11594656 (Fichna et al., 2012; Maier et al., 2009; Tang et al., 2015), rs2104286 (Maier et al., 2009; Tang et al., 2015), rs706778 (Kawasaki et al., 2009), rs3118470 (Fichna et al., 2012; Kawasaki et al., 2009), and rs7093069 (Borysewicz-Sańczyk et al., 2020) have been also reported to be associated with T1D. While, in another works, rs11594656 (Kawasaki et al., 2009), rs2104286 (Fichna et al., 2012), and rs7093069 (Fichna et al., 2012) polymorphism have not displayed any significant association with T1D.

3.3.2 Enviromental determinants

3.3.2.1 Breastfeeding versus cow's milk intake

The role of breastfeeding in type 1 diabetes is controversial. Although some studies have not found any association between the duration of breastfeeding and either risk of islet autoimmunity (Hakola et al., 2018) or type 1 diabetes (Hakola et al., 2018; Lund-Blix et al., 2019), others have been indicated that short term or absolute lack of breastfeeding constitue a risk factor favoring the occurrence of the disease (Patelarou et al., 2012). Furthermore, the vital role of breast milk as a protective factor against T1D has been supported by many studies (Abdulrahman et al., 2018).

Concerning cow's milk intake, it has been shown a causal relation between great consumption of cow 's milk and islet autoimmunity as well as progression to type 1 diabetes (Lamb et al., 2015). Moreover, some findings have been revealed that early introduction of cow's milk constitue a risk factor for type 1 diabetes (Awadalla et al., 2017).

3.3.2.2 Solid foods

In addition to breastmilk and formula substitutes, the infant is exposed to many other dietary antigens present in solid foods. Early (<4 months) exposure to gluten-containing foods was associated with the risk of progressing from islet autoimmunity to type 1 diabetes but not with the risk of islet autoimmunity. Late (>6 months) exposure to gluten-containing foods was not associated with islet autoimmunity nor wih T1D (Lund-Blix et al., 2019). Inconsistently, study by Frederiksen has found any association between the age at introduction of gluten and development of T1D (Frederiksen et al., 2013).

Similary, it shoud be added that maternal diet during pregnancy can also impact on the risk of developing T1D in their children. Study by Antvorskov et al. (2018) has shown that risk of T1D in the offspring increases with high intake of gluten containing foods during pregnancy. Neverthless, not all studies are in agreement as a study by Lund-Blix et al. (2019) have found no such relation.

3.3.2.3 Vitamin D

Due to the expression of 1,25(OH)2D3 receptor by both beta-cells and most cells of the immune system, several data points to a role of vitamin D in the pathogenesis of T1D (Altieri et al., 2017).

Concerning materal vitamin D status, it has been shown a strong link between lower 25(OH)D levels in women at late pregnancy and higher risk of developing T1D in the offspring (Sørensen et al., 2016). While, no such connection has found between first and second-trimester 25(OH)D concentrations and childhood T1D risk (Miettinen et al., 2012; Sørensen et al., 2016). A subsequent study carried out by Thorsen et al. (2018) has also demonstrated any association between serum 25(OH)D concentration in pregnancy and risk of type 1 diabetes in the child. In addition, Some evidences have not shown any association between neonatal 25(OH)D status and later risk of T1D (Mäkinen et al., 2019; Thorsen et al., 2018). However, a study by Tapia et al., (2019) has found that high 25(OH)D levels at birth may decrease T1D risk in children homozygous for the VDR rs11568820 G/G genotype.

Regarding the vitamin D intake and its status, lower levels of 25(OH)D are reported in patients with T1D compared to their matched controls (Nam et al., 2019; Shen et al., 2016).

Moreover, an inverse relation between the serum 25(OH)D concentration and the T1DM risk has been noted (Hou et al., 2021). Similary, both higher plasma concentration of 25(OH)D in childhood and vitamin D supplementation were associated with decreased risk of islet autoimmunity (Norris et al., 2018) and attenuation of natural history of T1D (Gregoriou et al., 2017), respectively, suggesting a protective role of vitamin D in this setting.

3.3.2.4 Obesity

The cases of type 1 diabetes rise parallely with rates of overweight / obesity (Craig et al., 2019). In fact, higher birth weight (Wang et al., 2021), weight gain in the first years of life (Magnus et al., 2018) , higher BMI (Ferrara et al., 2017) are all linked to increased risk of type 1 diabetes. Furthermore, paternal obesity and maternal obesity either before pregnancy (Magnus et al., 2018) or during pregnancy constitute a risk factor for the disease in the offspring .

Various mechanisms linking obesity and type 1 diabetes have been proposed. First, excess weight gain leads to insulin resistance. This latter along with rising blood glucose (glucotoxicity) accelerate beta cell apoptosis directly or indirectly (Rewers, 2012). The indirect way is mediated by increasing the demand for peripheral insulin thus leading to beta cell ER stress with consequent cytokine release (IFNα) and formation of neo-antigens (Brooks-Worrell & Palmer, 2019; Marroqui et al., 2017). Secondly, obesity induced low-grade inflammation is another mechanism that corresponds to the secretion of pro-inflammatory cytokines by stressed adipocytes undergoing apoptosis. This cytokines contribute to the release from islets cells of autoantigens and beta cell death (Buzzetti et al., 2020).

3.3.2.5 Viruses

Several data support the involvement of viral infections in the development of T1D. Viruses are known as potential inducing agents of islet autoimmunity and beta cell damage leading to insulin production deficiency hence to T1D (Principi et al., 2017). Likewise, they not only trigger islet autoimmunity but also accelerate disease onset in genetically susceptible individuals (Dunne et al., 2019). In addition, it should be highlighted that maternal viral infections during pregnancy constitute a risk factor for onset type 1 diabetes in children (Allen et al., 2018).

Mechanisms by which viruses lead to T1D are poorly understood, but various hypothesis have been suggested, such as molecular mimicry, inflammation, endoplasmic reticulum stress, bystander activation, and suppression of T cell (Dunne et al., 2019; Xia et al.,

2019). Although, it should be pointed that viruses can also protect the host against the incidence of T1D (Principi et al., 2017).

3.4 Cellular mechanism of autoimmune β-cell destruction

The precise mechanism underlying β-cell destruction is not fully defined. While, a possible chain of events is that β-cell specific autoreactive T cells initially evade negative selection within the thymus and reach the pancreatic lymph nodes. Then, it is speculated that the onset of an autoimmune reaction occurs after beta cell loss brought on by an enviromental agent. Subsequently, resident APC phagocytize these dying β-cellsprior to migrate to pancreatic lymph nodes, where they activate CD4+ T lymphocytes through the presentation of the processed β-cell peptides. In turn, the activated CD4+ Tcells allowthe activation of autoreactive CD8+Tlymphocytes which ultimately gain islets where they mediate β-celldestruction (DiMeglio et al., 2018; Sandor et al., 2019). It is well recognized that CD8+T cellsplay the primordial role in beta-cell destruction via different mechanisms including cytokine (IL-1, IFN-γ, TNF-α) production, perforin and granzyme release as well as direct contact with beta-cells via Fas-FasL interaction (Ichinose et al., 2007). A set of immune cells (macrophages, neutrophils and natural killer) that exerbate β-cell destructionare further recruted within the islets. This autoimmune attack is accentuated by Treg dysfunction which fail to inhibit the detrimental effect of these immune cells. In addition, B lymphocytes are also stimulated by $CD4^{+}$T cells in the pancreatic lymph nodes, however their contribution in the pathogenesis of T1D is thought to be minimal. Activated B cells secrete antibodies against β-cell proteins that are used as diagnostic markers for type 1 diabetes (DiMeglio et al., 2018). The development of atrophic inflammation within islets and their infiltration by immune cells occur months or even years before the clinical phase (Paschou et al., 2018).

4 Oxidative stress and type 1 diabetes

4.1 Reactive oxygen and nitrogen species: description and properties

The terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) are applied to both free radical and non-radical derivatives of oxygen and nitrogen, respectively (Liguori et al., 2018). Free radicals can be defined as any reactive chemical species (atoms or molecules) having one or more umpaired electron in their external orbital. This odd number of electron(s) confers to these entities some common properties including unstability, short life, and high reactivity. Free radicals attempt to donate or accept an electron from other molecules which they come in contact, thus acting as reductants or oxidants. While, the non-radical

derivatives does not possess any impaired electrons but they are able to trigger free radical reactions (Martemucci et al., 2022).

Table 1. Principal reactive nitrogen and oxygen species produced during metabolism (Martemucci et al., 2022)**.**

Reactive Oxygen Species (ROS)	
Radicals	**Non-Radicals**
Superoxide ($O_{2\cdot}$) Hydroxyl ($^{\bullet}OH$) Hydroperoxyl ($HO_2^{\bullet}$) Peroxyl ($ROO^{\bullet}$) Alkoxyl ($RO^{\bullet}$) Organic hydroperoxide (ROOH)	Hydrogen peroxide (H_2O_2) Ozone (O_3) Singlet oxygen (^1O_2Dg) Hypochlorous acid (HOCl) Peroxynitrite ($ONOO^-$)
Reactive Nitrogen Species (RNS)	
Radicals	**Non-Radicals**
Nitric oxide ($NO^{\bullet}$) Nitrogen dioxide ($NO_2^{\bullet}$) Nitrate radical ($NO3^{\bullet}$)	Nitrous acid (HNO_2) Nitrosonium cation (NO^+) Nitroxyl anion (NO^-) Peroxynitrite ($ONOO^-$) Dinitrogen trioxide (N_2O_3) Dinitrogen tetroxide (N_2O_4) Peroxynitrous acid(ONOOH) Nitryl chloride (NO_2Cl)

4.2 Sites and sources of RONS production

RONS can arise from both internal and external source (Pizzino et al., 2017). Certain of these sources are listed in the table 2.

4.3. Physiological role of reactive oxygen and nitrogen species

At low or moderate levels, both ROS and RNS are of crucial importance for human organism homeostasis, as they are involved in various physiological processes and serve as signaling molecules. The RNS released by endothelial cells are implicated in the regulation of numerous processes including: hemodynamics, thrombosis, vascular tone, angiogenesis, platelet aggregation, and relaxation and proliferation of vascular smooth cells. Similary, neurons and macrophages may also synthetize RNS that function as neurotransmitters and immunological mediators, respectively (Kumar & Pandey, 2015). In the other hand, ROS mediate the activation of redox-sensitive transcription factors, like AP-1, P53, NF-κB which regulate the expression of pro-inflammatory and certain cytokines as well as cell differenciation and apoptosis. Furthermore,ROS-induced activation of AP-1 and NF-κB

promote the destruction of invading pathogens.Besides,ROS are also essential in activating mitogen–activated protein kinases (MAPK) such ERK1/2,SAPK-JNK and p38involved in cell survival, proliferation and apoptosis (Bhattacharya, 2015; Burton & Jauniaux, 2011).

Table 2. Main sources of reactive nitrogen and oxygen species (Bhattacharya, 2015; Pizzino et al., 2017; Preiser, 2012)

Endogenous	Exogenous
• **Mitochondrial respiratory chain:** constitue the main source of ROS production. • **Xanthine oxidase** • **NADPH oxidase (NOX) family:** NOX2 in phagocytes (including neutrophils and macrophages), other types of NOX in various cell types (including lymphocytes, monocytes, fibroblastes and endothelial cells). • **Myeloperoxidase (MPO):** in neutrophils, monocytes, and eosinophiles. • **Nitric oxide synthetase (NOS):** nNOS in neurones, eNOS in epithelial cells, and iNOS in neutrophils, macrophages, endothelial cells, smooth muscle, and hepathocytes. • **Cytochrome P-450 oxidases:** present mainly in liver.	• Radiations. • Polluants. • Chemical solvents. • Tabacco. • Alcohol. • Heavy metals. • Foods such as smoked meats, fat, and waste oil. • Certain medications.

4.4. Antioxidant system

Antioxidants are compounds that can shield the body from oxidative damage. Based on their mechanism of action, we distinguished four subtypes: (a) Preventive antioxidants which inhibit radical species formation, (b) Radical scavenging antioxidants which block chain initiation, or prevent chain propagation reactions, (c) Repair or de novo antioxidants which repair or clear oxidized/damaged DNA, proteins, and lipids, (d) Adaptation-based antioxidants: in this category, the signal produced by free radicals triggers the formation and delivery of the adequate antioxidants to the proper location (Pisoschi et al., 2021). Based on their source, antioxidants are classified into two categories: endogenous, and exogenous. Exogenous antioxidants are received from diet and cannot be produced by the body. This category consist of trace elements (Se, Cu, Zn, Mn), vitamin E, vitamin C, and some phytochemicals. The endogenous antioxidants are produced within the organism. This

category comprises enzymes such as SOD, CAT, GPx, GR, non-enzymes like lipoic acid, glutathione, L-arginine, uric acid, bilirubin, melatonin, ubiquinone, as well as metal chelators like ferritin, lactoferrin, and caeruloplasmin (Martemucci et al., 2022; Pisoschi et al., 2021).

4.4.1. Superoxide dismutase (SOD)

Superoxide dismutases constitue a set of metalloenzymes that mediate the transformation of $O_2^{\cdot -}$ species into O_2 and H_2O_2 molecules via multiple oxidative and reductive cycles of metal ions (Cu, Zn, or Mn). According to the subcellular location and metal cofactor required for this reaction, three isoforms exist: cytoplasmic Cu, Zn-SOD (SOD1), mitochondrial Mn-SOD (SOD2) , and extracellular Cu, Zn-SOD (SOD3) (Sisein, 2014).

4.4.2. Catalase (CAT)

Peroxisomes contain catalase, an enzyme which reduces the production of hydroxyl radicals by breaking down H2O2 into water and oxygen molecules. This is important since excessive levels of hydrogen peroxide triggers HO • generation through Fenton and Haber-Weiss reactions. Additionaly, via the reduction of hydrogen peroxide, catalase mediates the detoxification of a number of compounds (Kumar & Pandey, 2015).

4.4.3. Glutathione peroxidase (GSH-Px)

The GSH-Px family is composed of two categories: the selenium-dependant GPx and the selenium-independent ones. Human have at least four distinct selemium-dependant GPx with different subcellular location: GPx1 (in the cytosol), GPx2 (in the gastrointestinal system), GPx3 (in the mitochondria and extracellular space), GPx4 (in membranes). Using GSH as a substrate, these GSH-Px catalyze the reduction of hydrogen peroxide and organic peroxides to water and alcohol, respectively (Kumar & Pandey, 2015; Sisein, 2014).

4.4.4. Glutathione Reductase (GR)

Glutathione Reductase is cytoplasmic enzyme responsible for the preservation of cellular GSH level. Glutathione Reductase uses flavin adenine dinucleotide (FAD) as a cofactor to reduce GSSH in the presence of NADPH, thereby promoting GSH regeneration (Sisein, 2014).

4.4.5. Glutathione

Glutathione is a thiol tripeptide that consist of cysteine, glutamate, and glycine residues. Glutathione is endougenously synthesized and exists in two forms: the reduced form, which is the predominant one, GSH, and the oxized form, GSSH. It is found in the nucleu, mitochondria, and cytoplasm where it primarly serve as an antioxidant. GSH exerts its

antioxidant activity via direct reaction of reactive oxygen species or indirectly via some detoxifying enzymes, protection of protein cystein residues from oxidation, repair of oxidatively damaged biomolecules, and renewal of other antioxidants like vitamin C and vitamin E (Sisein, 2014).

4.4.6. Vitamin C

Vitamin C also recognized as ascorbic acid is an hydrophilic vitamin with antioxidant properties. It exhibits its antioxidant function by giving an electron to lipid radicals to block lipid peroxidation chain reaction. Besides, it scavenge free radicals in a synergetic manner with vitamin E. Furthermore, vitamin C has the ability to recycle vitamin E to its reduced form (Nimse & Pal, 2015; Sailaja Rao et al., 2011).

4.4.7. Vitamin E

Alpha-tocopherol is the most active steroisomer of vitamin E in humans. This fat-soluble molecules functions mainly as an antioxidant that protects the cell membrane via the breakage of the lipid peroxidation chain. During this process, α-tocopherol scavenges peroxyl radicals generating tocopheroxyl, a relatively stable radical (Nimse & Pal, 2015).

4.5. Oxidative stress concept

The term “oxidative stress” describes a pathological state in which the balance between pro-oxidants and anti-oxidants is disturbed in favor of the former.This condition arises from an excess in free radical production or a decline in anti-oxidants levels, enabling free radicals to assault biomolecules and cause cell damage (Pisoschi & Pop, 2015).

4.6. Detrimental effects of Free Radicals on human health

As stated above, the excessive RONS gives rise to a status referred to us oxidative stress. This detrimental phenomenon induces severe oxidative damages in the main cellular macromolecules, such as proteins, DNA, and lipids. The byproducts of RONS attacks can serve as oxidative stress indicators (Liguori et al., 2018).

4.6.1. Lipids

Free radicals have the ability to cause lipid peroxidation in both cellular and organelar membranes when their concentration exceeds the homeostastic threshold. The unsaturated fatty acids are particulary susceptible to oxidation, resulting in generation of several type of harmful byproducts depending on the kind of UFAs being oxidized.The major lipoperoxidation products are 4-HNE, MDA, oxylipins, isoprostanes, and oxysterols. This process of lipoperoxidation distrupts the structure and fluidity of membranes and inactivates

membrane-bound receptors and enzymes, thus altering normal cellular functioning (Ghosh et al., 2018; Juan et al., 2021).

4.6.2. Protein

Proteins can also altered by free radicals in three distinct manners: by oxidizing particular amino acid residues, cleaving peptide chains, or conjugation with lipid-peroxidation products like MDA and 4-HNE to generate protein cross-linkage. Protein composition, structure, hydrophobicity/ hydrophibicity, charge, and folding are all affected as a resut of these oxidative modifications, which induce proteins to lose their ability to act as enzymes, receptors, membrane transporters, and many other biological fuctions (Aranda-Rivera et al., 2022; Mohammed et al., 2015). Moreover, these modified proteins are more vulnerable to proteolysis. Additionally, protein oxidation can also yield reactive byproducts like protein hydroperoxides, which can exerbate oxidative stress through producing more free radicals (Sharma, 2014).

4.6.3. DNA

Excessive ROS can also affect DNA structure through desoxyribose oxidation, nucleotide modification, and strand DNA breakage. These structural alterationsyield numerous mutations and DNA-protein crosslinks having as outcomes dysfunction or inactivation of proteins encoded by this genome. The primary mutagenic lesions issues from oxidative damage to DNA are: 2-hydroxyadenine, 8-oxoadenine, 5- hydroxycytosine, cytosine glycol, thymine, glycerol and 8′-hydroxy-2′-deoxyguanosine (Ghosh et al., 2018; Liguori et al., 2018). Indeed, mtDNA is more prone to oxidative damage due to its histone-free circular structure and its locationin mitochondria, which is the primary generator of ROS. Since mitochondria play a key part in apoptosis, oxidatively induced mutations in mtDNA may lead to a variety of disorders and diseases (Zhang et al., 2011).

4.7. Oxidative stress and type 1 diabetes

Numerous investigations have demonstrated the link between type 1 diabetes and oxidative stress El-Din Elshalkami et al., 2022). This connection is clearly evidenced by the altered oxidant / antioxidant balancein several studies performed on type 1 diabetes mellitus patients. A study carried out by Alghazeer et al.(2018) revealed a marked rise in lipid peroxidation which coincided with a significant decline in antioxidant capacity (SOD, CAT, GPx).This is corroborated by another recent study that found similar results along with a significant elevation of the pro-oxidant enzyme xanthine oxydase (Sobhi et al.,

2021).Oxidative stress has been suggested to be implicated in pancreatic β-cell destruction (Delmastro & Piganelli, 2011). β-cells are particularly more susceptible to the detrimental effects of oxidative stress due totheir limited antioxydant system and high endogenous generation of reactive oxygen species (Gurgul-Convey et al., 2016). The pathogenic effect of oxidative stress on beta cell is mediated to a large extentthrough the alteration of the key pathways essential for beta cell survival. Specifically, it induces the activation of AMP-activated protein kinase (AMPK) and c-Jun N-terminal kinase (JNK) while inhibiting mammalian target of rapamycin (mTOR). This ultimately leads to several downstream effects including beta cell apoptosis and beta cell mass decline. Furthermore, several oxidative stress inducers have been identified. First off, under diabetic conditions, the most prevalent feature of the disease, hyperglycemia, causes the production of ROS and their accumulation through various pathways including the mitochondrial electron transport chain (ETC), oxidative phosphorylation, polyol pathway activation, hexosamine pathway activation, increased intracellular advanced glycation end product formation, and protein kinase C(PKC) activation. Additionally, mitochondrial dysfunctionis another factor which provoks a state of oxidative stress via the direct induction of ROS production or through the induction of ER stress which ultimately aggravate ROS production (Eguchi et al., 2021).

5. Neutrophils and type 1 diabetes

5.3. Neutrophil life cycle

Neutrophils are a subtype of granulocytes that are generated in the bone marrow by granulopoisis. This process begins with the development of hematopoietic stem cells (HSCs) into multipotent precursors (MPPs) and subsequent differenciation stages: lymphomyeloids (LMPs), granulocyte-monocytes precursors(GMPs), myeloblasts, promyelocytes, myelocytes, metamyelocytes, band cells, and mature neutrophils (Bardoel et al., 2014).

Under steady state, a rate of approximately 10^{11} is produced every day. After their maturation, neutrophils leave the bone marrow to reach the bloodstream. These neutrophils joins either the circulating pool or the marginated pool. The first compartment is made up of free-flowing neutrophils, while the second one consists of neutrophils adhered to the capillary endotheluim commonly found in the lung, liver, and spleen. Under inflammatory conditions, these neutrophils exit circulation towards the inflammed tissue. The egress of neutrophils is strictly regulated by G-CSF, which promotes neutrophil release through downregulation of SDF-1 and VCAM-1on BM-stromal cells and BM-endothelial cells, respectively. In addition,

G-CSF favor the exxpression of the CXCR2 ligands in BM-endothelial cells. All these G-CSF effects distrupt the CXCRR4/CXCL12 and VLA-4/VCAM-1 interactions that are normally required for neutrophil retention in th bone marrow. Additionally, other factors like LTB4, C5a, and CXCL8 can also stimulate neutrophil realease upon inflammatory conditions (Bekkering, 2013). Neutrophils typially have a short half-life in the bloodstream. Thus, senescent circulating neutrophils upregulate CXCR4, which promotes their homing to the bone marrow, where they undergo apoptosis and subsequent clearance by stromal macrophages (Summers et al., 2010). Similary, the removal of apoptotic circulating neutrophils by reticular endothelial macrophages can also occur in the liver and spleen (Furze & Rankin, 2008).

5.4. Overview of neutrophil function: physiological and pathological role

Neutrophils, the most prevalent leukocytes in the bloodstream, constitue an important player in the first line of immune defense. They mediate the removal of invasive pathogens through a variety of processes, including: (1) pathogen phagocytosis and subsequent degradation inside the phagolysosome via ROS generation, (2) The release of antimicrobial peptides like MPO and elastase, (3) The release of NETs , structures with decondensed chromatin linked to granular enzymes, which sequester and neutralize invading pathogens (Njeim et al., 2020).

Beyond their pivotal role in innate immune defense, neutrophils may also modulate the adaptative immune response via the direction of both humoral and cellular immunity. Indeed, during inflammation, neutrophils migrate to the secondary lymphoid organs, where they deliver the phagocytic antigens to resident APCs (DCs and macrophages) or acquire features of APC by themeselves and interact directely with TCD4+. Analogously, neutrophils induce naïve TCD8+activation throughMHC-I-Ag presentation. Conversly, neutrophils can also supress T cell functions through the release of Arg-1 as well as the induction of Treg lymphocyte expansion. On the other hand, these neutrophils produce several cytokines like BAFF and APRIL that promote B cell development and differenciation. Furthermore, neutrophils induce IgG and IgA production in T-lymphocyte independent manner (Rosales, 2020).

5.5. Implication of neutrophils in T1D pathogenesis

The involvement of neutrophils in type 1 diabetes pathogenesis has been largely established. These cells play a prominent role in the different stages of this autoimmune

disease. Studies have revealed a decrease in the number of circulating neutrophils, which might be attributed to their infiltration and sequestration in pancreatic tissue. Importantly,T1D diabetic patients have displayed several features that may partially explain the mechanism by which neutrophils contribute in pancreatic beta cell autoimmunity. Firstly, it has been found that the peripheral protein level and activities of both NE and PR3 were dramatically raised, and that this rise was positively correlated with higher seropositivity of autoantibodies directed against β-cell antigens. Parallelly, there was an important increase in circulating MPO-DNA complexes. This evidence supports the role of NETs in beta cell death (Njeim et al., 2020). NETs are web-like structures extruded by neutrophils and are made up of decondensed chromatin associated with granular proteins. These structures are implicated in the neutralization of infections. Their aberrant function causes damage to the host tissue, leading to pathological immune-mediated conditions (Petrelli et al., 2022). Besides, neutrophil-released NE and PR3 proteins trigger TLR expression and activation, which are crucial mediators of insulitis and pancreatic β-cell destruction. These proteins can also contribute to T1D pathogenesis via the recruitment of neutrophils to the inflammatory site as well as the release of the cytokines such as IL-18, IFN-α, and IL-1β (Delgado-Rizo et al., 2017).

6. Theoretical rationale for the use of nicotinamide and curcumin in type 1 diabetes

6.1. Nicotinamide:

Nicotinamide is the amide form of vitamin B3 (niacin). It serves as a direct precursor to nicotinamide adenine dinucleotide (NAD+) and its phosphorylated form $NADP^+$ (Bogan & Brenner, 2008). In cells, nicotinamide supports NAD^+-dependent cellular processes including DNA repair via PARP and sirtuins, regulation of gene expression, antioxidant defense through NADPH regeneration, and mitochondrial function, thereby limiting oxidative damage and inflammation (Belenky et al., 2007; Cantó et al., 2015; Verdin, 2015).

Pancreatic β-cells undergo accelerated degradation in type 1 diabetes (T1D) as a consequence of autoimmune-mediated inflammation and oxidative stress, which induce DNA strand breaks and excessive activation of poly(ADP-ribose) polymerase-1 (PARP-1) (Burkart et al., 1999; Pieper et al., 1999). Persistent PARP activation leads to a marked depletion of intracellular NAD^+ pools, thereby impairing glycolysis, mitochondrial function, ATP production, and overall cell survival, ultimately amplifying β-cell apoptosis (Evans et al., 2002; Ying, 2008). Nicotinamide, a direct precursor of NAD^+, has been shown to preserve intracellular NAD^+ levels by limiting PARP-mediated consumption and by modulating

inflammatory and apoptotic pathways, providing a strong theoretical rationale for its therapeutic exploration in T1D (Kolb & Burmester, 1999).

6.1. Curcumin:

Type 1 diabetes is characterized by a complex interplay between oxidative stress, endoplasmic reticulum (ER) stress, and chronic inflammation, processes that collectively contribute to β-cell dysfunction and tissue damage (Evans et al., 2002; Donath & Shoelson, 2011). Neutrophils play a pivotal role in this inflammatory milieu, as their excessive activation promotes the overproduction of reactive oxygen species and the release of pro-inflammatory mediators, thereby amplifying cellular stress and injury (Donath & Shoelson, 2011). The choice of curcumin is based on its pleiotropic biological properties, which enable it to simultaneously target several key mechanisms involved in the pathophysiology of type 1 diabetes. Curcumin has been shown to effectively attenuate oxidative stress, modulate ER stress–associated signaling pathways, particularly those involving ATF4 and CHOP, and inhibit NF-κB activation, a central regulator of inflammatory responses (Hotamisligil, 2010; Aggarwal & Harikumar, 2009). Moreover, curcumin exerts immunomodulatory effects on neutrophils by limiting their excessive activation and inflammatory potential, thereby contributing to the preservation of cellular homeostasis (Menegazzi et al., 2016). The simultaneous targeting of oxidative stress, ER stress, and neutrophil-mediated inflammation confers curcumin a strong therapeutic relevance in the context of type 1 diabetes, thus fully justifying its use in the present study.

Material and Methods

1 Biological Material

1.1. Animals

Healthy female albino BALB/C mice were housed at the Animal Facility of Research Center of Biotechnology (CRBT) under controlled conditions with 12h light/ 12h dark schedule. Animals were kept in wire bottom cages with freely access to tap water and standard mice food (laboratory chow). Mice bedding (wood chips) in the cages was changedtwice a week. These mice, aged 10 weeks and weighing 28g were then used inexperiments. Animal care and handling were conducted following to the criteria and principles stated in the "Guidance document on the recognition, evaluationand use of clinical signs as human parameters for laboratory animals used in safety assessment"publishedby the Organization for Economic Cooperation and Development (OECD, Paris, 2000).

1.2. NIT-1 cell line

The NIT1 cell line (CRL-2055™) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and provided by BDcome, Algeria.

1.3. Human blood

Peripheral blood for neutrophil isolation was obtained via venipuncture from the antecubital vein of healthy, non-smoking adult volunteers who had not taken any medication for at least 15 days prior to sample collection.

2. Methods

2.1. Evaluation of molecules effects on mice pancreatic islets

2.1.1. Isolation and culture of mice pancreatic islets

Pancreatic islets were isolated from female albino Balb/c mice using the protocol described by Velasco et al. (2018), with minor modifications. All dissection tools were sterilized, and the working area was disinfected prior to the procedure. Mice were anesthetized with inhalation of diethyl ether, and the abdominal cavity was surgically opened. The pancreas was then perfused through the common bile duct with 5 mL of collagenase P solution (0.3 mg/mL) prepared in Hank's Balanced Salt Solution (HBSS).

Following perfusion, the swollen pancreas was excised and minced into ~2 mm fragments in ice-cold collagenase P solution. The tissue suspension was transferred into a conical tube and incubated in a 37°C water bath with gentle shaking for 5–10 minutes to facilitate enzymatic digestion. To terminate digestion, ice-cold supplemented HBSS(Sigma-Aldrich) was added, and the mixture was centrifuged at 185 × g for 5 minutes at 10°C. The resulting pellet was washed twice with ice-cold supplemented HBSS.

The final pellet was resuspended in HBSS, and the islets were manually isolated from remaining tissue debris under an inverted microscope using a Pasteur pipette. The purified islets were centrifuged again at 185 × g for 3 minutes at 10°C to remove residual HBSS. Finally, the islet pellet was resuspended and cultured in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS)(Sigma-Aldrich) and 1% penicillin-streptomycin(Sigma-Aldrich)for primary culture.

2.1.2. Determination of mice islet cell viability

After getting the mice islets of Langerhans, the latter were promptly suspended within the RPMI-1640 culture medium. This suspension was then afterwards distributed on a microplate and kept up for 24 hours in an incubator (The New Brunswick Innova® 42 incubator shaker) with 5 % O_2 and 95% CO_2 gases. The molecules (STZ(Sigma-Aldrich), curcumin(Sigma-Aldrich), and nicotinamide(Sigma-Aldrich)) whose impact on the viability of these islets is to be tested was included according to the following microplaque division: (a) control group, islets were kept untreated in their media; (b) STZ group, islets were incubated with streptozotocin 0.5 mM for 24h;(c) Cur group, islets were treated with curcumin 60 µg/ml for 48h; (d) Nc group, islets were incubated with nicotinamide 60 µg/ml for 48h;(e) STZ combined with Cur treatment group, islets were pre-treated with 60 µg/ml curcumin for 24h then 0.5 mM STZ for further 24h; (f) STZ combined with Nc treatment group, islets were pre-treated with 60 µg/ml nicotinamide for 24h then 0.5 Mm STZ for further 24h. The culture medium was subsequently thrown away whereas the islets are subjected to enzymatic digestion with trypsin(Sigma-Aldrich)-EDTA 0. 25% for 5 min. The activity of trypsin is then repressed by including a volume of RPMI-1640 prior to a centrifugation for 5 min to recoup a scattered pancreatic cell pellet. This pellet was at that point solubilized in RPMI-1640 medium. Hence, to assess the viability of these treated islets against an untreated control, a volume of this suspension is blended with a same volume of trypan blue 4%. Consequently, a drop of this blend is transferred to an hemocytometer

permitting the counting of the stained dead cells and the unstained living cells by applying the formula of the used hemocytometer (Velasco et al., 2018).

2.1.3. Determination of insulin secretion from isolated islets

To evaluate the effect of our tested molecules (curcumin and nicotinamide) on the amount of secreted insulin, the isolated islets were incubated in a microtitration plate with the tested molecules (60 µg/ml) in supplemented RPMI-1640 in a humidified atmosphere gassed with 95% CO_2 and O_2% for 24h. After that, to promote insulin secretion, the media was pourred off while the islet cells was re-incubated for 2h with DMEM low-glucose (1000 mg/l) media (Sigma-Aldrich). Next, the later media was also discarded and replaced by DMEM high-glucose (4500 mg/l) media(Sigma-Aldrich). After a final incubation of two other hours, aliquots of media were finaly harvested to quantify insulin hormone using an ELISA kit (Invitrogen). Aliquots should be conserved at -80 °C untill the insulin quantification assay.

2.2. Evaluation of molecules effects on NIT-1 cell line

2.2.1. Culture and maintenance of NIT-1 cell line

NIT-1 cell line was cultured in a T-75 flask and allowed to grow in RPMI-1640 culure media enriched with 10% FBS and 1% penicillin/streptomycin. The flask was kept at 37°C in an incubator (The New Brunswick Innova® 42 incubator shaker)gassed with 5% CO_2 and 95% O_2. The growth media was replaced by a fresh one every 2 days in order to eliminate the floating dead cells and supply necessary nutrient. Once the confluence reached 80%, a sub-culture was performed. For this, a single-cell suspension was produced by removing the cultured media, washing with PBS (1X) and reincubating the adherent cells with trypsin solution 0.25% EDTA for 3 to 5 min. After cell detachment, the trypsin action was stopped by addition of the RPMI-1640 medium containing fetal bovine serum (FBS) as inhibitor of trypsin. The resultant cell suspension was transferred to a conical tube and centrifuged for 5min at 900 rpm and 10°C. The supernatant was discarded while the cell pellet was suspendedin a supplemented RPMI-1640 media to the appropriate seeding concentration and used for different assays. All cell culture manipulations were carried out in sterile conditions.

2.2.2. Determination of NIT-1 viability

To evaluate the effect of the studied molecules (STZ, curcumin, and nicotinamide) on NIT-1 cell viability, the XTT kit was used. For this assay, NIT-1cell was cultured in 96-well plate at cell density of $1*10^5$ cell/ml in RPMI-1640 media. The plate was then kept at 37°C in a humidified incubator gassed with 5% CO_2 and 95% O_2 and the cells were given 24h to

adhere to the plate floor. Following period of incubation, NIT-1cells were divided into six distinct groups: (a) control group, cells were kept untreated in their media; (b) STZ group, cells were incubated with streptozotocin 3 mM for 24h; (c) Cur group, cells were treated with curcumin 60µg/ml for 72h; (d) Nc group, cells were incubated with nicotinamide 60 µg/ml for 72h ; (e) STZ combined with Cur treatment group, cells were pre-treatedwith 60µg/ml curcumin for 24h then 3Mm STZ for further 24h ; (f) STZ combined with Nc treatment group, cells were pre-treated with 60 µg/ml nicotinamide for 24h then 3Mm STZ for further 24h. Subsequently, the media containing tested molecules was thrown out. Afterward, adherent cells were rinsed with PBS (1X) and a fresh media was added. Next, the XTT solution (Sigma-Aldrich)was added to each well and permitted to re-incubate in the dark for 4 hours. This incubation allow the transformation of tetrazoluim salt XTT by viable cells to an orange formazan compound. Finally, the optical density of the formed dye was spectrophotometrically measured at a wavelength of 490 nm with a reference wavelenght of 660 nm. The number of viable cells was expressed as proportion compared to the negative control, which was considered to be 100%.

2.2.3. Measurement of reactive oxygen species production in NIT-1 cells

The level of ROS generation in NIT-1 cells was estimated using the oxidative-sensitive dye, H_2DCFDA(Sigma-Aldrich), as a ROS revealer. NIT-1 cells (at a density of $1x10^5$cell/ml) were first grown in the RPMI-1640 media on culture black multiwell plate. Cells were divided into the same groups described earlier for the viability assay. After that, cells were washed with PBS then loaded in PBS containing H_2 DCFDA (20µM) for 45min at 37° C protected from light. Ending this period, cells were rinsed twice with PBS, resuspended in 100 µl PBS (1X) and analyzed for DCF fluorescence (ʎex=485, ʎem=530) using a fluorescence multiwell plate reader. The results were expressed as the fold-increase in fluorescence compared to untreated cells or as percentage of the untreated cells. For microscopic visualization, the experiment was carried out as indicated earlier but in a transparent culture multiwell plate then the fluorescent images of NIT-1 were taken by Nikon inverted microscope using Nikon NIS Elements software.

2.2.4. Measurement of NIT-1 cell oxidative stress markers

As previously described, NIT-1 cells were plated at a density of $1x10^5$ cell/ml and incubated in 5% CO_2 humidified incubator at 37 $\overset{o}{C}$ in order to allow their adhesion and growth. After a period of 24 hours, the microtitration plate was divided into four conditions: (a) control group, cells were maintained untreated in their media for 24h; (b) STZ group, cells

were incubated with streptozotocin 3 mM for 24h; (c) Cur 30 group, cells were incubated with curcumin 30µg/ml for 24h; (d) Cur 60 group, cells were incubated with curcumin 60µg/ml for 24h. Subsequently, the supernatant of NIT-1 cells was then harvested and the oxidative stress markers were assayed**.**

2.2.4.1. Estimation of xanthine oxidase (XO) activity

The estimation of xanthine oxidase activity was performed following the modified procedure established by (Bergmeyer et al., 1974), which involves the measurement of uric acid productionat 290nm that results from xanthine oxidation in the presence of xanthine oxidase source (sample). The assay mixture comprises 666 µl potassium phosphate buffer (50 mM, pH=7.5), 333 µl xanthine (0.15 mM), and 33 µl of the sample. The production of uric acid is tracked by recording the change in absorbance at 290 nm over 1 minute against a blank that includes all the reagents except the sample, which was substituted with distilled water.The enzymatic activity of xanthine oxidase was determined using the formula below:

$$\textbf{Units/ml enzyme} = \frac{(\Delta\ \textbf{A290nm/min sample} - \Delta\textbf{A290nm/min Blank})\ (\textbf{3})}{(\textbf{12.2})\ (\textbf{0.033})}$$

Where, **3** = Total volume (in millilitres) of assay.

12.2 = Millimolar extinction coefficient of uric acid at 290nm.

0.033 = Volume (in millilitres) of NIT-1 supernatant.

2.2.4.2. Estimation of superoxide dismutase (SOD) activity

SOD activity was also deteminated in the NIT-1 supernatant following the method of (Subhangkar & Himadri, 2012) which implies the monitoring of pyrogallol autoxidation in presence of sample as a source of SOD. The reaction mixture is composed of 664 ul of Tris-EDTA buffer (Tris 50Mm pH=8; EDTA 10Mm), 25 µl of pyrogallol and 11 µl of sample. A blanc reaction without the sample was also prepared in the same conditions. The reaction was then photometrically monitored for 1 min at a wavelength of 420 nm.

2.2.4.3. Determination of Malondialdehyde (MDA) level

Membrane lipid peroxidation was also evaluated for the same supernatant by measuring the MDA level. The colorimetric method of Ohkawa et al. (1979) was followed to determine the MDA concentration. The principle of this method is based on the quantification of pink-colored complex, MDA–TBA2, by spectrophotometric reading at 532 nm. The reaction

mixture witch contain 125 µl sample, 125 µl TCA 20% and 250 µl TBA 0.67% was kept for 15 min in a boiling water, and then cooled to room temperature. Thereafter, 1 ml of n-butanol was added and the whole mixture was spun 3000 rpm/min x 15 min. Subsequently, the optical absorption was readed at 532 nm and used to estimate the MDA level.

2.2.4.4. Determination of Reduced gluthathione (GSH) level

Reduced gluthathione rate in the supernatant was estimated according to the method of Ellman (1959) based on the reaction between GSH and DTNB to form a yellow product, the thionitrobenzoic acid. Briefly, the assay was performed by mixing 20 µl of DTNB with 3 ml of the diluted sample « 25 µl of sample diluted with 5 ml of phosphte buffer pH=8». After incubation of 5 minutes at room temperature, the absorbance was measured spectrophotometrically at 412 nm against a blanc prepared with TCA 10 %. Exploiting the exintinction molar coefficient 14150 M^{-1} cm^{-1}, the GSH amount was calculted and the results were expressed as nmol/ml.

2.2.5. Measurement of NF-κB 1, ATF4, and CHOP expression

2.2.5.1. Total RNA extraction from cultured NIT-1 cells

To determine differences in gene level expression, NIT-1 cells were seeded in a microtitration plate at a density of $1*10^5$ cell/ml for 24h allowing cell adhesion. Cells were then treated with tested molecules: (a) Control untreated cells; (b) STZ (3 mM)-treated cells for 24h; (c) Cur (60 µg/ml)-treated cells for 24h, (d) Nc (60µg/ml)- treated cells for 24h; (e) Cells pre-treated with 60 µg/ml curcumin for 24h then 3 mM STZ for further 24h; (f) Cells pre-treated with 60 µg/ml nicotinamide for 24h then 3 mM STZ for further 24h.

After removing the culture media from all wells, total RNA extraction from NIT-1 cells was carried out using NucleoZol reagent (Macherey-Nagel) following the procedure described by the manufacturer. In brief, 1ml of NucleoZol was introduced into each well. This step must be accompanied by a strong mixing allowing cell lysis as well as the release of proteins from nucleic acids. This homogenate was allowed to incubate for 5 min at room temperature before being transferred to the Eppendorf tubes containing 200µl of chloroform. Subsequently, the mixture was robustly agitated for 15 seconds and left to incubate for further 3 min at room temperature. Then, a centrifugation of the mixture for 15 min (12,000 g and 4 °C) permit the formation of three layers: the lower layer contains phenol-chloroform, the intermediate layer contains proteins and the upper aqueous layer contains RNA molecules. The top layer was cautiously aspirated, transferred to the Eppendorf tubes containing 500 µl of isopropanol, and then the mixture was vigorously stirred and incubated for 10 min at room

temperature before being centrifuged for 10 min (12,000 g and 4 °C). The resultant supernatent was removed while the formed pellet was washed twice with cold ethanol 75% and centrifuged for 5min (7500g and 4 °C) to recuperate the RNA pellet. This latter was finally dried in a centrifugal concentrator (Eppendorf Concentrator plus) at 45°C for 15 min then solubilized in 20 µl of DEPC-treated water and re-incubated for 10 min at 55°C in an thermoMixer (Eppendorf).

2.2.5.2. Determination of RNA concentration and purity

The amount of isolated RNA as well as its purity was determinated by spectrophotometric measurement using the NanoDrop-8000 spectophotometer (Thermo Fisher Scientific). After cleaning the NanoDrop measurement areas with distilled water, a blank measurement was set using the DEPC-treated water, a solution in which RNA was solubilized. Then, 2 µl of the sample was put on the NanoDrop's measurement area and the absorbance was measured at 260 nm and 280 nm. Samples with a A260/A280 ratio between 1.8 and 2 and a A260/A230 ratio between 2 and 2.2 indicate the low quantity of protein and salt contaminants, respectively. Consequently, these RNA samples were directly used for the cDNA synthesis assay or kept at-80 °C for further use.

2.2.5.3. Digestion of contaminating DNA and RNA purification

Removal of residual DNA from isolated RNA was carried out by adding 5µl of DNase I (Sigma-Aldrich) solution to each RNA sample (30µl) with gentle mixing of the DNase I reaction tube. After an incubation of 30 min at 37°C, the activity of DNase I was stopped by adding 3µl of EDTA (0.5 M, pH= 8) with further incubation at 55 °C for 10 min. The RNA was then precipitated by adding 500 µl of isopropanol and 5µl of sodium acetate solution (3M) with an incubation of two hours at -20 °C. A centrifugation of 15 min (13000g, 4°C) was subsequently performed to discard supernatant. The obtained RNA pellet was rinsed with ice-cold ethanol 100% then spun for 10 min (13000g, 4°C) to remove ethanol. The purified RNA was ultimately dried for 15 min at 45 °C before suspending on 20 µl of DEPC-treated water. The prepared RNA was allowed to incubate for 10 min at 55 °C.

2.2.5.4. Reverse transcription and quantitative RT-PCR

Relative quantification of target gene expression was done in two steps. First, the extracted total RNA with high purity (A260/280) serves as a template in the reverse transcription reaction in which the first stranded cDNA was synthesized using the SuperScript® IV Reverse Transcriptase (Thermo Fisher Scientific). The reaction was run on

the SimpliAmp Thermal Cycler(Applied Biosystems/ Thermo fischer scientific). According to the manufacturer's instructions, RNA template was firstly annealed with 1 ml random hexamers (50 µM) in the reaction mixture containing dNTP mix (10 mM each) and completed by DEPC-treated water in a final volume of 13 ml. The mixture was subsequently heated for 5 min at 65 °C then cooled in ice for 1 min. Then, to perform extension, a mixture of 1ml SuperScript® IV Reverse Transcriptase (200 U/µL), 1ml RNaseOUT™ Recombinant RNase Inhibitor (40 U/µL), 1ml DTT (100 mM), and 4 ml 5× SSIV Buffer was added to first mixture and incubated for 10 min at 50-55°C.

The produced cDNA was subjected to the amplification by quantitative RT-PCR which was performed in 96-well plate with Applied Biosystems Quant Studio 5Real-Time PCR System (Applied Biosystems /Thermofisher scientific)using PowerUp™ SYBR™ Green PCR Master Mix kit (Thermo Fisher Scientific). Each reaction mixture was prepared in a final volume of 10 µl containing 5 µl of PowerUp SYBR Green Master Mix (2X), 10 ng cDNA, and 0.5 µM each specific primer. Primer sequences used for gene expression quantification are listed in the table 3. In this experiment, each condition was repeated thrice. β-actin was used as internal gene for normalization. The relative expression levels of target gene was estimated following the $2^{-\Delta\Delta CT}$ method.

ΔCT= CT (target gene)- CT (internal gene)

ΔΔCT= ΔCT (target sample)- ΔCT (control sample)

Table 3. Primers for qPCR analysis

Gene	Primers
β-actin	Forward: 5'-CATTGCTGACAGGATGCAGAAGG-3' Reverse: 5'-TGCTGGAAGGTGGACAGTGAGG-3'
ATF-4	Forward: 5′- CCCCCTTCGACCAGTCGGGT-3′ Reverse: 5′-CCGCCTTGTCGCTGGAGAACC-3′
CHOP	Forward: 5′-GTCCCTAGCTTGGCTGACAGA-3′ Reverse: 5′-TGGAGAGCGAGGGCTTTG-3′
NF-κB1	Forward: 5'-GCTGCCAAAGAAGGACACGACA-3' Reverse: 5'-GGCAGGCTATTGCTCATCACAG-3'

2.2.6. In silico study

To investigate the potential interactions between curcumin and genes involved in different processes inculding ER stress-induced apoptosis, the oxidative stress, and inflammation, a milti-step computational study was conducted using the Gene Ontology (GO) database, the STITCH database, GENMANIA, protein- protein interaction network (PPI), and the the string database (figure 4).

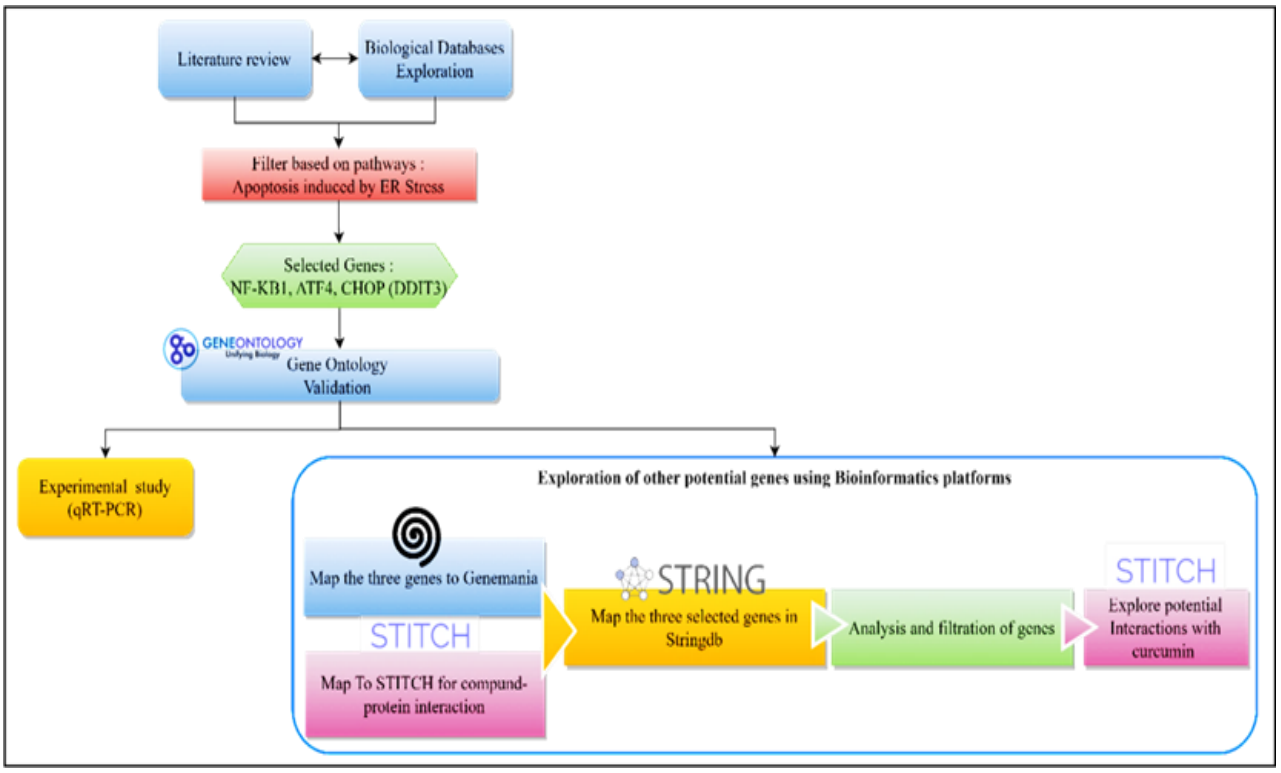

Figure 4. Workflow of the impact of curcumin in β-cell genes expression

2.2.7. Determination of insulin secretion from NIT-1 cells

NIT-1 cells at a density of $1x10^5$ cell/well were seeded in a culture multiwell plate for 24h, then exposed to molecules under study (curcumin 60 µg/ml, nicotinamide 60 µ/ml) for a further 24h. All incubations were carried out at 37°C in a humidified chamber with 5% CO_2 and 95 O_2%. At the end of this period, insulin secretion was stimulated with both low-glucose and high-glucose DMEM media as was earlier stated. Ultimately, the recovered media was then used for the dosage of this hormone using the sandwich ELISA kit.

2.3. Evaluation of molecules effects on neutrophils

2.3.1. Neutrophil isolation

Ten milliliters of blood were sampled from each healthy, non-smoker, non-diabetic donor. Blood samples were collected in centrifugation tubes containing lithium heparinate (5U/ml) as an anticoagulant. Erythrocytes were sedimented for one hour at room temperature using 2 ml of

dextran (10%). The collected leukocyte-enriched supernatant was centrifuged (400 g; 4 °C) for 25min on histopaque gradient to sediment neutrophils. Residual red blood cells in the neutrophilic pellet were eliminated through hypotonic lysis. This was achieved by adding 1 ml of cold, fresh distilled water to the suspension and mixing it gently for 30 seconds. Afterward, 5 ml of Ca^{2+} and Mg^{2+}-free HBSS media was added. The cell suspension was then centrifugated for 10min (400 g; 4 °C). This washing step was repeated until total removal of erythrocytes. The obtained neutrophilic pellet is then resuspended in 1ml of cold Ca^{+2} and Mg^{+2}-free HBSS1 media. The neutrophil number is subsequently determined by counting cells under the light microscope after cell coloration with Turk solution (Bouriche et al., 2016).

2.3.2. Determination of neutrophil viability

The effect of curcumin and nicotinamide on neutrophil viability was determined by a trypanblue exclusion test according to the protocol of Lucisano-Valim et al. (2002). Initially, 250 µl of neutrophil suspension was incubated for 10 minutes at 37° C with different concentrations of curcumin and nicotinamide. Then, an equal volume of trypan blue was added to both the control and the treated suspensions. Viable (non-stained) and non-viable (stained) cells were counted under a light microscope, which allowed for the calculation of the neutrophil viability rate using the following equation:

$$\textbf{Viability}(\%) = \frac{\textbf{Non} - \textbf{stained cell number}}{\textbf{Total cell number}} \times \textbf{100}$$

2.3.3. Determination of myeloperoxidase activity

The effect of curcumin and nicotinamide on MPO activity was assayed according to Wanikiat and its collaborators' method (2008). To extract the MPO enzyme, the isolated neutrophils were suspended in HBSS2 and fixed at a concentration of $5{,}5x10^6$ cells/ml. The suspension was then incubated at 37°C for 15 min with fMLP and cytochalasin B (10^{-6}/10^{-5}M) followed by centrifugation of 15 min (400g; 4°C). On a 96-wells microplate, 20 µl of supernatant containing the extracted MPO was then incubated for 10 min at 37° C with 30 ul

of different concentrations of curcumin and nicotinamide. Volumes of 25 µl of Tetramethylbenzidine (1.6 mM solubilized in DMSO) and 100µl of H_2O_2 (0.003 %prepared in PBS (50 mM, pH 5,4, 0,05% hexadecyltrimethylammonium bromide) were then added to each well. This enzymatic reaction was stopped by adding H_2SO_4(2N) after 5 min of incubation. The absorbances werethen read at a wavelength of 450nm. The following equation was used to estimate the rate of residual activity of MPO compared to the control which represents 100% of MPO activity.

$$\textbf{Activity}\ (\%) = \frac{\textbf{Absorbance sample}}{\textbf{Absorbance control}} \times \textbf{100}$$

2.3.4. Determination of elastase activity

The effect of curcumin and nicotinamide on the elastase activity was assayed following the procedure of Bouriche et al.(Bouriche et al., 2016). This method was performed in two steps. The first step consists in provoking the degranulation of neutrophils to recover the elastase. The degranulation was induced by incubating the neutrophils suspended in HBSS2 with fMLP(10^{-6}) and cytochalasin B (10^{-5}M). Then, a centrifugationwas carried out at 400 g for 15min at 4°C to separate the supernatant containing elastase. In the second step, the elastase activity assay was conducted in a 96-well microplate. A volume of 75 µl of the elastase-containing supernatant was incubated with 50 µl of varying doses of curcumin and nicotinamide for 10 minutes. Following this incubation, 75 µl of a specific substrateN-MeO-Suc- (Ala)2-Pro-Val-p-nitroanilide (5 x 10^{-4} M) was added. This substrate was initially prepared in methyl 2-pyrrolidone at a concentration of 5 x 10^{-2} M and subsequently diluted in a HEPES medium (0.1 M; pH=7.4) to achieve a concentration of 5 x 10^{-4} M.The reaction mixture was incubated for an additional 40 minutes at 37°C. Subsequently, the absorbance of the enzymatic product, the p-nitroallanine, was then measured at 405nm.The residual activity of the elastase enzyme was calculated using the next equation, with control wells represent 100% of elastase activity.

$$\textbf{Activity}\ (\%) = \frac{\textbf{Absorbance sample}}{\textbf{Absorbance control}} \times \textbf{100}$$

2.3.5. Molecular docking study

The high-resolution (2.00 Å) X-ray crystallographic structures of the MPO and Elastase enzymes were initially retrieved from the RCSB Protein Data Bank website (https://

www.rcsb.org) in the PDB format. Following this, these target enzymes were prepared for docking by removal of the native ligands, the undesired molecules, and water molecules using the VMD 1.9.3 (Visual Molecular Dynamics) software. Hydrogen atoms and Kollman charges were subsequently added to the structures, which were then saved in PDBQT format using Autodock tools. Similarly, the structure of the ligand under investigation, curcumin, was modeled in Chem3D software, subsequently subjected to energy minimization, and saved in PDB format. This ligand, along with the native co-crystallized ligands, underwent polar hydrogen addition and Kollman charge assignment before being saved in PDBQT format using Autodock tools. Molecular docking was then conducted using AutoDock software. For this process, the co-crystallized ligands:3Q77 (the native ligand of elastase) and 4C1M (the native ligand of myeloperoxidase) were redocked into their corresponding active sites. After successfully redocking the native ligands (with an RMSD value < 2.0 Å), the same parameters were applied for curcumin docking within the active sites of both MPO and elastase. Finally, the enzyme-ligand interactions in both 2D and 3D formats were visualized with VMD 1.9.3 software.

2.3.6. DFT computational study

The Gaussian 16 software package was used to optimize the molecular geometry of curcumin, employing the density functional B3LYP /6-31G(d,p) basis set (Danish et al., 2021). Using the optimized geometry of curcumin, we also investigated the molecular electrostatic potential (MEP), HOMO-LUMO energies, and several related characteristics, including chemical hardness, chemical softness, ionization potential, electron affinity, and electronegativity, all at the same level of theory.

2.4. *In vitro* inhibition of xanthine oxydase activity

To study the effect of nicotinamide on the activity of xanthine oxydase, the oskoueian and his colleagues' procedure (2011) was applied. For this, 30 µl of xanthine oxydase solution and 35 µl of nicotinamide were incubated for 10 min in the presence 635 µl of phosphate buffer, then 300 µl of xanthine was added to reaction mixture and the absorbance was controlled for one minute at 295nm. On the other hand, a negative control reaction with phosphte buffer, xanthine oxydase and xanthine, was monitored as mentionned above. Meanwhile, a blank reaction without the xanthine oxydase was also carried out. Finally, a positive control reaction using allopurinol was also conducted. The rate of xanthine oxydase activity was calculated using the measured absorbances and the formula below:

$$\textbf{Xanthine activity rate}\ (\%)\ = \frac{\textbf{A sample}}{\textbf{A control}} \times \textbf{100}$$

Δ**Asample**: Absorbance difference of the nicotinamide or allopurinol.

Δ**Acontrol**: Absorbance difference of the negative control.

2.5. Statistical analysis

Drawing of graphs and statistical analyses were accomplished using GraphPad Prism software version 8. All results were diplayed as mean± SD or as mean± SEM from at least three independent assays. Differences between means in two groups were evaluated with the Student's t- test. The one-way analysis of variance (ANOVA) and Tukey's post hoc test were followed to determinate differences between means in more then two groups. Differences are deemed as statistically significant at a P-value of 0.05 or less.

Results

1 Streptozotocin' s effect on mice Langerhans islets and NIT-1 cells

1.1. Effect of streptozotocine on the viability of Langerhans islets and NIT-1 cells

The effect of streptozotocine (0.5 mM) on the viability of mice-isolated pancreatic islets was assessed using trypan blue staining assay. As displayed in figure 5A, the incubation of streptozotocine (0.5 mM) for 24h with cultured isolated pancreatic islets significantly (**$p<0.001$) decreases the cell viability to ~80%.

Similary, to evaluate the STZ toxicity on pancreatic beta-cells, NIT-1 cells were exposed to streptozotocine 6mM for a duration of 24 hours. Then, the XTT viability assay was applied to measure cell viability. The obtained results indicates that streptozotcine at a concentration of 6 mM caused a significant (****$p<0.0001$) cell death of approximatively 50 % of NIT-1 cells (figure 5B). These findings are reflected by morphological observations conducted with an inverted microscope on cultured NIT-1 cells. As depicted in Fig.5C, untreated NIT-1 cells displayed an irregular polygonal shape, forming clusters that cling to the bottom of the microplate. Conversely, STZ-treated NIT-1 cells exhibited significant shrinkage, marked atrophy, and noticeable cell detachment, highlighting the cytotoxic effects of STZ.

1.2. Effect of streptozotocin on oxidative status in NIT-1 cells

The present results displayed in figure 6A show that streptozotocine (3mM) induced a marked increase (**$p<0.01$) in ROS generation which was accompanied with statistically significant elevation (*$p<0.05$) in the activity of the pro-oxidant enzyme xanthine oxydase (XO) as well as the activity of the antioxidant enzyme SOD (*$p<0.05$). The excessive ROS production in STZ-treated NIT-1 was evidenced by microscopic observation of these cells which exhibited high level of DCF fluorescence compared to control non-treated NIT-1cells (figure 6B). Concurrently, MDA level was significantly raised in STZ-treated NIT-1 cells (****$p<0.0001$). While, the GSH content exhibited a significant drop (***$p<0.001$) after STZ-treatment (figure 6C).

Xanthine oxidase is a key source of reactive oxygen species (ROS), catalyzing the reduction of molecular oxygen to superoxide (O_2•−) and hydrogen peroxide (H_2O_2) (Todorov et al., 2021). The noted elevation in ROS level and xanthine oxidase activity may be ascribed to the induction of xanthine oxidase reaction by streptozotocine, resulting in reactive oxygen

species generation. This explanation is supported by previous studies which have reported the ability of streptozotocine to enhance xanthine oxidase reaction as well as the production of superoxide radicals in XO system(Belviranli et al., 2012). On the other hand, the elevated superoxide activity (SOD) could be attributed to its induction by the rise in superoxide production. This is frequently an attempt to shield the β-cells from the damaging effects of excessive superoxide anions generation (Erejuwa et al., 2010). Similarly, the marked increase in the level of MDA may be due to an increased lipid peroxidation following ROS-induced NIT-1 cell damage. While, the observed decrease in GSH level after STZ exposure is probably due to its increased consumption by NIT-1 during their attempt to remove lipid peroxides. Overall, these findings point to a state of oxidative stress brought on by streptozotocine exposure.

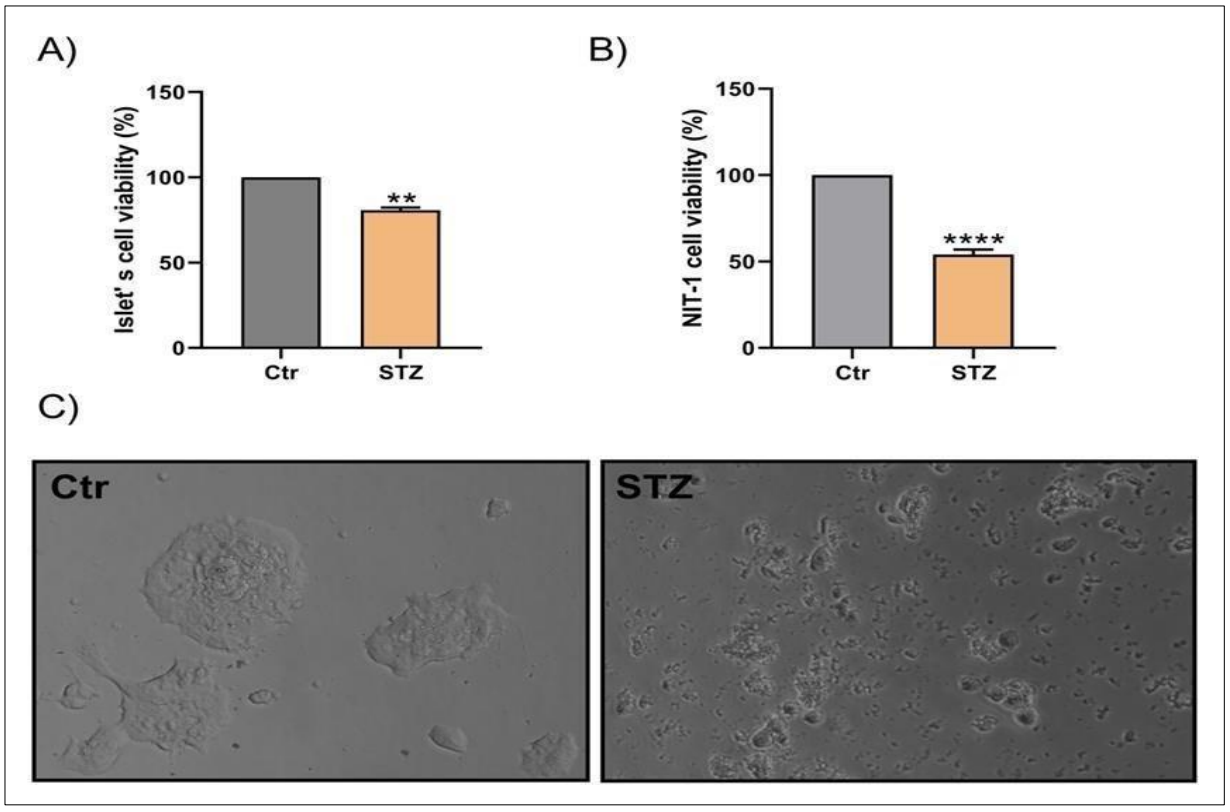

Figure 5. Effect of streptozotocine treatment on the viability of mice-isolated pancreatic islets and NIT-1 cells.(A)Viability rate of islet's cells. Ctr: control untreated islets, STZ: streptozotocine (0.5 mM)-treated islets for 24h. (B) Viability rate of NIT-1 cells. Ctr:control untreated NIT-1cells, STZ: streptozotocine (6 mM)-treated NIT-1 cells for 24h. (C) Morphological observation of control and streptozotocine-treated NIT-1 cells under inverted microscope (Nikon, EclipseTs2) at 10x magnification. The all data are expressed as mean ± SEM of three independent assays. **$p<0.01$ significant relative to the untreated pancreatic islets, ****$p<0.0001$ significant relative to the control untreated NIT-1 cells.

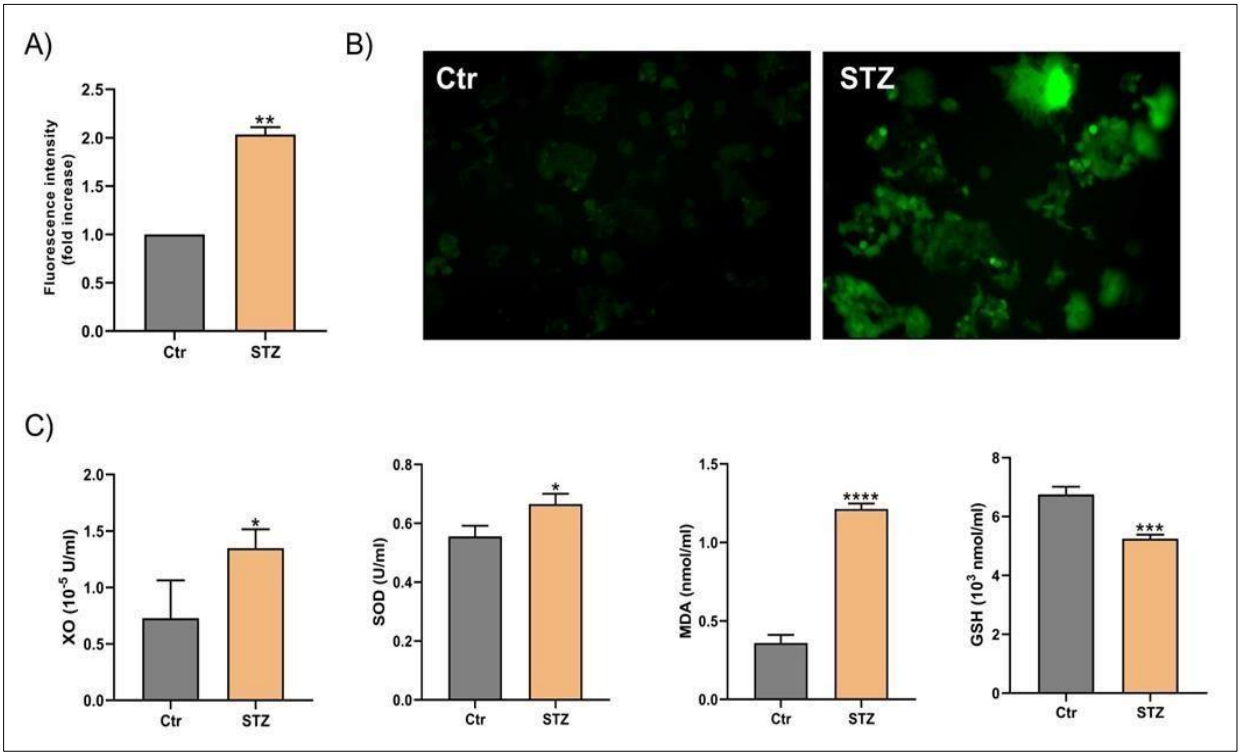

Figure 6. Effect of streptozotocine on redox status in NIT-1 cells.

(A) Relative quantification of ROS generation using DCFH-DA fluorescent. The bars display the fold-increase in ROS level related to control untreated cell group which was set to 1. **(B)** Microscopic observation of NIT-1 cells treated with DCFH-DA fluorescent. **(C)** Levels of oxidative stress markers. All data are displayed as mean± SD of three independent assays. Asteriks indicate the significant differences (*p<0.05 , **p<0.01, ***p<0.001,****p <0.0001) relative to the control untreated NIT-1 cells. **Ctr:**control untreated NIT-1 cells. **STZ:** streptozotocine-treated NIT-1 cells.

1.3. Effect of streptozotocine on NF-kB1, ATF4, and CHOP genes expression in Nit-1 cells

As shown above, streptozotocine provoked a marked beta-cell death. Thus, we attempt to explore the molecular events underlying STZ-induced toxicity against these cells. After a comprehensive literature review and a GO pathway enrichment, we have selected three genes (NF-κB1, ATF4, and CHOP) for analyzing their expression level under STZ-treatment condition. The Figure 7 represents GO pathway enrichment, where these genes are obsiously associated with oxidative stress and apoptosis.

Our RT-qPCR results demonstrate a significantly higher level of NF-κB1, ATF4, and CHOP expression in STZ-treated NIT-1 cells when compared to the control non-treated cells. Importantly, STZ up-regulated NF-κB1, ATF4, and CHOP in NIT-1 cells by approximately 16-, 8-,and 2-fold, respectively (Figure 7).

Previous research have founded that these three genes may be linked and that their simultaneous overexpression triggers ER stress-induced apoptosis and the UPR response implicating the PERK pathway (Yamaguchi & Wang, 2004). Hence, our current findings holds significant value since it obviously show that streptozotocine toxicity against beta cells arises from its impact on NF-κB pathway gene induction, which triggers β-cell apoptosis. Theses outcomes partially explain the molecular mechanism involved in streptozotocine-mediated β-cell death.

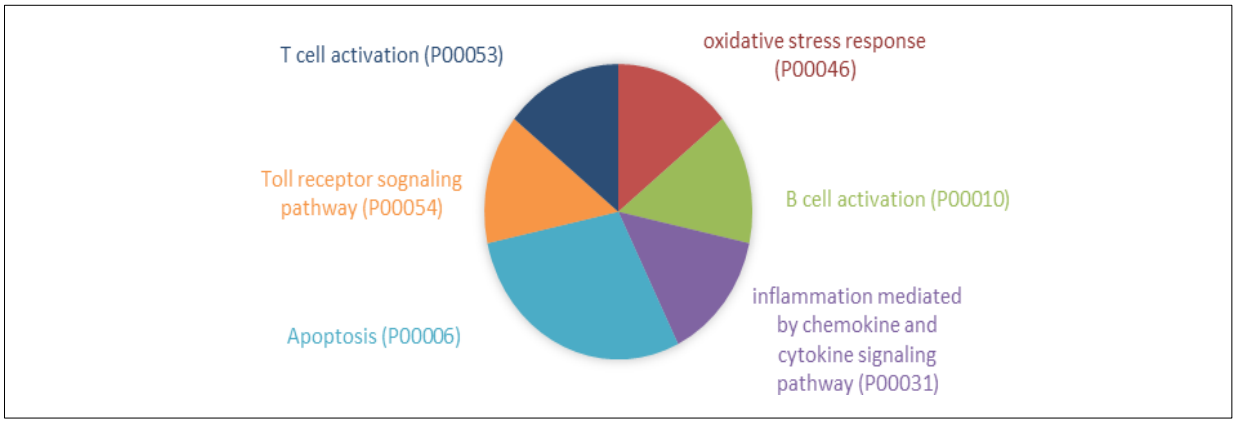

Figure 7.GO pathway enrichment of three filtred genes: pathways related to NF-κB1, ATF4, and CHOP.

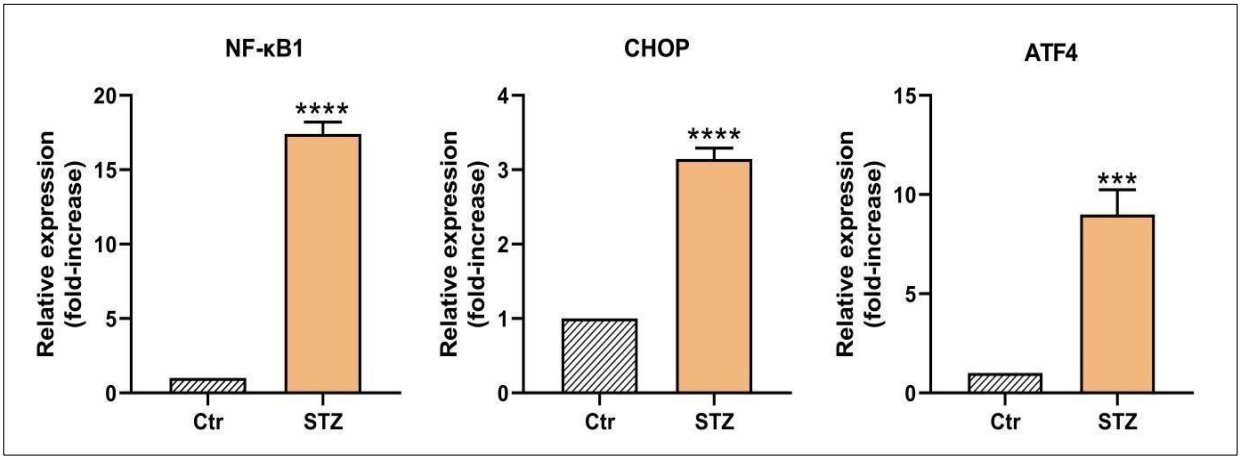

Figure 8. Effect of streptozotocine on expression level of NF-κB1, ATF4, and CHOP genes in NIT-1 cells.

The Ct values were normalized using β-actin as the endogenous gene. The relative expression values are presentedas mean ± SEM of three replicates. The bars represent the fold-increase related to control non-treted group which was set to 1. Asterisks indicate significant difference (***p ≤0.001, ****p ≤0.0001) relative to the control untreated NIT-1 cells.

2. Effects of curcumin and nicotinamide on Langerhans islets and NIT-1 cell line

2.1. Effects of curcumin and nicotinamide on Langerhans islets and NIT-1 cell line viabilities

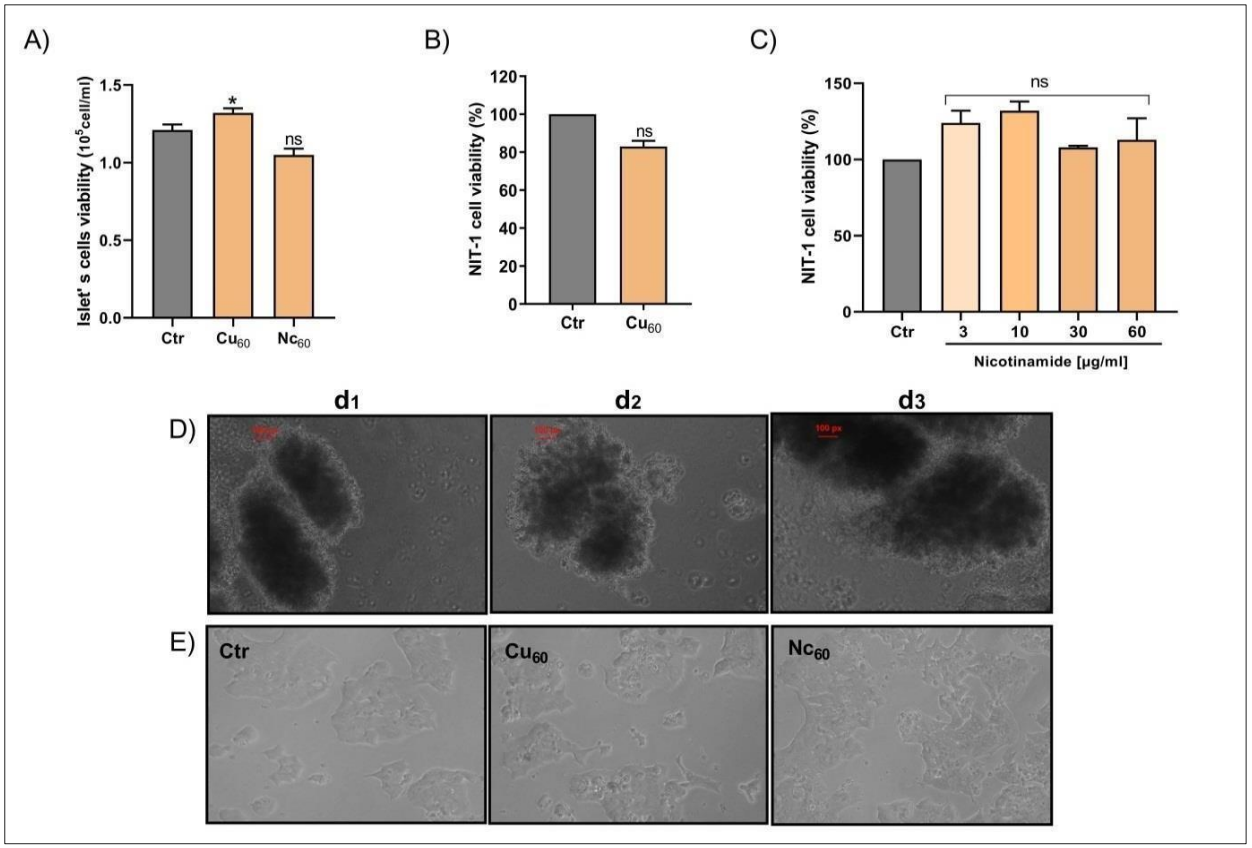

Figure 9. Effect of curcumin and nicotinamide on Langerhans islets and NIT-1 cell line viabilities.

(A) Viability rate of islet's cells treated with curcumin and nicotinamide for 48h**.** Ns: not significant, *p< 0.05 signifiacant related to untreated islets. **(B)** Viability rate of NIT-1 cells treated with curcumin for 72h. Ns: not significant related to untreated NIT-1cells. **(C)**Viability rate of NIT-1 cells treated with nicotinamide for 72h. Ns: not significant related to untreated NIT-1 cells. The all data are expressed as mean ± SEM of three independent assays. (D) Morphlogical observation of mice isolated pancreatic islets using an inverted microscope (Nikon, EclipseTs2) at 10x magnification. d1:control untreated-islets, d2:curcumin (60µg/ml)-treated islets, d3: nicotinamide (60µg/ml)-treated islets.(E) Morphological observation of NIT-1 cells using an inverted microscope (Nikon, EclipseTs2) at 10x magnification. Ctr: control untreated-NIT1 cells, Cu_{60}: curcumin(60µg/ml)-treated NIT1 cells, Nc_{60}: nicotinamide(60µg/ml)-treated NIT1 cells.

Initially, the cytotoxicties of both molecules on mice isolated islets were evaluated using the trypan blue colorimetric method. Figure 9A obviously shows that curcumin at 60

µg/ml not only lacked a cytotoxic effect toward islets cells but it also significantly (*p<0.05) improved the viability of these laters. Likewise, the exposure of islets cells to 60 µg/ml nicotinamide for 24 hours had no significant cytotoxic effect on these islets cells.

On the other hand, assessment of toxicity of these molecules on NIT-1 cells was also performed by measuring cell viability after 72h –incubation with these molecules. As can be seen in figure 9A, the colorimetric XTT assay revealed that curcumin at 60 µg/ml had a slight and insignificant cytotoxic effect on these cells. while, nicotinamide was devoid of cytotoxic effect at the applied doses (3, 10, 30, 60 µg/ml) (figure 9C).

2.2. Protective effect of curcumin and nicotinamide on NIT-1 cells and on pancreatic islet cells against STZ-induced death

The protective effects of both molecules against the cytotoxicity of streptozotocine on pancreatic islet cells were also tested. The two molecules were applied to isolated mice pancreatic islets for 24h with previous STZ-treatment (0.5 mM). The viability results showed a statistically marked improvement of cell viability with both curcumin and nicotinamide treatments (60 µg/ml) with p-value of *p< 0.05 and **p< 0.01, respectively (10A). Hence, as for beta-cells, nicotinamide offers the highest degree of protection for pancreatic islets. Similary, to examine whether curcumin and nicotinamide protect β-cells from death, we artificially induced cell death in NIT-1 cells by exposing them to streptozotocin (6 mM). We then assessed cell viability in the presence and absence of these molecules. Results from the XTT assay clearly show that treatment of NIT-1 cells with streptozotocin (6 mM) alone significantly reduced cell viability to 54 %. While, pre-incubation of NIT-1 cells with either curcumin (30 µg/ml, 60 µg/ml) or nicotinamide (30 µg/ml, 60 µg/ml) significantly reversed the viability decline caused by streptozotocin exposure. Cell viability of 54% obtained after the STZ treatment rose to 71% (at 30 µg/ml) and 78% (60 µg/ml) with curcumin, and to 82% (at 30 µg/ml) and 94% (60µg/ml) with nicotinamide (1B). Therefore, nicotinamide pre-treatment provides the greatest level of β-cell protection. Likewise, microscopic observation showed that the atrophy, striking distortion in cellular morphology, and cell detachment of NIT-1cells induced by streptozotocin were noticeably recovered following curcumin and nicotinamide pretreatment (10C).

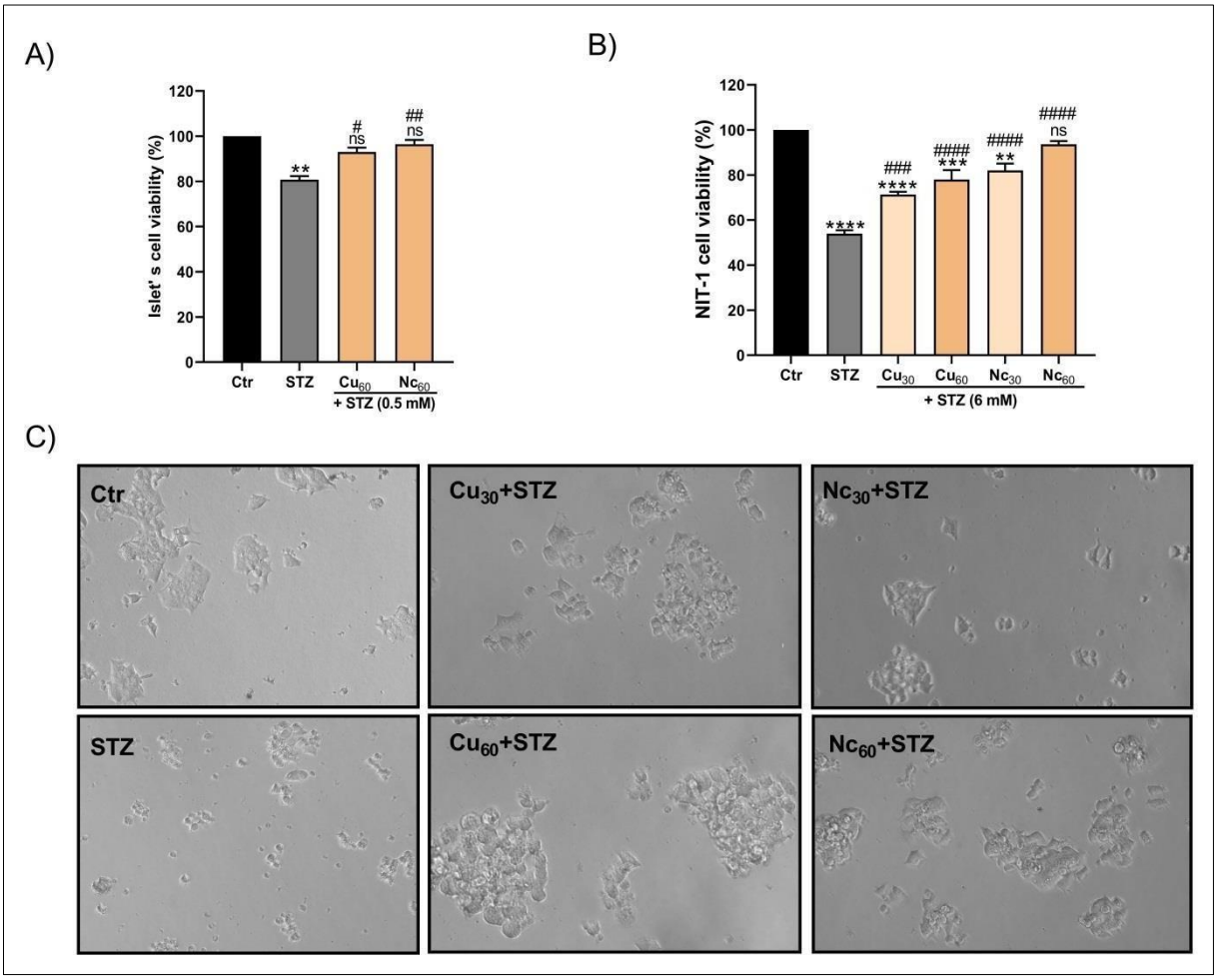

Figure 10. Protective effect of curcumin and nicotinamide against streptozotocine-induced death in mice isolated pancreatic islets and NIT-1 cells.

(A) Viability rate of islet's cells. Ctr: control untreated islets, STZ: streptozotocine(0.5 mM)-treated islets for 24h,Cu_{60}+STZ(0.5mM):islets treated with curcumin 60µg/ml for 24h then streptozotcine 0.5mM for further 24h.Nc_{60}+STZ(0.5mM): islets treated with nicotinamide 60µg/ml for 24h then streptozotocine 0.5mM for further 24h.Asteriks indicate the significant difference (**P< 0.01) related to control untreated islets. Ns indicate the absence of significant difference related to control untreated islets.Thesign # indicate the significant difference($^{\#}p<0.0.5$,$^{\#\#}p<0.01$) related to streptozotocine treated islets. **(B)** Viability Rate of NIT-1 cells.Ctr:control untreated NIT-1 cells,STZ: streptozotocine(6 mM)-treated NIT-1 cells for 24h ,Cu_{30}+STZ (6mM):NIT-1 cells treated with curcumin 30µg/ml for 24h then streptozotcine 6mM for further 24h, Cu_{60}+STZ (6mM):NIT-1 cells treated with curcumin 60µg/ml for 24h then streptozotcine 6mM for further 24h. Nc_{30}+STZ(6mM):NIT-1 cells treated nicotinamide 30 µg/ml for 24h then streptozotocine 6mM for further 24h.Nc_{60}+STZ(6mM):NIT-1 cells treated nicotinamide 60 µg/ml for 24h then streptozotocine 6mM for 24h. Asteriks indicate the significant difference (**p< 0.01,***p< 0.001, ****p<,0.0001) relative to control untreated cells. Ns indicate the absence of significant difference relative to untreated NIT-1 cells.The sign # indicate the significant difference ($^{\#\#\#}$ p< 0.001, $^{\#\#\#\#}$ p<,0.0001) relative to streptozotocine treated NIT-1cells. **(C)** Morphological observation of NIT-1 cells under different conditions using an inverted microscope at 10x magnification .All data are displayed as mean ± SEM of three independent assays.

2.3. Effects of curcumin and nicotinamide on oxidative stress status

Our results showing the protective effects of curcumin and nicotinamide against STZ-induced cytotoxicity prompted us to reveal the mechanism driving this protection. Therefore, using a DCFDA ROS-indicator, we explored whether this protective effects were attributed to the ability of these molecules to reduce ROS level provoked by STZ exposure. Quantitative analysis of intracellular ROS demonstrate that treatment with streptozotocine (3 mM) markedly (**p<0.01) enhanced ROS level in NIT-1 cells. Contrastly, pretreatment with both curcumin (60 µg/ml) and nicotinamide (60 µg/ml) significantly ($^{\#}$p<0.05) decreased STZ-provoked ROS level (fig.11A). Microscopically captured images also support the earlier findings showing that cells pretreated with both curcumin and nicotinamide have a lesser proportion of green fluorescence compared to STZ-treated group (fig.11B). These suggest that these molecules protect NIT-1 cells against STZ cytotoxicity at least through the decrease of ROS level.

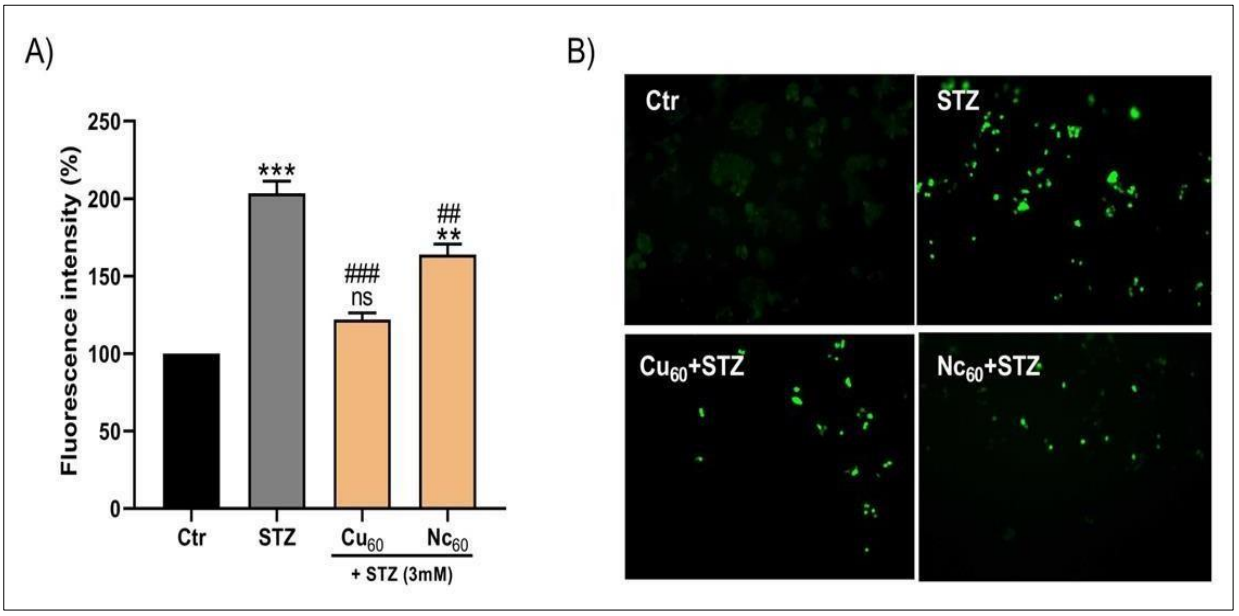

Figure 11. Effect of curcumin and nicotinamide on streptozotocine-induced ROS generation in NIT-1 cells.

(A) Relative quantification of ROS generation using DCFH-DA fluorescent**.** The bars display the rate-increase in ROS level related to control untreated cell group which was set to 100%. Ctr: control untreated NIT-1 cells, STZ: streptozotocine(3 mM)-treated NIT-1 cells for 24h , Cu_{60}+STZ (3mM): NIT-1 cells treated with curcumin 60µg/ml for 24h then streptozotcine 3 mM for further 24h, Nc_{60}+STZ(3 mM):NIT-1 cells treated nicotinamide 60 µg/ml for 24h then streptozotocine 3mM for 24h. Asteriks indicate significant difference(**p<0.01,***p<0.001) relative to the control untreated NIT-1 cells. Ns indicate the absence of significant difference relative to the control untreated NIT-1 cells. The sign # indicate the significant difference ($^{\#\#}$p<0.01,$^{\#\#\#}$p<0.001) relative to the streptozotocine-treated NIT-1 cells. All data are expressed as mean ± SD from three independent assays.**(B)** Microscopic observation of NIT-1 cells treated with DCFH-DA fluorescent.

2.4. Effect of curcumin on GSH and MDA levels and SOD activity

In this section, we examined curcumin's antioxidant activity in β-cells (Figure 12). These findings offer a more thorough comprehension of the oxidative stress state. The results show that curcumin at 30 µg/mL does not provoke oxidative stress, as evidenced by the low MDA and SOD levels. Curcumin managed to slightly lower baseline MDA level compared to control condition. At the same concentration, the level of reduced glutathione (GSH) was found to be significantly (***p<0.001) increased in curcumin-treated NIT-1cells compared to the control non-treated cells, reflecting a state of cellular redox equilibrium in β-cells. However, at a dose of 60 µg/mL, curcumin triggered oxidative stress. This was demonstrated by a notable increase in superoxide dismutase (SOD) activity, along with a rise in malondialdehyde (MDA) levels, even though this marker's elevation was statistically insignificant. In contrast, glutathione level exhibited a significant drop, indicating a depletion of the cell's antioxidant capacity at this concentration.

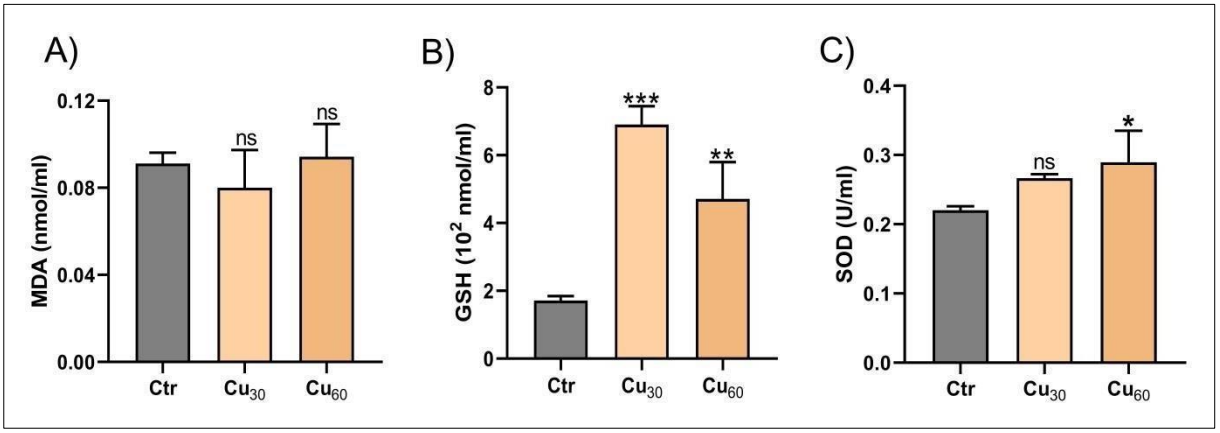

Figure 12. Effect of curcumin on redox status in NIT-1 cells.

(A) Malondialdehyde level.**(B)** Reduced glutathione level.**(C)** Superoxide dismutase activity. All parameters were measured in the NIT-1 cell supernatant and all data are displayed as mean± SD of three independent assays. Asterisk indicate the significant differences (*p<0.05 , **p<0.01, ***p<0.001) relative to the control untreated NIT-1 cells. Ns indicate the absence of significance relative to the control untreated NIT-1 cells . Ctr: control untreated NIT-1 cells group. Cu_{30}: curcumin (30µg/ml)-treated NIT-1 cells for 24h. Cu_{60}: curcumin (60µg/ml)-treated NIT-1 cells for 24h.

2.5. Effect of curcumin and nicotinamide on Nf-κB1, ATF4, and CHOP genes expression in NIT-1 cells

In order to get profound insight into the toxic effects of the studied molecules on pancreatic beta-cells, a quantitative gene expression analysis was conducted. For this, NIT-1 cells were incubated for 24h with both curcumin (60 µg/ml) and nicotinamide (60 µg/ml),

separately. Cells were then collected for RT-qPCR analysis. Results indicates that the expression of NF- κB1, ATF4, and CHOP was significantly up-regulated upon curcumin as well as nicotinamide treatments. This significance is obviously revealed by the ****p< 0.0001 value. On the other hand, results given in figure 13 reveal the synergistic effect of curcumin and streptozotocine on the up-regulation of these genes. The ####p <0.0001 and ****p <0.0001 values showed that all three genes' expression had increased in a highly significant way. While, the synergistic effect of nicotinamide and streptozotocine was only observed with ATF4 (figure 14) .

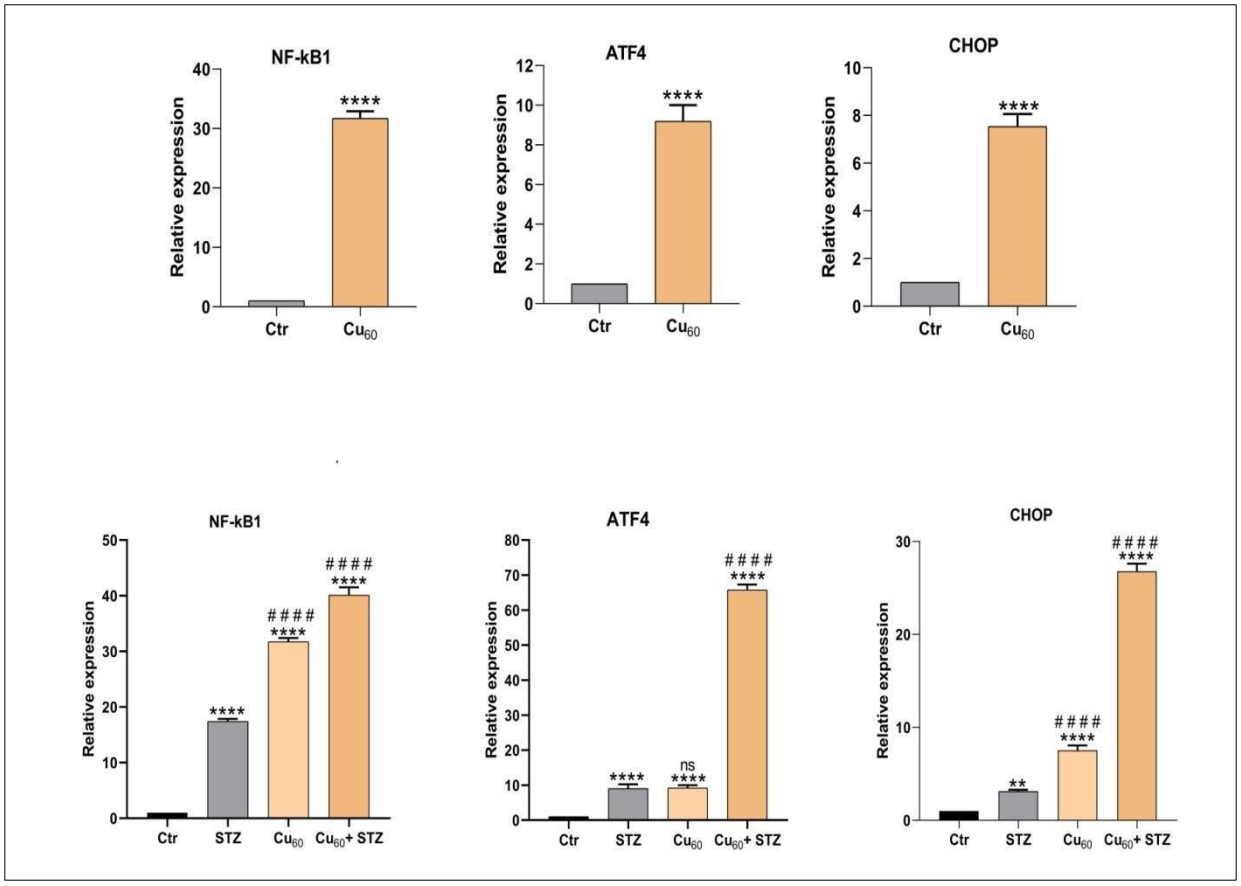

Figure 13. The mRNA expression level of NF-κB1, ATF4, and CHOP genes in NIT-1 cells under curcumin treatment.

The Ct values were normalized to β-actin as the internal gene. The relative expression values are presented as mean ± SD of three replicates. The bars represent the fold-increase related to control non-treted group which was set to 1. Asterisks indicate significant difference (**p ≤0.01, ****p ≤0.0001) related to control untreated NIT-1cells. The sign # indicate significant difference (####p ≤0.0001) related to STZ-treated NIT-1 cells. Ns indicate the absence of significant difference related to STZ-treated NIT-1cells. Ctr: control untreated NIT-1 cell group. STZ: streptozotocine (3mM)-treated NIT-1 cell for 24h. Cu60: curcumin(60 µg/ml)-treated NIT-1 cell for 24h. Cu60+STZ: NIT- 1 cells treated with curcumin 60 µg/ml for 24h then streptozotocin (3mM) for 24h.

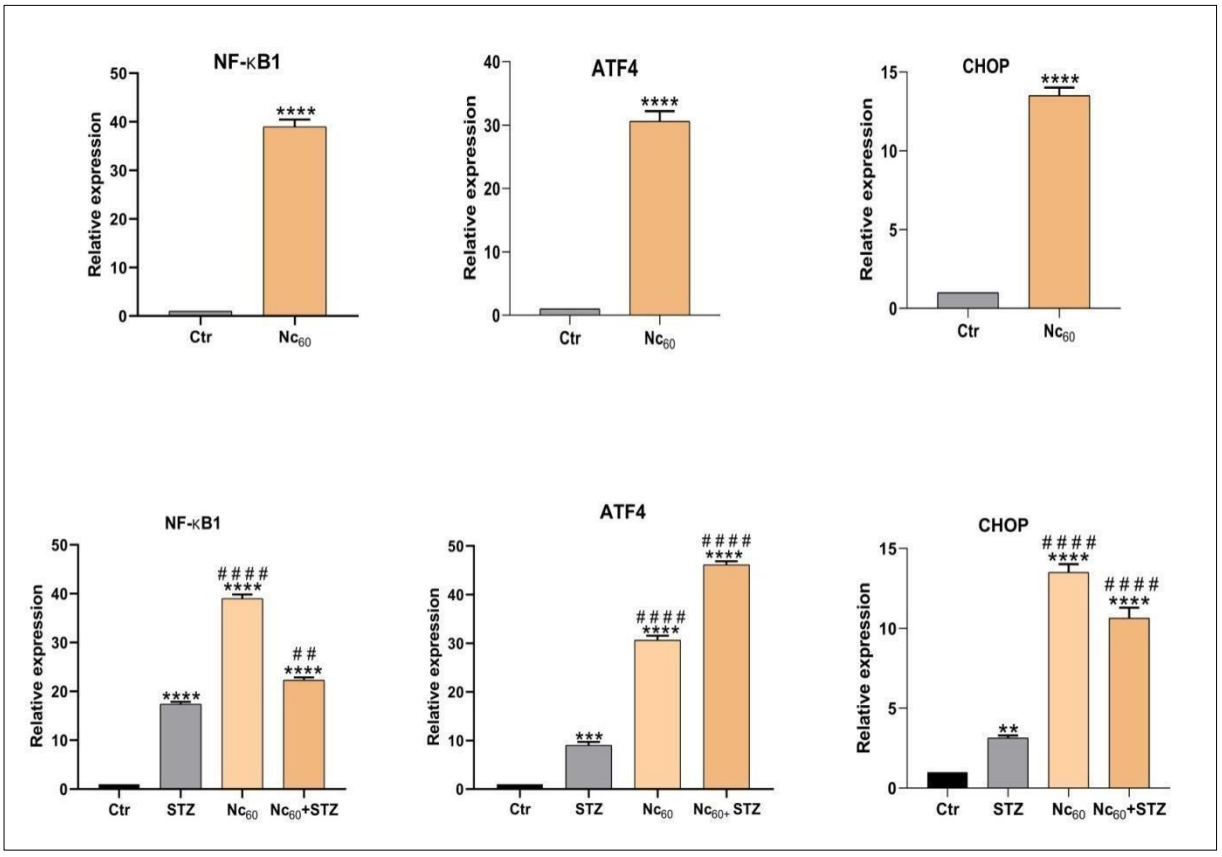

Figure 14. The mRNA expression level of NF-κB1, ATF4, and CHOP genes in NIT-1 cells under nicotinamide treatment.

The Ct values were normalized to β-actin as the internal gene. The relative expression values are presented as mean ± SD of three replicates. The bars represent the fold-increase related to the control non-treated group which was set to 1. Asterisks indicate significant difference (**p ≤0.01,***p ≤0.001,****p ≤0.0001) related to the control untreated NIT-1cells. The sign # indicate significant difference ($^{\#\#}$p ≤0.01,$^{\#\#\#\#}$p ≤0.0001) related to STZ-treated NIT-1 cells. Ctr: control untreated NIT-1 cell group. STZ: streptozotocine(3mM)-treated NIT-1 cell for 24h.Nc_{60}: Nicotinamide (60µg)-treated NIT-1 cell for 24h. Nc_{60}+STZ: NIT- 1 cells treated with nicotinamide60 µg/mlfor 24h then streptozotocin (3mM) for further 24h.

2.6. In silico investigation of curcumin interaction with NF-κB1, ATF4, and CHOP proteins

To explore the compound-protein interaction between curcumin and the proteins NFBK1, ATF4, and CHOP, we conducted a query in the STITCH database focused on these three genes. The generated interconnection graph clearly illustrates the sequential influence of curcumin on these genes, as depicted in Figure 15A. It is evident that curcumin has a direct connection to the CHOP (DDIT3) protein, which is further interconnected with both

ATF4and ATF3, as well as the NFBK1 genes. Concurrently, curcumin is directly linked with the NF-κB signaling pathway via the proteins NFKBIA and IKBKB, which are in turn directly linked to NFKB1.

2.7. In silico determination of NF-κB1, ATF4, and CHOP related genes and curcumin implication

This stage of study was dedicated to investigate the effect of curcumin on genes related to NFKB1, ATF4, and CHOP. This was accomplished by mapping these three genes inGENMANIA , recovering 20 genes, and combining them with the ones derived from the compound-protein interaction in the preceding step. All of these final genes were filtered by a second mapping in the protein-protein interaction network that was created in the string database. This latter along with GO pathway enrichment allowed for the removal of all instances that did not relate to apoptosis, oxidative stress pathways, or the pathways involved in type 1 diabetes, nor did they have any interconnection within the protein-protein interaction network (PPI). The following set of genes: Gabbr2, Batf, and Kpna1, Rps3a, 1Ankrd42, and Akap8 were eliminated during the filtration process.While, the remaining 14 genes along with NFKB1, ATF4, and CHOP were mapped to the STITCH database in order to analyze compound-protein interactions with curcumin. The findings of the protein-compound interconnection network in Fig.15D.reveal that curcumin directly interacts withJund, Ddit3, Dapk3, Cebpa, and the NF-kB pathway via NF-κB. Furthermore, it obvious that curcumin has interactions with a specific set of proteins related to inflammation, oxidative stress response, and apoptosis. Curcumin triggers apoptosis by interacting with stress-related proteins (CHOP, also called Ddit3) and AP-1 components (Jun and Jund). Curcumin, on the other hand, decreased oxidative stress by modulating the NFkB pathway as well as other stress response pathways, such as Atf3 and Atf4. Additionally, by influencing proteins involved in inflammatory signaling (the NF-κB pathway, CEBP family), it can manage inflammation.

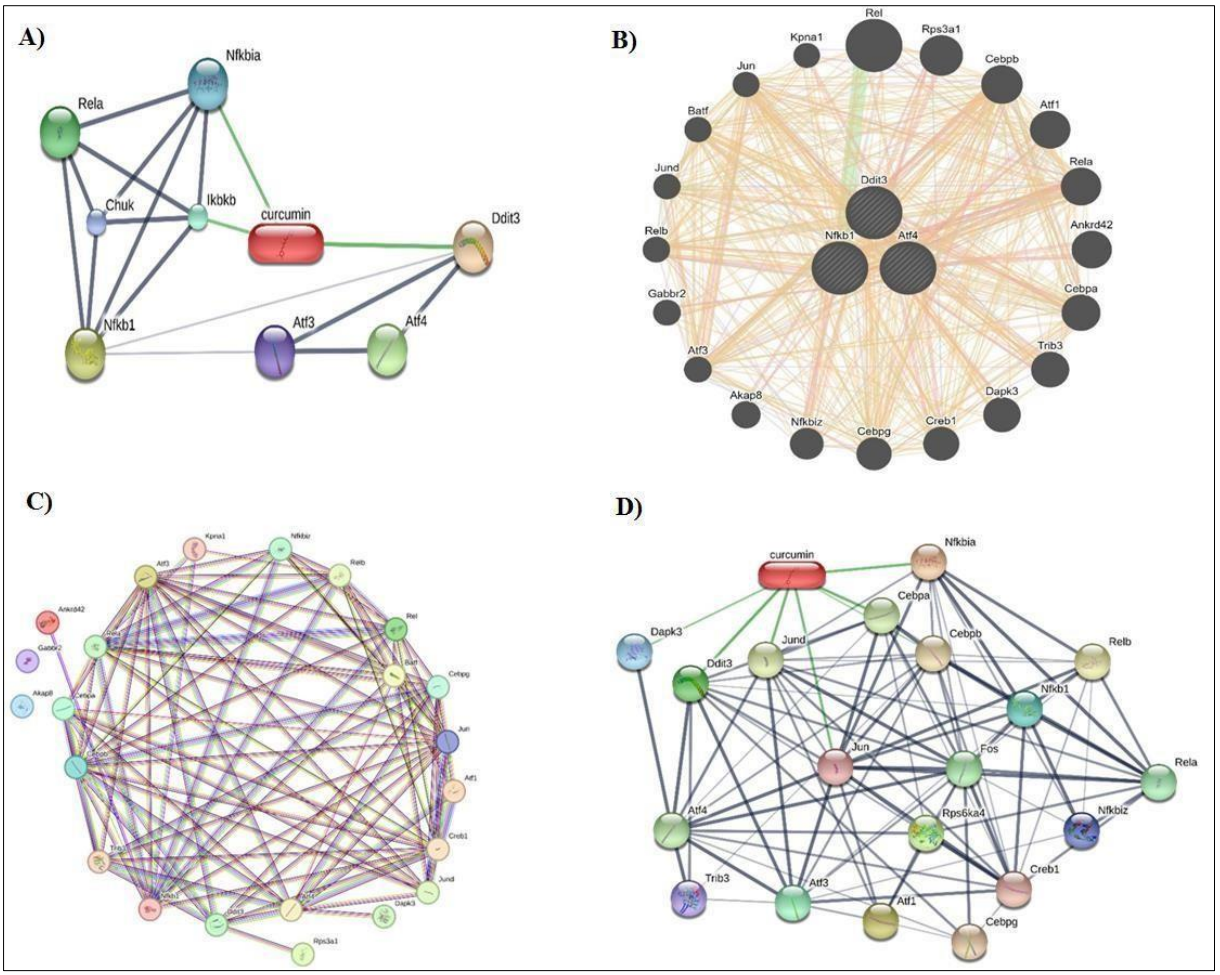

Figure 15. Results from Computational studies.

(A) Curcumin and Proteins interaction in STITCH database. **(B)** NFKB1, ATF4, and CHOP related genes. **(C)** Protein-Protein Interaction network. **(D)** Curcumin-Proteins Interaction mapped by STITCH database.

2.8. Effect of curcumin and nicotinamide on insulin secretion in Langerhans islets and NIT-1 cells

To examine whether curcumin and nicotinamide can improve beta-cell function, an insulin secretion assay was carried out. The amounts of secreted insulin from mice-isolated islets and NIT-1 cells are depicted in Figure 16. The incubation of mice pancreatic islets (10^5 cell/ml) with either nicotinamide or curcumin at 60 µg/ml for 24 hours significantly stimulated insulin secretion compared to non-treated islets, with p-value of ***$p<0.001$ and *$p< 0.05$, respectively. Specifically, nicotinamide at 60 µg/ml increased glucose-stimulated insulin secretion from mouse pancreatic islets by up to eleven-fold (***$p<0.001$) (Figure 16A).

On the other side, the effects of the molecules on insulin release in NIT-1 cells were also assayed. The exposure of NIT-1 cells to nicotinamide (60 µg/ml) for 24h induced a slight

but not significant stimulatory effect on insulin secretion compared with the control group. While, treatment of these cells with curcumin (60 µg/mL) for 24h resulted in a statistically significant decrease in insulin secretion in comparison to control NIT-1 cells (Figure 16B).

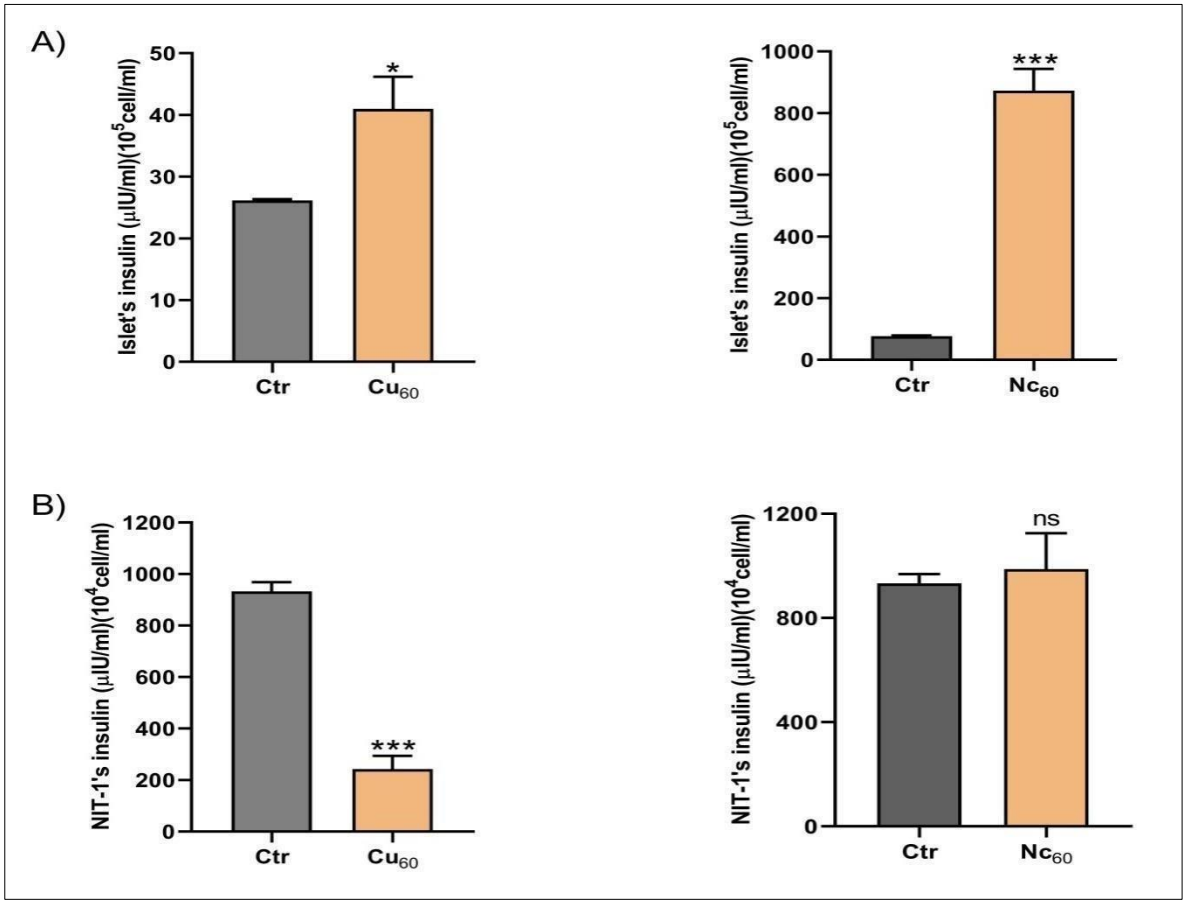

Figure 16. Effect of curcumin and nicotinamide on glucose-induced insulin secretion in mice-isolated pancreatic islets and NIT-1 cells.

(A) Insulin secretion level in Langerhans islets treated with curcumin (60 µg/ml) and nicotinamide (60 µg/ml) for 24h. **(B)** Insulin secretion level in NIT-1cells treated with curcumin (60 µg/ml) and nicotinamide (60 µg/ml) for 24h.Data were expressed as mean± SEM of three independent assays. Asterisk indicate significant difference (*p<0.05,***p<0.001) relative to the control untreated islets or untreated NIT-1 cells. Ns indicate the absence of significant difference relative to untreated NIT-1 cells. Ctr: control untreated- islets/ -NIT-1 cells. Cu_{60}: islets/ NIT-1 cells treated with curcumin (60 µg/ml).Nc_{60}:islets/ NIT-1 cells treated with nicotinamide (60 µg/ml).

3. Effects of curcumin and nicotinamide on neutrophils

3.1. Cytotoxicity of molecules on neutrophils

To determine the effect of the studied molecules on human neutrophil viability, we performed the trypan blue staining assay. As displayed in figure 17A, treatment of human isolated neutrophils with curcumin (0.5, 1, 3, 5, 10, 30, 50, 100 µg/ml) significantly decreases

their viability. Its cytotoxic effect was exerted in a dose-dependent manner with a calculated IC_{50} value of 25.60 ± 7.88 µg/ml.

However, the incubation of neutrophils with nicotinamide (100 µg/ml) did not affect neutrophil viability and no cytotoxic effect has been observed on these cells figure 17B.

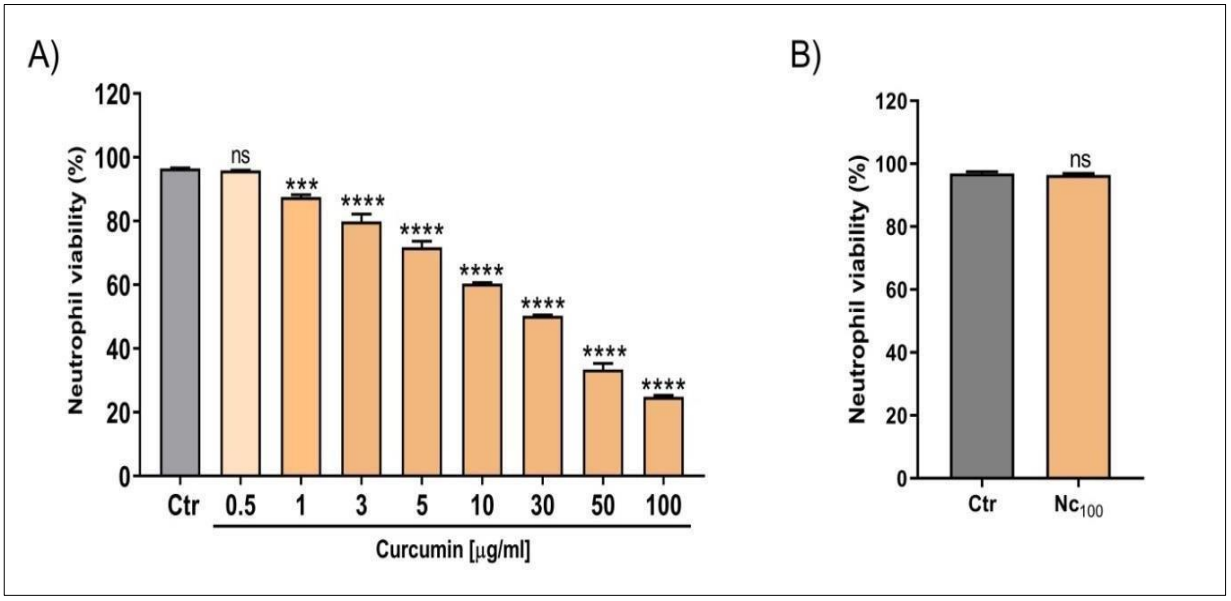

Figure 17.Effect of curcumin (A) and nicotinamide (B) on neutrophil viability.

The all data are expressed as mean ± SEM (n=3).ns:indicate the absence of significant difference relative to the control untreated neutrophils.Asterisks indicate significant differences (***p<0.001; ****p<0.0001) relative tothe control untreated neutrophils. Ctr: control untreated neutrophil group. Nc100: neutrophils treated with 100µg/ml of nicotinamide.

3.2. Effect of molecules on MPO activity

The effects of molecules on MPO activity were assessed by measurement the inhibition of coloration resulting from TMB oxidation by MPO. Figure 18A demonstrates that the pre-incubation of curcumin (5, 10, 20, 50 µg/ml) with MPO significantly reduces its activity in a dose-dependent manner (IC_{50}=14.41 ± 1.74 µg/ml) in presence of TMB as MPO substrate. The maximal levels of inhibition was 79.59 % at a concentration of 50 µg/ml. In contrast, curcumin at a dose of 0.5 µg/ml did not exhibit any significant inhibitory effect.

Unlike curcumin, pre-treatment of MPO with nicotinamide (0.5, 1, 5, 10, 20, 50, 100 µg/ml) did not exert any inhibitory effect on its activity Figure 18B.

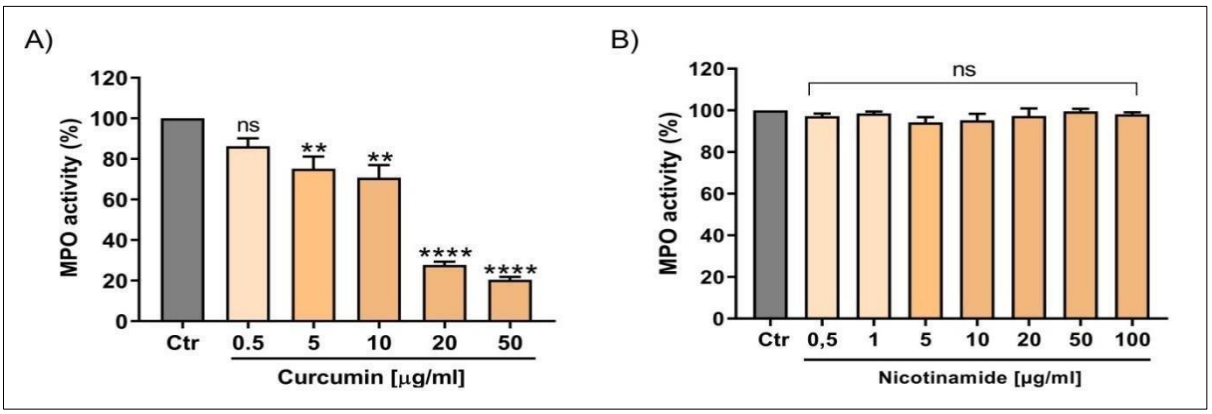

Figure 18. Effect of curcumin (A) and nicotinamide (B) on human neutrophil myeloperoxidase (MPO) activity.The all data are expressed as mean ± SEM (n=3). ns: indicate the absence of significant difference relative to untreated group. Asterisks indicate significant differences (**p<0.01; ****p<0.0001) relative to control untreated group.

3.3. Effect of molecules on HNE activity

As for the MPO, elastase-containing supernatant was pre-incubated with the studied molecules in the presence of elastase substrate to evaluate their effects on elastase activity. The obtained results presented in figure 19A showed that curcumin (1, 5, 10, 50 µg/ml) significantly decrease elastase activity in a dose-dependent manner with a IC_{50} value of 6.06 ± 3.67 µg/ml. The maximal elastase activity reduction exerted by curcumin was about 68 % at a concentration of 50 µg/ml.

While, the co-incubation of elastase and nicotinamide (0.5, 1, 5, 10, 20, 50, 100 µg/ml) did not affect the activity of the studied enzyme Figure 19B.

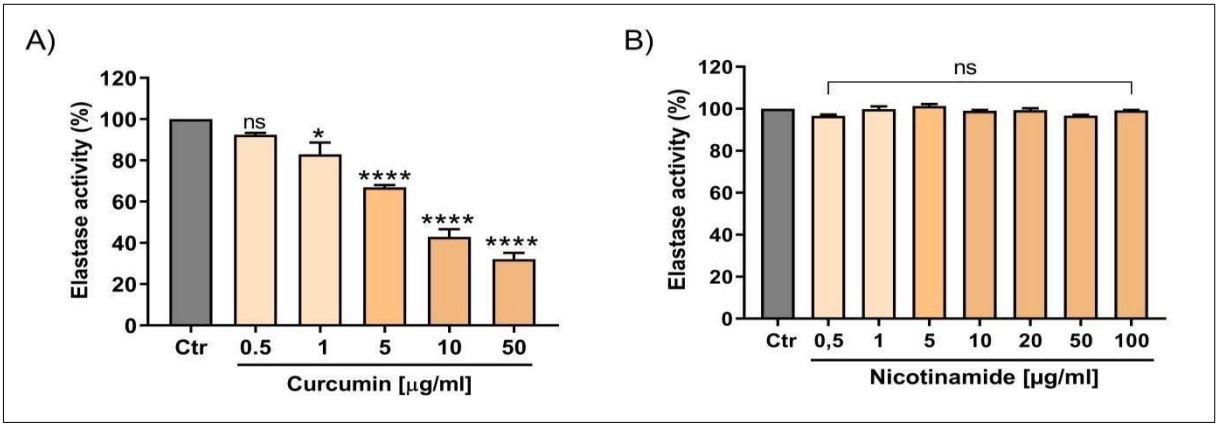

Figure 19. Effect of curcumin (A) and nicotinamide (B) on human neutrophil elastase (HNE) activity.The all data are expressed as mean ± SEM (n=3). ns: indicate the absence of significant differences relative to control untreated group.Asterisks indicate significant differences (*p<0.05; ****p<0.0001) relative to control untreated group .

3.4. Molecular docking analysis

In the current study, pure curcumin was subjected to molecular docking analysis in order to predict its binding affinities and the possible binding modes with the active sites of both HNE and elastase.

3.4.1. Molecular docking of curcumin with elastase

Initially, the native ligand of elastase, 2HY, was re-docked within the active site of elastase to evaluate the suitability of the protocol for ligands docking. The RMSD value of re-docked co-crystallized ligand was determined as 0.93 A°. Then, curcumin, which is determined in vitro as elastase inhibitor, was docked inside the active pocket of the same enzyme. Consequently, curcumin showed a binding energy of -7.41 kcal/mol which was comparable to that of the native ligand (-8.037 kcal/mol). The relative binding affinity of curcumin compared to the native ligand was calculated as 92.19 %. In the term of binding modes, the native ligand interacts with elastase through: three conventional hydrogen bonds established with Ser 195, Ser 214, and Val 216; two hydrogen-carbon bonds established with His 57 and Tyr 94; and two alkyl interaction formed with Leu 99B and Val 190 residues. While, the binding contact between the docket curcumin and elastase is mainly attributed to the formation of hydrogen bonds with Phe 41(2.07 A°), Tyr94(2.03 A°), Ser 195(3.32 A°), Ser 214(2.15 A°), Val 216 (3.08 A°) residues. Besides, other stabilizing non-covalent interactions like pi-pi stacking interaction, pi-slfur interaction, and alkyl interaction were established with His 57, Cys 42, and Leu 99B, respectively.

3.4.2. Molecular docking of curcumin with myeloperoxidase

Similar, the redocking of the co-crystal ligand (NIH) into myeloperoxidase active pocket results in an RMSD value of 0.83 A° reflecting an interesting ligand-enzyme overlap. It was also possible to observe ligand-myeloperoxidase binding interactions with a binding energy of -7.31 kcal/mol. This co-crystal ligand exhibited three hydrogen bonds with Thr 238, Arg 239, and Gln 91 residues. It also displayed a Pi stacked interaction with HEM 605. Likewise, curcumin was docked in the myeloperoxidase-binding cavity, yielding a binding energy of -7.87 kcal/mol, which was to be greater compared to that of corresponding co-crystal ligand (the relative binding energy was 107.6 %). The analysis of binding mode showed that curcumin had a similar binding interaction compared to co-crystalized ligand via the formation of H-bond with Gln 91 residue. Additionally, curcumin formed two other H-bonds with Thr 100 and Glu 102 residues and interact with HEM 605 to generate a pi anion.

Overall, the molecular docking results clearly revealed that curcumin had significant interaction with the myeloperoxidase and elastase.

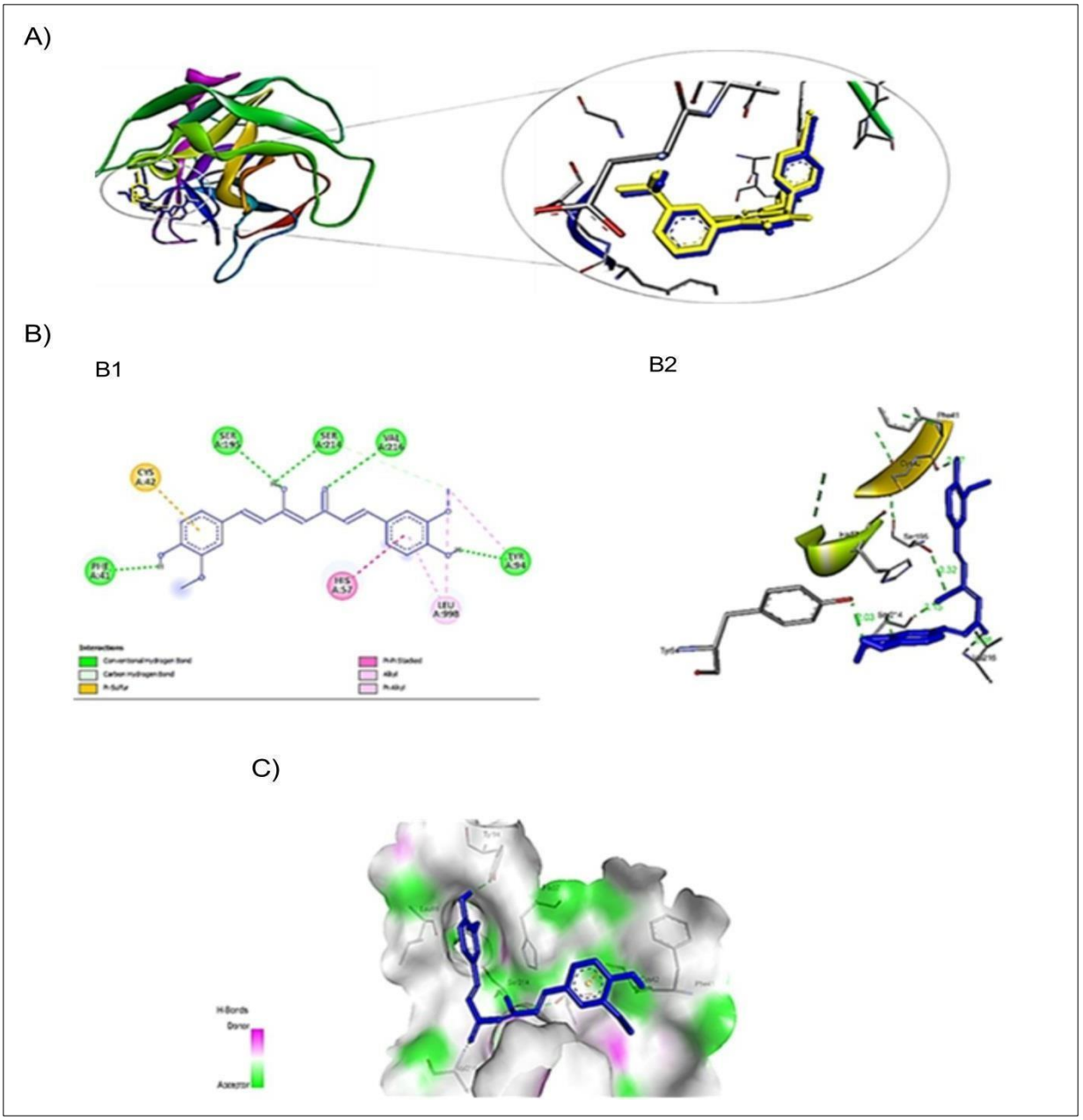

Figure 20. Molecular docking results of curcumin to HNE enzyme.

A: Overlap between the docket ligand pose (blue) and the reference 2HY obtained from PDB (yellow) for elastase enzyme. **B:** Interaction of curcumin with the active site of HNE presented in 2D (B1) and 3D (B2) diagram hydrogen bonds interaction in green, pi stacked in pink, alkyl bonds in light pink, and pi sulfur in orange. **C:** Hydrogen bonds interaction of curcumin ligand with HNE.

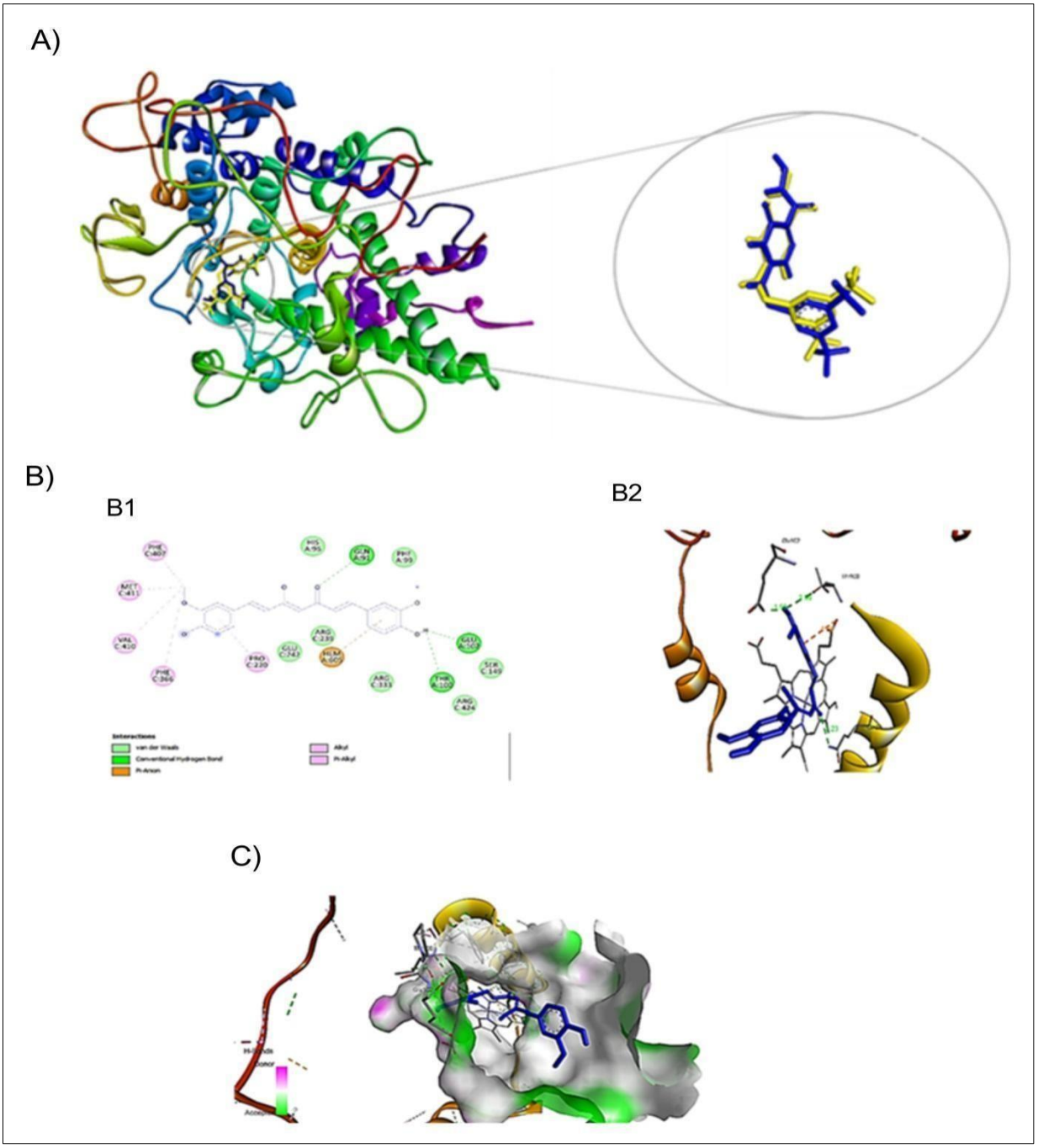

Figure 21. Molecular docking results of curcumin to MPO enzyme.

A: Overlap between the docket ligand pose (blue) and the reference NIH obtained from PDB (yellow) for myeloperoxidase enzyme. **B:** 2D (B1) and 3D (B2) interaction diagram of curcumin ligand with myeloperoxidase active site, hydrogen bonds interaction in green, pi stacked in pink, alkyl bonds in light green, and pi anion in orange. **C:** Hydrogen bonds interaction of curcumin ligand with MPO active site, acceptor bond in green, donor bond in pink.

Table 4. Molecular binding energy and protein-ligand interaction of refrence ligands and curcumin in the catalytic sites of HNE and MPO enzymes.

Target enzyme	Ligand	Binding energy (kcal/mol)	Relative ΔG% (kcal/mol)	Interacting residues	Interaction type
Elastase (3Q77)	Reference molecule (2HY)	-8.037	100	Ser 195, Ser 214, Val 216	Hydrogen
				His 57, Tyr 94	Carbon hydrogen
				Leu 99B, Val 190	Alkyl
	Curcumin	-7.41	92.19	Phe 41, Tyr94, Ser 195, Ser 214, Val 216	Hydrogen
				His 57	Pi-sigma
				Leu 99B	alkyl
				Cys 42	pi-sulfur
Myeloperoxidase (4C1M)	Reference molecule (NIH)	-7.31	100	Thr 238, Arg 239, Gln 91	Hydrogen
				HEM 605	Pi stacked
	Curcumin	-7.87	107.6	Thr 100, Glu 102, Gln 91	hydrogen
				HEM 605	pi anion

*Relative binding energy % (acquired by dividing the binding energy (ΔG) of curcumin by the binding energy of the corresponding co-crystallized ligand).

3.5. Density Functional Theory (DFT) analysis

3.5.1. Geometry optimization and molecular reactivity analysis

Following a successful evaluation of curcumin's in vitro inhibitory activity on MPO and HNE enzymes, we planned to use density functional theory calculations to get insight about the molecular structure, chemical reactivity, and reactive nature of curcumin. The geometry of curcumin was initially optimized at B3LYP/6-311++G(2d, 2p) level of DFT theory. The optimized structure of curcumin is displayed in figure 22A.

A frontier molecular orbital analysis was then conducted. Since the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are the main participants during molecular interactions, valuable informations about the molecular behavior, properties, and chemical reactivity of molecules can be deduced from these orbitals, and their energy gap. HOMO and LUMO are regarded as electron donor and electron acceptor orbitals, respectively (Aman et al., 2020). HOMO and LUMO spacial plots for the present molecule are given in Figure 22C, with red denoting a positive electron density and green denoting a negative electron density. Curcumin, with HOMO energy of -5.182688 eV and LUMO energy of -1.972272 eV,exhibited a relatively low band gap energy |EHOMO-ELUMO| of 3.210416 eV, suggesting its good chemical reactivity. Such finding explains the ability of curcumin to inhibit MPO and HNE enzymes.

In order to gain a thorough understanding of the chemical behavior of curcumin, the global chemical reactivity descriptors like chemical hardness, chemical softness, chemical potential, electronegativity, electron affinity, and ionization potential were computed according to Koopmans' Theorem (Koopmans, 1934) and their values are displayed in table 5. Specially, the larger values of ionization potential (5.182688 eV) and electronegativity (3.57748eV) reflect the high reactivity of curcumin molecule. Thus, according to the gap energy as well as the chemical reactivity parameters, curcumin is chemically reactive to function as an effective inhibitor toward MPO and HNE.

Table 5. Frontier molecular orbital energies and global chemical reactivity descriptors (GCRD) for curcumin.

Parameters	**eV**
E_{HOMO}	-5.182688
E_{LUMO}	-1.972272
Energy band gap $\|E_{HOMO}-E_{LUMO}\|$	3.210416
Ionization potential (I = EHOMO)	5.182688
Electron affinity (A = -ELUMO)	1.972272
Chemical hardness ($\eta = (I-A)/2$)	1.605208
Chemical softness ($\zeta = 1/2\eta$)	0.3114861127
Electronegativity ($\chi = (I+A)/2$)	3.57748
Chemical potential ($\mu = -(I+A)/2$)	-3.57748

3.5.2. Molecular electrostatic potential (MEP) analysis

Molecular electrostatic potential mapping, which is associated with electron density, is an effective tool for getting insight into the chemical reactivity of molecules through the determination of electrophilic (electron-accepting) and nucleophilic (electron-donating) sites within these formers. MEP highlights electronegative atoms in red as hydrogen bonding acceptors and electron-poor atoms in blue as hydrogen bonding donors. Neutral atoms are displayed in a gradient from green to yellow as contributors in hydrophobic interactions (Alesawy et al., 2021).

The MEP surface of the optimized curcumin is depicted in figure 22B, where the different colors code for distinct electrostatic potential values. The color code of the studied molecule is ranged from -7.720e-2a.u.(deepest red) to 7.720e-2a.u(deepest blue). According

to fig. 22B, the blue patches with positive electrostatic potential are covered by protonated O-H bonds reflecting electrophilic abilities. On the other hand, curcumin display three red patches visible around the carboxylate group oxygen atoms -O16, -O1, and -O25. This highlights the high electron density and the negative electrostatic potential in these areas indicating nucleophilic capabilities. Furthermore, the presence of these three red patches explains also the high binding energies of curcumin (-7.41 kcal/mol with elastase, and -7.87 kcal/mol with MPO), and its ability to form five and three hydrogen bonds with both elastase and MPO, respectively, during the molecular docking process described above.

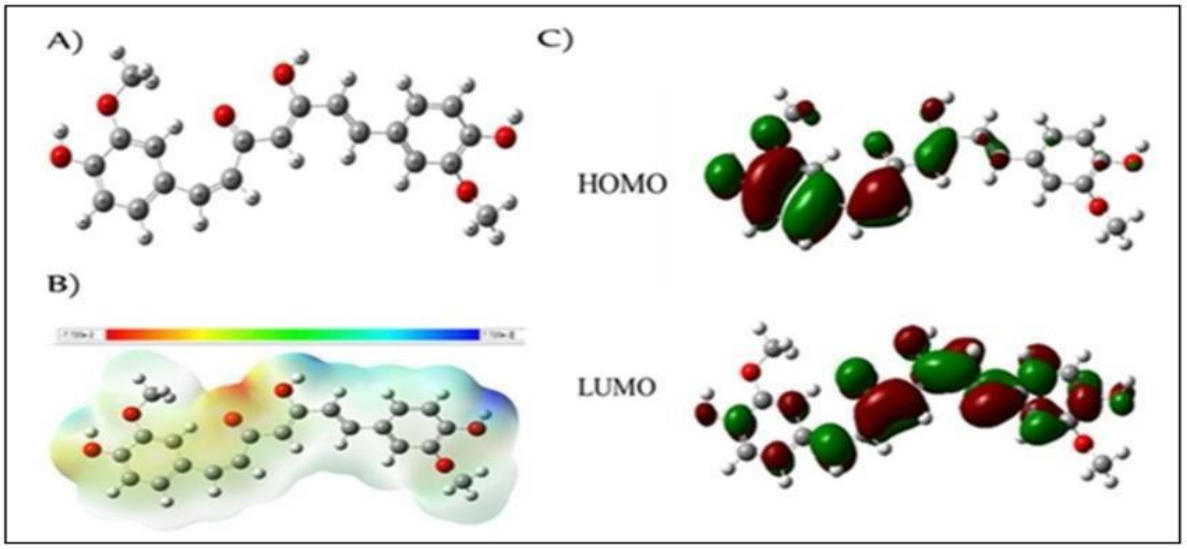

Figure 22. Curcumin DFT analysis.

A: DFT optimized structure of crystalline curcumin. **B:** Molecular electrostatic potential (MEP) surface of the curcumin molecule. **C:** The pattern of HOMO and LUMO frontier molecular orbital surfaces of curcumin.

4. *In vitro* inhibition of xanthine oxidase

The study of nicotinamide effect on xanthine oxidase activity was evaluated by monitoring the uric acid production. The results of in *vitro* xanthine oxidase presented in figure 23 showed that nicotinamide significantly inhibited xanthine oxidase (XO) in dose-dependent manner, with an IC_{50} value of 0.032 ± 0.003 mg/ml. It showed a maximum inhibition of 62.05% at a concentration of 0.08 mg/mL, while the minimum observed inhibition is 24.41% at 0.01 mg/mL. In comparison, allopurinol, a standard XO inhibitor, showed a lower IC_{50} value of less than 0.01 mg/ml, with a maximal inhibitory effect of 93.03% at 0.08 mg/ml and a minimum of 62.54% at 0.01 mg/ml.

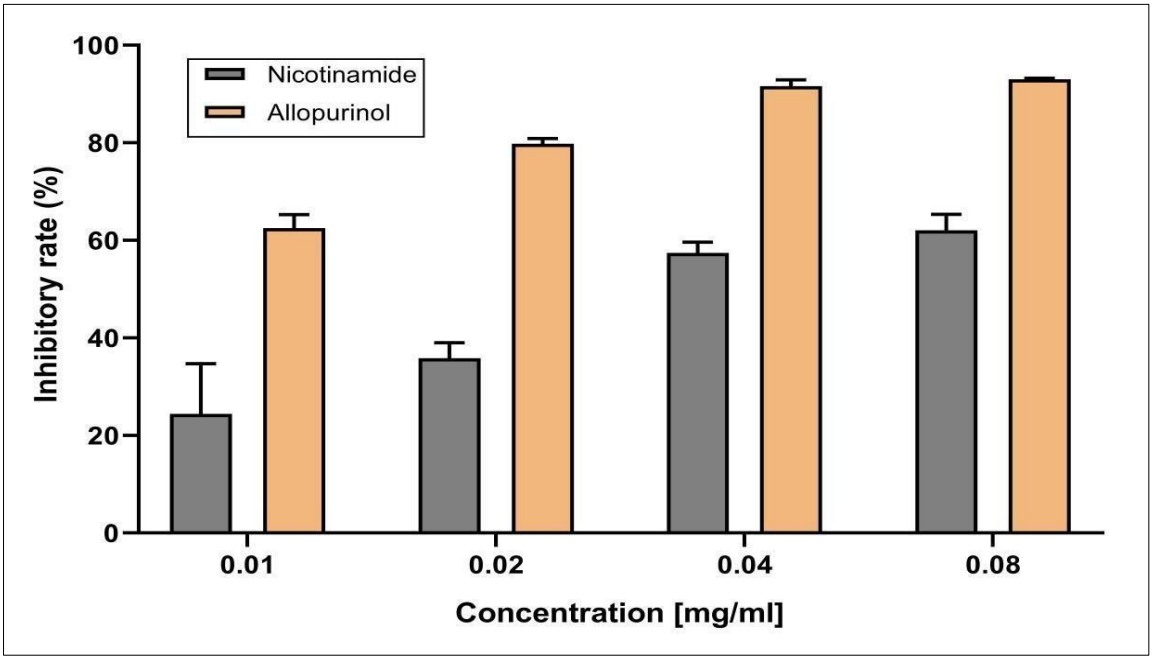

Figure 23. The inhibitory effects of nicotinamide and allopurinol on xanthine oxidase.Data were expressed as mean±SD from three independent assays.

Discussion

For many years, it was thought that autoimmune processes that mediate the destruction of pancreaticbeta-cells is the cause of type 1 diabetes. There is growing evidence that this disease develops as a result of autoreactive T lymphocytes activation that attack the insulin-producing beta cells.Although, this concept remains holds, various observations in recent years point to the implication of pancreatic beta-cells in their own demise beyond being a victim of an autoimmune destruction (Roep et al., 2021). Hence, in order to partially understand how beta cells may participate to their own death,we have used an experimental model to examine the early dysregulations and intracellular events that occurin β-cells prior to the autoimmune reactionthat results in type 1 diabetes development. The utilized experimental model was the mouse insulinoma cell line NIT-1 treated with streptozotocine. The pancreatic NIT-1 cell line was established from transgenic NOD/Lt mouse (Hamaguchi et al., 1991).The non-obese diabetic (NOD) mice are one of the most commonly used models for studying type 1 diabetes. This is because they can spontaneously develop autoimmune diabetes. Furthermore, this model shares several genetic and immunological characteristics with the human form of the disease(Chen et al., 2018).

The environment is also an important factor, we, therefore, used streptozocin as an exogenous factor that can harm β-cell without the intervention of an autoimmune reaction. Thus, the STZ-treated NIT-1 cells constitute an excellent model for studying β-cell's early dysregulations of pancreatic β-cell that precede the autoimmune response associated with type 1 diabetes as this model gathers both genetic predisposition to type 1 diabetes and the absence of autoimmune response.

In addition to the viability reduction of mice-isolated pancreatic islets and β-cells following streptozotocine treatment, the STZ-treated NIT-1 cells model had yielded two main outcomes concerning the dysregulations that affect the pancreatic β-cells: (a) β-cell in a state of oxidative stress, and (b) β-cell in a state of endoplasmic reticulum stress.The first disorder was manifested by an increase in reactive oxygen species accumulation, the increase in the activity of the xanthine oxydase and superoxide dismutase, and a rise in the malondialdehyde level, as well as adecrease in reduced glutathione level. While, the state of endoplasmic stress was reflected by an up-regulation of three genes related to ER-stress associated death: NF-κB1, ATF4, and CHOP. Research has revealed that there may be a connection between these three genes and that their concurrent overexpression triggers apoptosis brought on by ER stress and the PERK pathway-related UPR response (Yamaguchi & Wang, 2004).

Overall, these results are of great importance, as it clearly and partially explain the mechanisms of contribution of pancreatic β-cell to its own death before the intervention of the immune system. These mechanisms include both the induction of oxidative stress and endoplasmic reticulum stress. Although NF-κB1, ATF4, and CHOP are primarily involved in cellular stress and death of pancreatic β-cells, the present findings highlight an additional role for these genes as potential genetic biomarkers. Their early and significant modulation under metabolic stress conditions suggests that that may serve as early indicators of β-cell dysfunction, detectable before irreversible β-cell damage. Transcriptomic quantification , particularly the combined analysis of NF-κB1, ATF4, and CHOP, could therefore define a molecular signature relevant for the early diagnostic, pending further clinical validation. Based on these informations that we have today, a complete understanding of a state of gene expression and proteins related to ER stress and oxidative stress in β-cell biology and their consequences on β-cell integrity will have a direct impact on future T1D management.

Although the wide range of synthetically developed medications, substances of natural origins including those derived from plants, animals, and microorganisms have gained a great interest and have served as the backbone of diseases prevention and treatment since ancient times. This is mainly due to their extraordinary diversity and their large spectrum of pharmacological properties (Gielecińska et al., 2023; Wangchuk, 2018). Hence, in this second section, we primary focus to explore the beneficial effects of two natural molecules, specifically curcumin and nicotinamide in managing type 1 diabetes (T1D) using a combination approaches that target both pancreatic β-cells and the immune system.

Curcumin is a natural lipophilic polyphenol derived from the Curcuma longa. It is utilized as a natural food coloring additive due to its intense yellow color. Furthermore, curcumin is widely recognized for its significant pharmacological properties including antioxidant, anti-inflammatory, antiviral, chemopreventive, chemotherapeutic, antinociceptive, proapoptotic, antiproliferative, antimalarial effects, andanti-parasitic (Urošević et al., 2022).

Nicotinamide is the amide derivative of vitamin B3 which can be sourced directly from a range of food products like meats, fish and eggs (Fricker et al., 2018). Nicotinamide possesses valuable medicinal effects, including anti-inflammatory (Yanez et al., 2019), anti-oxidant (Rehman et al., 2021), and anti-cancer (Al-Gayyar et al., 2019) properties. Moreover, nicotinamide has shown its efficacy against multiple disorders such as: skin lesions (Nouh et al., 2023), heart failure (Abdellatif et al., 2021), and neuronal injuries (Peterson et al., 2015).

First, the molecules under investigations could be toxic at higher doses. Therefore, it is crucial to assess the toxicity of a compound before its consideration as preventive or therapeutic agent for human use. We showed that curcumin and nicotinamide did not exhibit cytotoxic effects against mice-isolated pancreatic islets. We also showed that nicotinamide did not exert cytotoxic effect against pancreatic β-cells (NIT-1 cells) while curcumin induce a slight insignificant cytotoxic effect toward these cells. These outcomes show that the tested doses of curcumin and nicotinamide could be safely used in the ensuing tests.

The loss of beta cell mass is inevitable in the pathogenesis of type 1 diabetes (Subramanian et al., 2024). So, the discovery of safe agents that can protect pancreatic beta cells from death by targeting the β-cell itself would be a helpful approach to prevent and cure T1D at an early stage. Previous findings reported that streptozotocine induce β-cell death (Goyal et al., 2016). We therefore experimentally induced β-cell death using streptozotocine and then evaluated the possible protective effects of curcumin and nicotinamide against streptozotocine-induced mice isolated islets/beta-cells (NIT-1 cells) death. We demonstrated that the viability rates of mice isolated islets and NIT-1 cells were remarkably improved, suggesting that curcumin and nicotinamide prevent the death of mice isolated pancreatic islets and beta cells (NIT-1 cells).

Oxidative stress is one of the mechanisms underlying β-cell dysfunction and damage leading to progression of T1D as well its complications (Zhang et al., 2020). Furthermore, β-cells are the most sensitive cells to oxidative stress and damage by ROS due to their lower antioxidant capacity in terms of endogenous antioxidant enzyme such as SOD1, SOD2, Gpx1, and catalase (Darenskaya et al., 2021). Therefore, β-cell protection from oxidative stress-induced damage would also be a successful strategy to manage T1D. Hence, to evaluate the antioxidant activities of curcumin and nicotinamide, we experimentally used streptozotocine to induce reactive oxygen species production in beta-cells (NIT-1). STZ is a nitrosamine generated by the soil bacterium *Streptomyces achromogenes*. Its structure resembles that of glucose, enabling it to preferentially enter pancreatic beta cells through the GLUT-2 glucose receptor and induces their death. This death is primarily mediated by the generation of reactive oxygen species (ROS) (Goyal et al., 2016). Our results demonstrate that pre-treatment of beta-cells with both curcumin and nicotinamide markedly reduced ROS accumulation in these cells, indicating the antioxidants abilities of curcumin and nicotinamide. Based on these findings and on the mechanism of STZ-induced beta cell death, which include ROS production, we speculate that the protective effects of curcumin and nicotinamide against STZ-induced beta-cell death may be partly due to their anti-oxidants

properties that protect beta cells from oxidative stress.

Additionally, a further analysis of cellular oxidative status following β-cell (NIT-1 cells) treatment with low and high doses of curcumin allowed us to speculate that this molecule act as antioxidant in low doses and as pro-oxidant in high doses. This fact is reflected by the improvement of antioxidant defense system (SOD and GSH) and reduction of lipid peroxidation product (MDA) in β-cells under treatment with 30µg/ml curcumin. While, treatment with 60 µg/ml dose reduce the SOD activity and GSH level with an increase in MDA level compared to β-cells treated with low dose of curcumin (30 µg/ml).

To further investigate the antioxidant abilities of nicotinamide, we evaluated the possible in vitro inhibitory effect of nicotinamide on xanthine oxidase activity. This latter is a pro-oxidant enzyme that catalyze the last steps of purine catabolism. It mediate the oxidation of hypoxanthine to xanthine and subsequently to uric acid. The two breakdown reactions are accompanied by superoxide anions ($O2\cdot-$) and hydrogen peroxide (H_2O_2) production (Kostić et al., 2015). Therefore, the hyperactivity of XO results in an hyperuricemia state as well as excessive generation of reactive oxygen species: $O2\cdot-$and H_2O_2, a state known as oxidative stress (Ito et al., 2019). It should be noted that hyperuricemia is also involved in the induction of beta-cell death and dysfunction (Lu et al., 2020). Our present results showed that nicotinamide exert an efficient inhibitory effect on xanthine oxidase activity. These findings indicate not only the potential of nicotinamide to act as antioxidant agent but also its ability to prevent hyperuricemia.

In conjunction with attempts to protect beta cells from death, strategies to promote insulin secretion in glucose-dependent manner from beta cells would be a useful tool in treating type 1 diabetes. Currently, US Food and the Drug Administration (FDA) has approved only two classes of molecules as insulin secretagogues for the treatment of type 1 diabetes: glinides and sulphonylureas. Nevertheless, these molecules function in glucose independent manner, raising the risk of harmful hypoglycemia (Vetere et al., 2014). Hence, we tempt in our current study to improve insulin secretion in glucose-dependent manner by using curcumin and nicotinamide as modulators. Our present results evidently demonstrated a significant improvement of glucose-induced insulin secretion from mice-isolated pancreatic islets following exposure to curcumin and nicotinamide. However, we didn’t observe such an effect when treating pancreatic beta cells (NIT-1 cells) with these molecules. The ability of these molecules to enhance glucose-stimulated insulin secretion (GSIS) from Langerhans’ islets, but not from NIT-1 cells, may be attributed to the complex cellular communication

between the different cell types within the pancreatic islets which modulate the β-cell behavior. In contrast, NIT-1 cells function in independent manner. Previous research support this hypothesis, highlighting that normal insulin secretion is not solely a product of β-cell function but also relies on the harmonious collaboration of these cells with their surrounding other cell types within the islets. This synergy is crucial for maintaining proper insulin secretion (Lernmark, 1974; Svendsen et al., 2018; Van Der Meulen et al., 2015).

Despite the wide spectrum of beneficial properties of curcumin and nicotinamide founded in this study, it has to be kept in the mind that no chemical agent can be guaranteed as toxicity-free. Hence, a throughout research was conducted to confirm that these compound are biologically safe and devoid of cytotoxic effects toward pancreatic β-cells. Importantly, a molecular analysis performed in this current study showed that high dose (60µg/ml) of both curcumin and nicotinamide induce an up-regulation of three genes (NF-κB1, ATF4, CHOP) that are implicated in endoplasmic reticulum stress-induced death, indicating at some extent the negative effects of the overdose of these molecules on beta-cells. To avoid long term toxicity from these molecules in beta cells, several strategies prove beneficial. First, low sub-toxic doses (Aldini, 2020), validated via gene and function tests to prevents NF-κB1, ATF4, CHOP activation. Moreover, combining these molecules with ER stress relievers such as chemical chaperons TUDCA or 4-PBA effectively reduces UPR toxicity while boosting cell survival (Da Silva, 2019; Xing, 2022). Additinally, slow-release nanoparticles control exposure to prevent toxic peaks (Sanna, 2021). Finally, regular biomarkers monotoring ensure precise dose adjustments over time. In addition, the assessment of genetic expression of other genes implicated in cellular death pathways, cellular stress and cellular survival may provide deep insights and well understanding into safety of these molecules on beta-cells.

A further in silico study was conducted to explore the interactions of curcumin with proteins and biological pathways. The findings clearly demonstrated that curcumin interacts with proteins involved in oxidative stress response, inflammation, and apoptosis. These results clarify at some extent the experimental effects of curcumin. However, this bioinformatics study did not provide information into the dose-effect relationship.

An alternative approach for preserving pancreatic β-cells from autoimmune attacks in type 1 diabetes is to follow an immunomodulatory intervention. Such intervention may selectively target specific immune cells and mediators. Importantly, the strong involvement of neutrophils in T1D pathogenesis and pancreatic β-cell destruction (Obeagu & Obeagu, 2023) has led us to search neutrophil-targeted therapies. NETs extrusion is a mechanism by which

neutrophils are involved in the pathogenesis of T1D. NETs have the ability to stimulate inflammation and the subsequent initiation of a T cell-mediated immune response in type 1 diabetes (Parackova et al., 2020). Indeed, NETs formation is regulated by two key enzymes, neutrophil elastase (NE) and myeloperoxydase (MPO), that are stored in primary granules of neutrophils (Papayannopoulos et al., 2010). In addition, the serum level of MPO and NE were markedly increased in T1D (Klocperk et al., 2021; Wang et al., 2014). Furthermore, a study by Shu et al. (2020) has demonstrated the role of elastase in the onset and progression of β-cell autoimmunity via the induction of innate immune responses in pancreatic islets. Considering these informations, neutrophil viability, along with the activities of human neutrophil elastase (HNE) and myeloperoxidase (MPO), may serve as important targets for immunomodulation to attenuate pathological inflammation driven by neutrophils in order to prevent or at least alleviate the severity of type 1 diabetes. In this connection, our current results have demonstrate the potent ability of curcumin to induce neutrophil death in the one hand, and to reduce the activity of MPO and elastase in the other hand. Further, the results from the DFT analysis of curcumin and the molecular docking analysis support the inhibitory potential of curcumin against elastase and myeloperoxidase. These results are in line with previous works, which have reported that curcumin possess potent anti-inflammatory properties (Bisset et al., 2021). While, our present results have also shown the failure of nicotinamide to induce neutrophil viability reduction and the MPO and elastase activity inhibition. These finding indicate the inability of nicotinamide to act as a modulator of neutrophil lifespan and the activity of MPO and elastase.

Conclusion and perspectives

This research aimed to explore early pancreatic β-cell disorders preceding the autoimmune onset of Type 1 Diabetes to reveal novel therapeutic targets, while evaluating the beneficial properties of curcumin and nicotinamide in managing the disease.

Using streptozotocin-treated NIT-1 cells as a study model to investigate β-cell's early dysregulations, the results have revealed valuable informations concerning this disorders. It has been shown that beta-cell was under states of oxidative stress and an up-regulation of a set of genes (NF-κB1, ATF4, and CHOP) implicated in reticulum endoplasmic stress-induced death.

The evaluation of curcumin and nicotinamide highlights their potential as novel antidiabetic agents or adjuvants. Curcumin exhibits a versatile profile, acting as an immunomodulator by inhibiting neutrophil elastase (HNE) and MPO, and reducing neutrophil viability, findings supported by DFT and Molecular Docking analysis. It further protects NIT-1 cells from STZ-induced death and oxidative stress, specifically improving oxidative status at 30 µg/ml (decreased MDA/SOD, increased GSH) and decreasing ROS accumulation. Conversely, the benefits of nicotinamide are primarily mediated by its potent antioxidant activity, specifically through the reduction of ROS accumulation in NIT-1 cells and the inhibition of xanthine oxidase. Furthermore, nicotinamide protects NIT-1 cells from STZ-induced death and enhances insulin secretion in isolated pancreatic islets.

Despite these benefits, the study highlights the critical importance of dosage in the administration of these compounds. While nicotinamide showed no significant cytotoxicity, curcumin exhibited a slight pro-oxidant effect and minor NIT-1 cytotoxicity at higher concentrations (60 µg/ml). Furthermore, the observation that both molecules can, to some extent, induce the expression of genes (NF-κB1, ATF4, CHOP) related to ER stress-induced death warrants a cautious approach.In addition, In silico study showed the interaction of curcumin with proteins related to inflammation, oxidative stress, and apoptosis pathways.

In conclusion, while curcumin and nicotinamide represent viable candidates for the development of novel pharmaceuticals or adjuvants in the management of Type 1 Diabetes, their therapeutic integration requires rigorous dose optimization to ensure a balance between cytoprotective efficacy and safety. The findings of this study warrant further profound

investigations regarding in particular:

- ✓ The conduction of a more in-depth research using genomics, transcriptomics, and proteomics are crucial to fully understand pancreatic β-cell's dysregulations in order to validate new preventive and therapeutic targets for T1D.
- ✓ The *in vivo* evaluation and confirmation of the potentially valuable effects of curcumin and nicotinamide for managing type 1 diabetes.
- ✓ The investigation of curcumin and nicotinamide delivery system in order to avoid the issue of low bioavailability that may restrict the effectiveness of natural compounds.
- ✓ A thorough investigation into the pharmacokinetics and pharmacodynamics of curcumin and nicotinamide to define the optimal dose for both prevention and treatment of type 1 diabetes.
- ✓ In-depth and long-term studies on the toxicity of curcumin and nicotinamide *in vitro* and *in vivo* to determine the safe doses with no or minimum toxicity, that is a necessary step before any clinical application.
- ✓ The combination of curcumin and nicotinamide in hope of improving the effectiveness of these natural molecules as a result of their potential additive or synergistic effects.
- ✓ Expanding the scope of beneficial effects of curcumin and nicotinamide by the use of these molecules to target additional immune pathways involved in the pathogenesis of type 1 diabetes on the one hand, and by testing the ability of these molecules to induce pancreatic β-cell regeneration from residual β-cells in the other hand.

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الملخص

تهدف هذه الدراسة إلى الكشف عن بعض الاضطرابات المبكرة في خلايا بيتا البنكرياسية، التي تُعدّ أحد العوامل الرئيسية في تطور داء السكري من النوع الأول، بالإضافة إلى تقييم التأثيرات البيولوجية لكل من الكركمين والنيكوتيناميد كمركبات يمكن أن يكون لها دور علاجي لهذا المرض. تم استخدام الخلايا NIT-1 المعالجة بالستربتوزوتوسين (STZ) كنموذج تجريبي لمحاكاة خلل الخلايا بيتا. وقد تبين أن معالجة الخلايا NIT-1 والجزر البنكرياسية المعزولة من الفئران بالستربتوزوتوسين (STZ) أدى إلى موت كل هذه الخلايا، مصحوبة بزيادة في التعبير الجيني لكل من ATF4 ، وNF-κB1، وCHOP على مستوى الخلايا NIT-1، وهي جينات مرتبطة بإجهاد الشبكة الإندوبلازمية، إلى جانب مؤشرات واضحة للإجهاد التأكسدي، بما في ذلك انخفاض مستوى الجلوتاثيون (GSH)، وارتفاع مستويات المالوندیالدهید (MDA)، وتراكم أنواع مركبات الأكسجين التفاعلية (ROS)، وزيادة أنشطة أكسيداز الزانثين (XO) وسوبر أكسيد ديسميوتاز (SOD). بعدها تمت" دراسة تأثيرات كل من الكركمين والنيكوتيناميد الواقية لخلايا NIT-1 والجزر البنكرياسية. حافظ النيكوتيناميد على سلامة الخلايا المذكورة، في حين وبعد المعالجة بالكركمين، تمت ملاحظة سمية خلوية طفيفة في الخلايا NIT-1 من غير أن تكون لها دلالة إحصائية. وكتأثيرات إيجابية عزز كل من المركبين إفراز الإنسولين في الجزر البنكرياسية بشكل كبير. من جهة أخرى أدى الكركمين إلى انخفاضه في خلايا NIT-1، بينما زاد النيكوتيناميد من إفراز الأنسولين في هذه الخلايا. أما في ما يخص موت الخلايا فقد أسهم المركبان في إبقاء الخلايا على قيد الحياة رغم معالجتها بالستربتوزوتوسين (STZ) وقللا من تراكم ROS بشكل واضح. أظهر الكركمين بتركيز منخفض (30 ميكروغرام/مل) خصائص مضادة للأكسدة تمثلت في رفع مستويات GSH وخفض مستويات MDA وSOD، بينما أدى استخدامه بتركيز عال„ (60 ميكروغرام/مل) إلى الإجهاد التأكسدي. أما النيكوتيناميد، فقد أظهر نشاط"ا (في الزجاج) مضا"دا للأكسدة يتمثل في تثبيط إنزيم أكسيداز الزانثين (XO) بطريقة تتعلق بالجرعة. من جهة أخرى، تمت دراسة تأثير المركبين على تعبير الجينات NF-κB1 و ATF4 وCHOP، إذ تبين أن الجرعات العالية (60 ميكروغرام/مل) لكلا المركبين ترفع من مستويات التعبير عن هذه المورثات. أما الدراسة البيولوجية الحاسوبية (bioinformatics) فقد أظهرت قدرة الكركمين على الارتباط بمجموعة من البروتينات المتدخلة في الالتهاب والإجهاد التأكسدي والموت المبرمج للخلايا. كما تم تقييم تنظيم النشاط المناعي لكل من الكركمين والنيكوتيناميد على العدلات البشرية (neutrophils) ، حيث قلل الكركمين بشكل واضح من عمر هذه الخلايا، ونشاطي الإيلاستاز والميلوبيروكسيداز بطريقة تتعلق بالجرعة، بينما لم يظهر النيكوتيناميد أي تأثير مناعي ملحوظ. أخير"ا، بينت التحليلات الحاسوبية (computational studies) الكفاءة التثبيطية للكركمين تجاه إنزيمي HNE وMPO من خلال تحليل التحام الجزيئات وحسابات نظرية الكثافة الوظيفية وتحليل الجهد الكهروستاتيكي، كما كشفت عن قوة ألفة ارتباطه بالمواقع الفعالة، إلى جانب خصائصه الإلكترونية والطاقوية المتميزة.

الكلمات المفتاحية: مرض السكري من النوع الأول، الخلايا بيتا البنكرياسية، الجزر البنكرياسية، الخلايا المتعادلة، السمية الخلوية، إفراز الأنسولين، نشاط مضاد للأكسدة، إنزيم أكسيداز الزانثين، إنزيم الميلوبيروكسيديز، التعديل المناعي، إنزيم الايلاستاز، كركمين، نيكوتيناميد.

Abstract

This study aims to identify certain early pancreatic beta cell's dysregulations responsible for type 1 diabetes development a nd to evaluate certain biological effects of curcumin and nicotinamide that may be effective in the management of type 1 diabetes. Initially, β-cell disorders were studied using streptozotocin-treated NIT-1 cells as an experimental model. Streptozotocin significantly induced the death of both β-cells and mice-isolated pancreatic islets. Streptozotocine-treated NIT-1cells showed a set of disorders including an up-regulation of three genes expression related to ER-stress (ATF4, NF-κB1, and CHOP), and a state of oxidative stress which was observed as a decrease in GSH level and an increase in ROS accumulation, MDA level, and the activities of XO and SOD. Secondly, the potential beneficial effects of curcumin and nicotinamide on pancreatic beta-cells (NIT-1 cell line) and pancreatic islets (isolated from mice) were evaluated. Nicotinamide was non-toxic to both islets and NIT-1 cells, while curcumin showed a minor insignificant toxicity to NIT-1 cells .Curcumin and nicotinamide significantly enhanced insulin secretion in pancreatic islets. Curcumin significantly reduced insulin secretion in β-cells, whereas nicotinamide slightly increased this secretion. Both molecules significantly protected β-cells against the STZ-induced death and STZ-induced ROS accumulation. Curcumin at low dose (30µg/ml) exerted an antioxidant effect in β-cell as reflected by high level of GSH and low levels of MDA and SOD, while at high dose (60µg/ml), it induced oxidative stress. The antioxidant activity of nicotinamide was confirmed *in vitro* by evaluating its effect on xanthine oxydase activity. Nicotinamide showed remarquable and dose-dependent inhibition of XO activity. The possible side effects of these molecules on pancreatic β-cell was also verified by assessing their impact on the expression of NF-κB1, ATF4, and CHOP genes. Both molecules at high dose (60µg/ml) increased the expression of these genes. In silico study showed the interaction of curcumin with a set of proteins involved in apoptosis, oxidative stress, and inflammation.The immuno-modulatory activity of curcumin and nicotinamide on human neutrophil was also assessed. Curcumin significantly decreased neutrophil viability, elastase activity, and myeloperoxidase activity in a dose-dependent manner, while nicotinamide showed no immunomodulatory effects on these parameters. Curcumin's potential as a HNE and MPO inhibitor was confirmed through computational studies, including molecular docking analysis, DFT calculations, and MEP analysis, which revealed its high affinity with HNE and MPO enzyme active sites and provide informations on the electronic, energetic, and elecroteostatic characteristics of curcumin.

Keywords: Autoimmune type 1 diabetes, pancreatic β-cells, pancreatic islets, Neutrophils, Cytotoxicity, Insulin secretion, Antioxidant activity, Xanthine oxidase, Myeloperoxidase, Elastase, Immunomodulation, Curcumin, Nicotinamide.

Résumé

Cette étude vise à identifier certains dysrèglements précoces de cellules bêta pancréatiques responsables du développement du diabète de type 1 ainsi qu'à évaluer certains effets biologiques de la curcumine et du nicotinamide qui peuvent être efficaces dans la gestion du diabète de type 1. Initialement, les troubles des cellules bêta ont été étudiés en utilisant des cellules NIT-1 traitées à la streptozotocine comme modèle expérimental. La streptozotocine a induit significativement la mort des cellules bêta et les îlots pancréatiques isolés de souris. Les cellules NIT-1 traitées par la streptozotocine ont montré un ensemble de troubles, y compris une augmentation de l'expression de trois gènes liés au stress du réticulum endoplasmique (ATF4, NF-κB1, et CHOP), et un état de stress oxydatif qui s'est manifesté par une diminution du niveau de GSH et une augmentation du niveau de MDA, et des activités de XO et de SOD. Deuxièmement, les effets bénéfiques potentiels de la curcumine et du nicotinamide sur les cellules bêta pancréatiques (la lignée cellulaire NIT-1) et les îlots pancréatiques (isolés des souris) ont été évalués. La nicotinamide n'était pas toxique pour les îlots et les cellules NIT-1, tandis que la curcumine présentait une toxicité mineure et insignifiante pour les cellules NIT-1. La curcumine et la nicotinamide ont augmenté significativement la sécrétion d'insuline dans les îlots pancréatiques. La curcu mine a réduit significativement la sécrétion d'insuline dans les cellules bêta, alors que la nicotinamide augmente légèrement cette sécretion. Les deux molécules ont significativement protégé les cellules bêta contre la mort induite par la streptozotocine et contre l'accumulation des espèces réactives de l'oxygène induite par la streptozotocine. La curcumine à faible dose (30 µg/ml) a exercé un effet antioxydant dans les cellules bêta , ce qui s'est traduit par des niveaux élevés de GSH et de faibles niveaux de MDA et de SOD, tandis qu'à forte dose (60 µg/ml), elle a induit un stress oxydatif. L'activité antioxydante du nicotinamide a été confirmée *in vitro* en évaluant son effet sur l'activité de XO. La Nicotinamide a montré une inhibition remarquable et dose-dépendante de l'activité de XO. Les effets secondaires possibles de ces molécules sur les cellules bêta pancréatiques ont été également vérifiés en évaluant leur impact sur l'expression des gènes NF-κB1, ATF4 et CHOP. Les deux molécules à forte dose (60 µg/ml) ont augmenté l'expression de ces gènes. Une étude in silico a montré l'interaction de la curcumine avec un ensemble de protéines impliquées dans l'apoptose, le stress oxydatif et l'inflammation. L'activité immunomodulatrice de la curcumine et de la nicotinamide sur les neutrophiles humains a été également évaluée. La curcumine a significativement diminué la viabilité des neutrophiles, l'activité de l'élastase et l'activité de la myéloperoxydase de manière dose-dépendante, tandis que le nicotinamide n'a montré aucun effet immunomodulateur sur ces paramètres. Le potentiel de la curcumi ne en tant qu'inhibiteur de HNE et de MPO a été confirmé par des études computationelles, notamment une analyse d'amarrage moléculaire, des calculs de Théorie de la fonctionelle de la densité, et une analyse de potentiel électrostatique moléculaire , qui ont révélé sa forte affinité avec les sites actifs des enzymes HNE et MPO et ont fourni des informations sur les caractéristiques électroniques, énergétiques et électrostatiques de la curcumine.

Les mots clés: Diabète auto-immun de Type 1, Cellules bêta pancreatiques, îlots pancréatiques, Neutrophiles, Cytotoxicité, Sécrétion d'insuline, Activité antioxydante , Xanthine oxydase, Myéloperoxydase, Élastase, Immunomodulation, Curcumine, Nicotinamide.